HOST-PLANT ACCEPTANCE BY THE CARROT FLY: SOME UNDERLYING MECHANISMS AND THE RELATIONSHIP TO HOST-PLANT SUITABILITY

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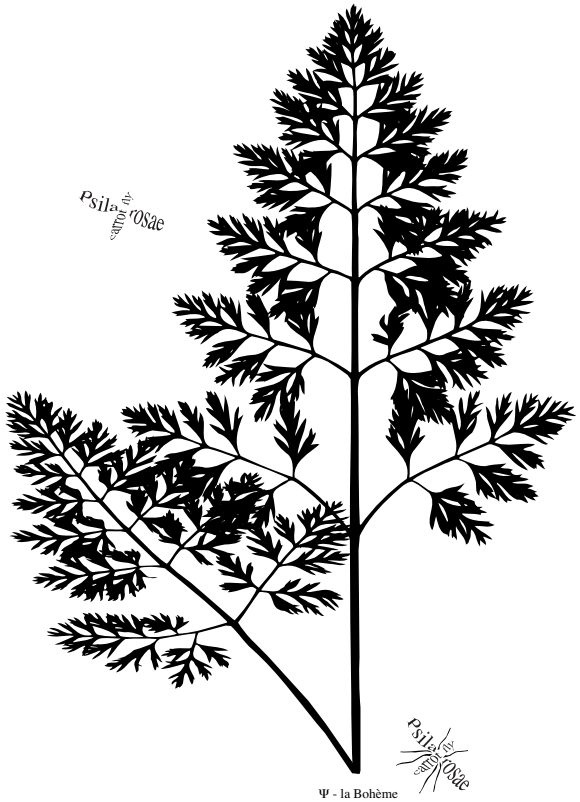

Ψ - la Bohème

Psiladies, you don't adore the perfume of roses
as anyone who knows just your name inevitably supposes.
Instead you better like to smell with your antennal noses
the scents of those humble umbel bearers even in minute doses.
They unwillingly attract you with these essences meant as defences
against their adversaries, but you – malign – use all your senses
to find your victims far or close: olfaction as well as vision,
and no surprise if also taste is involved in your decision.
But may the how of the latter still be mysterious to us
the why of your choosiness is even more so – alas!
Of course, as mothers you are supposed to bother
first of all about your progeny's nutritional need,
as your child cannot easily move to another
far away suitable host on which to feed.
But mothers do not always know best,
on some occasions you fail the test
and lay some eggs beneath a root
which is no good for your brood.
But foliage is what you inspect
and the access is only indirect
to information about the part
hidden in the soil, so it's art
to make the right selection
based on your predilection.
Yet, if your choice is good,
there will be proper food
for the future generation,
the larvae in consideration.
With their solid upper lip
larva they'll commence to rip
the carrot's skin
larva at the
root
larva tip
tip
larva tip larva
tip
tip
tip

Dedicated to

my Mother, my Father, my Sister and my little Niece

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Summary

The larvae of the carrot fly, *Psila rosae* (F.) (Diptera: Psilidae), attack the roots of numerous wild and cultivated plants belonging to the family Umbelliferae (= Apiaceae). Since they have only a limited capacity to move within the soil and to find suitable host plants on their own, their survival depends largely on the host choice by the ovipositing female flies. In a previous study, characteristic allelochemicals in the leaf surface of carrots were shown to elicit oviposition. However, these oviposition stimulants could not explain the preference of the carrot fly for particular carrot cultivars. In this thesis I therefore expanded the investigations, which had so far focused on carrot, *Daucus carota*, the economically most important host plant, to a wider range of host species differing considerably in susceptibility. This study was intended to be a further step towards the identification of the physical and chemical plant traits that account for the host-plant preferences of the carrot fly. This should eventually result in a better understanding of the mechanisms of host-plant resistance to this pest insect.

As a basis for the subsequent investigations into chemical plant cues mediating host acceptance, I determined the preference hierarchy of the carrot fly for 30 umbelliferous host-plant species respectively varieties and 6 non-hosts. To this end, foliage of the test plants was presented to caged populations of carrot flies in dual and multiple choice oviposition assays together with leaves of a standard plant, the susceptible carrot cultivar "Danvers". Only two species, *Anthriscus cerefolium* (garden chervil) and *Carum carvi* (caraway), proved to be more acceptable than the standard plant, about half of the species received significantly fewer eggs. *Pimpinella major* (greater burnet saxifrage) was the least preferred umbelliferous species and was ranked within the non-hosts. Differences in post-alighting, pre-ovipositional behaviour of the female flies on the leaves, i.e. in the proportion of exploratory runs leading to oviposition, were responsible for a major part of the variation recorded in the number of eggs laid. A nearly identical set of plant species was in addition tested for the suitability to support larval development. I collected the pupae and non-pupated third instar larvae that had grown on potted plants inoculated with a constant number of eggs. Both the number and weights of individuals produced varied widely among the plant species. *Pimpinella major* was the only umbelliferous plant included that did not give rise to any carrot flies. The two Compositae (= Asteraceae) *Cichorium intybus* (chicory) and *Tanacetum vulgare* (tansy) likewise failed to support larval development. The acceptability of various plant species to ovipositing carrot flies was only weakly, but significantly correlated with the suitability of the respective plants for larval development. Both adult host-plant preferences and larval performance accounted for a part of the variation in susceptibility among the plants observed in the field. Across the whole set of plant species examined, antixenosis contributed more substantially to resistance than antibiosis, whereas the reverse appeared to be true for carrot cultivars.

To assess the effect of non-chemical plant traits on the oviposition behaviour, the carrot flies were exposed to various surrogate leaves made of paper, that differed in colour, shape and surface characteristics. In the presence of host-plant extracts, physical factors strongly influenced oviposition. Green, yellow and orange three-dimensional models similar in shape to host-plant leaves and covered with a thin layer of paraffin wax were most acceptable to the females. In a comparative study, the preference for pinnate leaves, which are typical of the umbelliferous hosts, was shown to be specific to the carrot fly, as two other oligophagous flies, the cabbage root fly and the onion fly, showed diverging ovipositional responses to the same set of precise imitations of real host and non-host leaves.

Extracts prepared from undamaged carrot foliage with various extraction methods were compared in choice assays regarding their effectiveness in stimulating oviposition. Surrogate leaves treated with a warm hexane surface extract, which was produced with a new microwave-assisted procedure, were almost as acceptable to the flies as real host leaves. This hexane extract elicited about twice as much oviposition than the previously used dichloromethane surface extract and the diethyl ether fraction of an extract that was obtained by a short immersion of the leaves into almost boiling water. Egg-laying in response to crude methanol and hot water extracts was only weak owing to the presence of yet unidentified polar deterrent compounds.

The ovipositional responses of the carrot fly to foliage of various host-plant species were compared with the responses to the corresponding leaf surface extracts. The stimulatory activity of dichloromethane extracts and the diethyl ether fraction of hot water extracts did not reflect accurately the differential acceptability observed among the respective intact leaves. The preference ranking for the warm hexane extracts seemed to match better the ranking for real leaves. Two out of five silicagel column fractions of this crude hexane extract were shown to stimulate oviposition: the diethyl ether and the methanol fraction. The highly active diethyl ether fraction was analysed by GC-MS for the previously identified oviposition stimulants: phenylpropenes (*trans*-methylisoeugenol, *trans*-asarone), furanocoumarins (xanthotoxin, bergapten) and polyacetylenes (falcarinol, falcarindiol). The various plant species exhibited widely differing profiles of these stimulatory compounds, which cautions against generalizing results obtained from a single typical host plant of an oligophagous insect. Only fractions that contained moderate to high levels of at least two of these compound groups elicited strong ovipositional responses (e.g. phenylpropenes and polyacetylenes in carrot, *Daucus carota*, furanocoumarins and polyacetylenes in hogweed, *Heracleum sphondylium*, and hemlock, *Conium maculatum*). The contents of the known stimulants thus accounted in a synergistic manner for the variable activity of the diethyl ether fraction, but could not explain adequately the preference of the carrot fly for particular host-plant species (e.g. *Anthriscus cerefolium*). The ranking of the species according to the variable activities of the methanolic fraction, which must be attributed to yet unidentified stimulatory compounds, corresponded much better with the preferences of the carrot fly for the intact leaves. Furthermore, the water fractions of several hot water extracts were shown to reduce egg-laying below surrogate leaves that were pre-treated with a stimulatory extract. This inhibitory effect was particularly strong in the non-preferred species *Pimpinella major*, which suggests that yet unidentified polar deterrents may also contribute to differences in the acceptability of host plants.

The findings of this thesis confirm the hypothesis that semiochemicals in the leaf surface are central to the understanding of host acceptance in the carrot fly. As in other herbivorous insects, the oviposition behaviour of the carrot fly is influenced by complex mixtures of stimulatory and probably also inhibitory compounds, which are perceived by different chemosensory organs (olfactory sensilla at the antennae and tarsal contact chemoreceptors). This study sets the stage for further characterisations of behaviourally active compounds.

Zusammenfassung

Die Larven der Möhrenfliege, *Psila rosae* (F.) (Diptera: Psilidae), befallen die Wurzeln von zahlreichen wilden und kultivierten Pflanzen aus der Familie Umbelliferae (= Apiaceae). Weil sie aufgrund ihrer geringen Fortbewegungskapazität im Boden nur beschränkt aus eigener Kraft geeignete Wirtspflanzen auffinden können, hängt ihr Überleben entscheidend von der Wirtswahl der Fliegen bei der Eiablage ab. In früheren Untersuchungen wurde gezeigt, dass charakteristische sekundäre Pflanzenstoffe in der Blattoberfläche von Karottenpflanzen bei den Weibchen die Eiablage auslösen. Diese Eiablagestimulanzien konnten jedoch die Präferenz der Möhrenfliege für bestimmte Karottensorten nicht erklären. Ich dehnte darum die Untersuchungen, die sich bisher auf die wirtschaftlich bedeutendste Wirtspflanze, die Karotte, konzentrierten, auf ein breiteres Spektrum von Wirtsarten mit stark unterschiedlicher Befallsanfälligkeit aus. Diese Studie stellt einen weiteren Schritt zur Identifizierung der chemischen und physikalischen Pflanzeneigenschaften dar, welche die Wirtspräferenzen der Möhrenfliege massgeblich beeinflussen. Damit soll schliesslich ein besseres Verständnis der in den Wirtspflanzen gegen dieses Schadinsekt wirksamen Resistenzmechanismen erreicht werden.

Als Grundlage für die darauffolgenden Untersuchungen über den Einfluss von chemischen Pflanzensignalen auf die Wirtswahl, wurde die Rangfolge von 30 Umbelliferenarten bzw. Varietäten und 6 Nichtwirtspflanzen in der Eiablagepräferenz der Möhrenfliege bestimmt. Zu diesem Zweck wurden Blätter von Testpflanzen zusammen mit solchen einer Standardpflanze, der Karottensorte "Danvers", in Zweifach- und Mehrfachwahlversuchen den in Käfigen gehaltenen Fliegen zur Eiablage angeboten. Nur zwei Arten, *Anthriscus cerefolium* (Garten-Kerbel) und *Carum carvi* (Kümmel), wurden der Standardpflanze vorgezogen. Bei etwa der Hälfte der Arten wurden signifikant weniger Eier als beim Standard abgelegt. *Pimpinella major* (Grosse Bibernelle) war die am wenigsten beliebte Umbelliferenart und überschnitt sich in der Rangfolge mit Nichtwirtspflanzen. Unterschiede im Verhalten der Fliege nach dem Landen auf den Blättern, d.h. im Anteil der Blattläufe, die zur Eiablage führten, waren verantwortlich für einen Grossteil der Variation in der Anzahl der abgelegten Eier. Eine nahezu identische Auswahl von Pflanzenarten wurde zusätzlich auf ihre Eignung für die Larvalentwicklung der Möhrenfliege hin getestet. Dazu wurden Topfpflanzen mit einer konstanten Anzahl Eier inokuliert. Sowohl die Anzahl als auch die Gewichte der dabei erhaltenen Möhrenfliegenindividuen – Puppen und nicht verpuppte L3-Larvenstadien – variierten stark in Abhängigkeit von der Pflanzenart, auf der sie sich entwickelt hatten. *Pimpinella major* war die einzige Umbelliferenart, die gar keine Möhrenfliegen hervorbrachte. Letzteres traf auch auf die beiden Compositen *Cichorium intybus* (Wegwarte, Zichorie) und *Tanacetum vulgare* (Rainfarn) zu. Die Eiablagepräferenz der Imagines stimmte nur in groben Zügen mit der relativen Eignung der jeweiligen Pflanzenarten für die Larvalentwicklung überein. Beide Faktoren, die Wirtswahl und Wirtseignung, erklärten einen Teil der im Feld beobachteten Variation in der Anfälligkeit der Pflanzen. Antixenosis trug stärker zur Resistenz der Pflanzen bei als Antibiosis, wenn die gesamte Bandbreite der untersuchten Arten betrachtet wurde, währenddem für Karottenvarietäten das Umgekehrte zu gelten schien.

Um den Einfluss von nicht-chemischen Pflanzenmerkmal auf das Eiablageverhalten abzuschätzen, wurden den Möhrenfliegen diverse künstliche Blätter aus Papier angeboten, die sich in Farbe, Form und Oberflächeneigenschaften unterschieden. In Gegenwart von Wirtspflanzenextrakten als chemische Reize übten diese physikalischen Faktoren eine grosse Wirkung auf die Eiablage aus. Die Fliegenweibchen bevorzugten grüne, gelbe und orange dreidimensionale Blattmodelle, die eine ähnliche Form wie Wirtspflanzenblätter aufwiesen und mit einer dünnen Paraffinschicht überzogen waren. In einer vergleichenden Studie konnte gezeigt werden, dass die Vorliebe für gefiederte Blätter, also für die typische Blattform von Umbelliferen, spezifisch für die Möhrenfliege ist, da sich zwei andere oligophage Fliegenarten, die Kohlfliege und die Zwiebelfliege, durch andere Präferenzen bei der Eiablage auszeichneten, wenn ihnen dieselbe Auswahl an genauen Imitationen von Wirts- und Nichtwirtsblättern dargeboten wurde.

Mit verschiedenen Verfahren hergestellte Extrakte von unbeschädigten Karottenblättern wurden in Wahlversuchen auf ihre eiablagestimulierende Wirkung hin miteinander verglichen. Mit einem warmen Hexanoberflächenextrakt behandelte Blattmodelle waren für die Fliegen beinahe so akzeptabel wie echte Wirtspflanzenblätter. Dieses Hexanextakt löste die Eiablage etwa doppelt so wirksam aus wie das früher verwendete Methylenchloridoberflächenextrakt und die Diäthylätherphase eines Extraktes, das durch kurzzeitiges Eintauchen der Blätter in fast siedendes Wasser gewonnen wurde. Rohe Methanol- und Heisswasserextrakte hatten nur eine schwach stimulierende Wirkung, da sie vermutlich noch eiablagehemmende Stoffe enthielten.

Die Eiablagepräferenzen der Möhrenfliege für Blätter von verschiedenen Wirtsarten wurden verglichen mit den Präferenzen für die entsprechenden Blattoberflächenextrakte. Die stimulierende Aktivität von Methylenchloridextrakten und von der Ätherphase von Heisswasserextrakten widerspiegelte die unterschiedliche Beliebtheit der jeweiligen Blätter nur ungenau. Eine bessere Übereinstimmung in dieser Hinsicht wurde mit den warmen Hexanextrakten erzielt. Zwei von fünf Kieselgelsäulenfraktionen von diesem Hexanrohextrakt erwiesen sich als eiablagestimulierend: Die Diäthyläther- und die Methanolfraktion. Die hochaktive Diäthylätherfraktion wurde mit GC-MS auf die schon bekannten Stimulanzien hin analysiert: Phenylpropene (*trans*-Methylisoeugenol, *trans*-Asaron), Furanocumarine (Xanthotoxin, Bergapten) und Polyacetylene (Falcarinol, Falcarindiol). Die verschiedenen Pflanzenarten zeigten stark unterschiedliche Muster in der Verteilung dieser Sekundärstoffe, was zur Vorsicht mahnt bei der Verallgemeinerung von Ergebnissen, die nur von einer einzigen typischen Wirtspflanze eines oligophagen Insektes erhalten wurden. Nur Fraktionen, die mittlere bis hohe Mengen von mindestens zwei dieser Substanzgruppen enthielten, lösten bei der Möhrenfliege starke Eiablagereaktionen aus (z.B. Phenylpropene und Polyacetylene in der Möhre, *Daucus carota*, Furanocumarine und Polyacetylene in Wiesen-Bärenklau, *Heracleum sphondylium*, und Fleckenschierling, *Conium maculatum*). Die Gehalte an Eiablagestimulanzien waren somit in synergistischer Weise für die Aktivität der Diäthylätherfraktion verantwortlich, konnten aber die Vorliebe der Möhrenfliege für bestimmte Wirtsarten (z.B. *Anthriscus cerefolium*) nicht angemessen erklären. Die unterschiedliche Aktivität der Methanolfraktionen, die noch nicht identifizierten Stimulanzien zugeschrieben werden muss, korrelierte viel besser mit der Eiablagepräferenz der Möhrenfliege, die mit intakten Blättern ermittelt wurde. Ausserdem konnte gezeigt werden, dass die Wasserphase mehrerer Heisswasserextrakte die Eiablage unterhalb von Blattmodellen reduzierte, die mit einem stimulierenden Extrakt vorbehandelt wurden. Diese eiablagehemmende Wirkung war besonders ausgeprägt bei *Pimpinella major*, einer sehr unbeliebten Art, was darauf hinweist, dass noch unbekannte abschreckende Stoffe möglicherweise auch zu den Unterschieden in der Beliebtheit der Wirtspflanzen beitragen.

Die Resultate dieser Untersuchung stützen die Hypothese, dass Signalstoffe in der Blattoberfläche entscheidend für das Verständnis der Wirtswahl der Möhrenfliege sind. Wie bei anderen herbivoren Insekten wird das Eiablageverhalten der Möhrenfliege durch komplexe Gemische von stimulierenden und wahrscheinlich auch hemmenden Pflanzenstoffen bestimmt, welche durch verschiedene Sinnesorgane (Riechsensillen an den Antennen und Geschmackshaare an den Tarsen) wahrgenommen werden. Diese Arbeit bildet die Grundlage für die Identifikation von weiteren verhaltensaktiven Verbindungen.

General Introduction

Evolutionary aspects of insect-plant interactions

Interactions among phytophagous insects and higher plants undoubtedly rank amongst the ecologically most important relationships between living beings as judged by both the number of species and the number of individuals involved and by the fact that many more organisms belonging to higher trophic levels (predators, parasites) directly or indirectly depend on the insects feeding on plants (Strong et al., 1984). Diet breadth is one of the most striking distinctive feature in nutritional ecology between insect and vertebrate herbivores (Crawley, 1989). Only very few exceptional cases of food specialists can be found among herbivorous vertebrates (e.g. giant panda, koala bear). In contrast, among herbivorous insects, polyphagous species – consuming a wide range of host plants belonging to several families – are far outnumbered by their monophagous and oligophagous counterparts, i.e. insects eating only a few species within a single plant genus or several species belonging to a single plant family, respectively (see for example Chapman, 1982). The driving evolutionary forces behind this predominance of restricted host ranges are a central topic in the study of insect-plant interactions. If we look at the plant's side, we are confronted with a bewildering array of secondary metabolites, which are inhomogeneously distributed over the taxa and mostly have no obvious physiological purpose.

Already more than a century ago, Stahl (1888) in a pioneering treatise about snails feeding on herbs postulated that the profound influence of animals on the formation of plant characteristics, which is generally accepted for mutualistic relationships (e.g. among pollinators and flowers), may as well be effective in the antagonistic interactions among herbivores and plants. He suggested that secondary plant substances much like morphological peculiarities such as thorns confer a protection – if only relative – to the plant from being attacked, which has evolved in response to selection pressure exerted by their natural enemies, notably by herbivorous animals. Seventy years later, these ideas were forcefully restated by Fraenkel (1959), who claimed that reciprocal adaptive evolution has occurred in the feeding habits of insects and in the biochemical properties of plants. In his view the main function of secondary substances – indeed their *raison d'être* – is defence against phytophagous insects. Arguing that most plants are nutritionally equal to insects, he asserted that food specificity is based solely on the presence or absence of specific secondary metabolites. While the majority of unspecialized insects is supposed to be deterred by these defensive compounds, some species, which have managed to overcome the physiological or behavioural hurdle, eventually may even require particular allelochemicals as a means to find and identify their host plants. In their very influential essay promoting the concept of coevolution, Ehrlich & Raven (1964) followed up a similar line of arguments. Starting from the observation that related butterflies often feed on related plants and inferring that secondary compounds are responsible for these host associations, they drew a scenario of a stepwise pattern of coevolutionary stages: plants that happen to produce new chemical compounds reducing their palatability to phytophagous animals gain a selective advantage. Once relieved of most of their former enemies, they are more competitive, spread into new habitats and may eventually speciate. In an analogous manner, insects that succeed in colonizing such a resistant plant group enter a new adaptive zone and are free to diversify in the absence of competitors. This process has also been termed "escape and radiate coevolution". Later on, further concepts diverging to some extent from this scheme have been put forward, in particular reciprocal or pairwise coevolution and diffuse coevolution (for a survey see Futuyma & Keese, 1992; Rausher, 1992).

In spite of the considerable research efforts stimulated by Ehrlich and Raven's (1964) hypothesis, unequivocal empirical evidence for coevolutionary interactions among herbivorous insects and plants remained scarce so far. A fairly convincing example is provided by the umbellifers (Apiaceae) and their affiliated insects to which belongs also the carrot fly, the organism studied in this thesis. The biosynthetic pathways leading to different coumarins apparently have been acquired in umbelliferous plants in the following sequence: hydroxycoumarins, linear furanocoumarins, angular furanocoumarins (Berenbaum, 1983). Linear coumarins are more toxic to polyphagous insects than their hydroxycoumarin precursors

(Berenbaum, 1978). The still more "derived" angular furanocoumarins, which occur only in a few advanced genera, are noxious to adapted oligophagous insects able to tolerate linear furanocoumarins (Berenbaum, 1981; Berenbaum & Feeny, 1981). This pattern has been interpreted as a case of escalation in a coevolutionary arms race (Berenbaum & Feeny, 1981; Berenbaum, 1983).

The coevolutionary theory has been criticized because its underlying premises have not been substantiated persuasively in many cases (for a possible exception see Berenbaum, 1991): a) phytophagous insects reduce plant fitness; b) insect attack selects for resistance in plants; c) secondary plant substances have evolved as defences against herbivores; d) there is interspecific competition among insects. According to Jermy (1976; 1984), the evolutionary interactions between plants and phytophagous insects are rather asymmetric insofar as the evolution of insects follows that of the plants ("sequential evolution") mostly without any major feedback on plant evolution. Speculation about the role of secondary metabolites continues (Haslam, 1994/1995), but the hypothesis is still upheld that secondary metabolism evolved under the selection pressure of a competitive environment including herbivores amongst other plant antagonists like pathogenic microorganisms (e.g. Berenbaum, 1995; Hartmann, 1996). Whatever may be their original function, secondary plant compounds undeniably can have a profound negative effect on survival and growth of phytophagous insects (e.g. Berenbaum, 1978; Blau et al., 1978; Johnson et al., 1996). Clearly, lack of selection by insects on plant chemistry still leaves open the possibility that secondary metabolites account for the prevalence of narrow host ranges (Courtney, 1988; Thompson, 1988).

Polyphagy intuitively appears to be an advantageous strategy in terms of resource availability. Its relative rarity implies that there must be costs to this kind of feeding habit. It usually has been assumed that adaptation to plants with a specific set of secondary compounds impairs the ability to cope with chemically different hosts. However, such trade-offs in performance could only rarely be detected. Also there seem to be many examples of plant compounds that deter insects without exhibiting any harmful post-ingestive effects (Bernays & Chapman, 1987). On the basis of these observations, Bernays & Graham (1988) argued that chemical coevolution has been overemphasized as a cause of diet specialization. As an alternative explanation they proposed that generalist natural enemies provide a major selection pressure for restricted host ranges. In associated commentaries, the first notion was only rarely rejected (Ehrlich & Murphy, 1988; Schultz, 1988), but mostly welcomed (e.g. Barbosa, 1988; Janzen, 1988). However, the statement that predators are the dominant factor in the evolution of narrow host ranges has not received wide acceptance (e.g. Courtney, 1988; Jermy, 1988). It emerges from the still ongoing debate that along with plant chemistry a multitude of other selective forces must be considered as potential determinants of the degree of diet specificity: insect size, resource availability (host abundance, predictability, apparency), plant phenology, sexual interactions ("sexual rendez-vous hypothesis"), predators and parasitoids ("enemy-free space") and interspecific competition (for surveys see Jaenike, 1990; Futuyma & Keese, 1992; Rausher, 1992; Bernays & Chapman, 1994). It is often difficult to judge whether these correlates are cause or effect of narrow host ranges. Some may be a prerequisite for the evolution of specialization (e.g. host plants are abundant), while others may tend to maintain a once given host affiliation (e.g. crypsis or sequestration of host-plant toxins against predators). The relative importance of the above mentioned factors for host specificity is still an unresolved problem and clearly may depend strongly on the insect group concerned (Bernays & Chapman, 1994). For example, it is hardly imaginable that natural enemies or mate finding – sexual encounters usually do not take place on host plants – could have prompted the carrot fly to confine its host range to umbelliferous plants.

The predominance of oligophagous species among insect herbivores does not necessarily indicate that oligophagy is in most cases the best strategy regarding the balance between costs and benefits, but may simply reflect the irreversibility of specialization. In a provocative contribution, Jermy (1993) questioned the often implicitly made assumption that insect-plant relationships are the result of unconstrained selection. In his opinion, novel food preferences originate mainly from heritable changes (mutations *sensu lato*) in the insects' chemosensory system and hence in host recognition. Thus, the evolution of host specificity is channelled primarily by genetic constraints and secondarily by selection.

This argumentation tightly connects the question "why" with the question "how" and thus eventually leads us to the issue which this thesis deals with: the mechanisms of host selection!

The mechanisms of host-plant selection

While the ultimate causes of diet specialization in phytophagous insects still remain largely enigmatic, there has been substantial progress in our understanding of the proximate causes, i.e. of the factors that govern host choice in feeding or ovipositing insects. As stated by Schoonhoven (1996), the general principles of host-plant selection have been elucidated. It is believed that acceptance or rejection of a potential host as food or egg-laying site depends on an interplay between excitatory and inhibitory inputs both from inside the insect and from outside via the sensory organs (Miller & Strickler, 1984). Age, circadian rhythms, nutritional and reproductive status (e.g. food deprivation, egg-load, mating status) as well as prior experience are important internal factors, which may influence the readiness to respond to a given external stimulus (Städler, 1992). The stimuli originating from the plant clearly are of chemical or physical nature. As mentioned above, the primacy of plant chemistry as a factor explaining the evolution of diet specialization has been challenged. In contrast, there is a consensus that allelochemics (for definitions see Nordlund, 1981) play a preeminent role in mediating host choice at the behavioural level. Nonetheless, studies with butterflies (e.g. Rausher, 1978) and flies (e.g. Harris & Miller, 1984; Roessingh & Städler, 1990) have demonstrated that oviposition may be appreciably affected by leaf shape, colour and surface characteristics. This cautions against totally neglecting non-chemical traits when seeking for factors that determine host-plant acceptability.

Verschaffelt (1910) was the first to provide experimental evidence for the involvement of specific plant chemicals in the host-selection process. He showed that non-host plants become acceptable as food to caterpillars of *Pieris* butterflies, when they are impregnated with sinigrin, a glucosinolate characteristic of the cruciferous host plants. Since these days, insect-crucifer associations remained among the most widely studied and best understood systems. Umbellifer specialists have also received attention relatively early in the studies of Dethier (1941) about chemical factors influencing host choice by *Papilio* larvae. Examples like the glucosinolates, to which crucifer-feeding insects seem to respond universally (Städler, 1992), may have given birth to the idea that host selection is mediated solely by a few characteristic secondary compounds, which work as key or "token" stimuli for the recognition of the host plants (e.g. Fraenkel, 1959). This simplistic view has largely been abandoned in favour of more refined explanations based on complex combinations of chemical stimuli ("Gestalt"). Host-choice in herbivorous insects is now seen as a process that is based on an integration of manifold positively and negatively interpreted signals originating from the plant.

Since a sequence of behavioural events precedes the consummatory action, i.e. feeding or oviposition, the semiochemicals involved have been classified according to the step during which they are effective (Dethier et al., 1960). Attractants and repellents are volatile compounds which influence host finding by causing the insect to make oriented movements towards the host plant respectively away from it. They are perceived by olfactory sensilla. Under natural conditions, anemotactic flight, an odor-stimulated up-wind movement towards the host, normally does not seem to occur over great distances, probably 10 m at the most (Städler, 1992). Hence, landing on host plants apparently is often at least in part a chance event. Usually, host recognition and the "decision" to use a plant for feeding or oviposition are made only in close vicinity or upon direct contact to the plant. Before egg-laying, many insects display special behaviours aimed at assessing host quality, e.g. exploratory runs in flies. As this sampling generally does not cause any damage to the foliage – leaf drumming by butterflies occasionally might be an exception – , behaviourally active compounds located in the surface waxes or in the boundary layer surrounding the leaf are of particular importance (Städler, 1986; Städler & Roessingh, 1991). The balance between inhibitory and stimulatory compounds at the leaf surface can determine whether a plant is acceptable or not (Renwick, 1989). Stimulants and deterrents are mostly non-volatiles or compounds of low volatility which influence host acceptance by eliciting respectively inhibiting feeding or oviposition. They are sensed by gustatory sensilla (contact chemoreceptors) or – less frequently – by olfactory sensilla.

Owing to the advent of powerful methods for chemical analysis (GC, HPLC, MS) and of electrophysiological techniques (electroantennograms, tip recording), there has been an enormous progress in the identification of plant compounds affecting insect behaviour. Thus, we know now a plethora of semiochemicals involved in a variety of insect-plant interactions (see for example the surveys in Städler, 1986; Städler, 1992). These behaviourally active substances may be secondary plant metabolites or nutrients (Kennedy, 1965). Though, substances originating from the primary metabolism (e.g. amino acids, carbohydrates) are seemingly uncommon as oviposition stimulants. The knowledge of a few identified active compounds mostly is not sufficient to adequately explain the host choice of a herbivorous insect. When the semiochemicals are specific (e.g. the glucosinolates), they possibly account for the general host range (e.g. the host-plant family), but often not for the preference ranking among different hosts. Moreover, many of the isolated stimulants are non-specific and therefore much like some physical plant traits (e.g. leaf colour and shape) would not allow the insects a "fail-safe" host recognition (Städler, 1992). In conclusion, only a minority of insect-plant relationships has been comprehensively studied. As Feeny (1992) pointed out in his review of the developments in the field of chemical ecology: "Thus we know a little bit about a lot of interactions, but understand a lot about relatively few of them". The carrot fly-umbellifer association arguably counts among the former category, as will be elaborated in the following section.

Host-plant selection in the carrot fly

Apart from some popular and thus thoroughly investigated butterflies (e.g. the swallowtail butterflies, the monarch butterfly), most insect-plant relationships studied so far concern herbivorous insects that are of economic importance in agriculture or forestry. This holds also true of the carrot fly, *Psila rosae* (F.) (Diptera, Psilidae), which is a serious pest of umbelliferous (= apiaceous) crops in the northern hemisphere. Damage is caused by the larvae, which attack the roots of the host plants. That is why the impact is especially critical on vegetables, of which the edible part is affected, above all on carrots, but for example also on celeriac and parsnip. Losses are mainly due to a decrease in quality rather than in yield, as the mines produced by the maggots can render the roots unmarketable.

Conventional methods to control the carrot fly are problematic for several reasons. The application of insecticides has become less efficient in some cases due to microbial degradation in the soil or to the development of tolerance by the fly. It is also no longer desirable because of the residues that may accumulate in the crop and in the environment. As a consequence, there have been efforts to diminish the amounts of pesticide applied by complementing chemical control with cultural practices (e.g. choice of sowing and harvest time, crop rotation, crop covers, intercropping) and pest forecasting systems (Dufault & Coaker, 1987; Finch, 1993; Rämert & Ekbom, 1996). Another promising approach to reduce the dependence on insecticides has been to exploit plant resistance. Extensive screening of different carrot varieties has led to the identification of cultivars that are less susceptible to carrot fly attack (e.g. De Ponti & Freriks, 1980; Ellis & Hardman, 1981). Subsequent breeding programs tried to achieve carrot lines with still higher levels of resistance, but had only limited success (Ellis et al., 1985; Ellis et al., 1991).

Increasing interest in the biology of the carrot fly can be seen in the above context. Particularly, host-plant resistance and its underlying mechanisms have been recognized as important targets for investigations. In the very long term, studies are aimed at identifying resistance factors to provide reliable criteria for the selection of less susceptible carrot cultivars or for introducing genes that control these factors either by traditional breeding methods or with modern molecular techniques. Three major components of host-plant resistance can be distinguished (Painter, 1951). *Antixenosis* refers to plant properties that elicit negative behavioural reactions ("non-preference") or total avoidance by the larva or by the adult fly. *Antibiosis* refers to adverse post-ingestive effects, which result in reduced growth, survival and fecundity in the carrot fly larvae. *Tolerance* is a basis of resistance in which the plant shows an ability to grow and reproduce itself or to repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host. Research into the mechanisms of resistance has focused chiefly on the first aspect, the process of host selection.

With the availability of mass rearing methods, it was possible to conduct detailed laboratory studies in the host-choice behaviour of the carrot fly. Bohlen (1967) described the exploratory runs over the host foliage performed by the females prior to egg-laying, which takes place in the soil surrounding the plants. Städler (1971; 1972) worked on the orientation of the first instar larvae towards the roots and on plant cues influencing host selection in adult carrot flies, which resulted in the isolation of the first oviposition stimulant from carrot leaves, *trans*-methylisoeugenol (Berüter & Städler, 1971). These investigations marked the beginning of research into the chemical ecology of this insect-plant relationship, which proceeded along two separate lines: Semiochemicals involved in larval host-finding (e.g. Ryan & Guerin, 1982) and chemical factors mediating host finding and acceptance in the adult fly. *Trans*-methylisoeugenol and the related *trans*-asarone were shown to act not only as oviposition stimulants, but also as host-plant attractants for the carrot fly in the field (Guerin et al., 1983). Along with these two phenylpropenes, Städler & Buser (1984) isolated four additional stimulatory compounds from the surface waxes of carrot leaves: the polyacetylene falcarindiol, the substituted coumarin osthol and the furanocoumarins xanthotoxin and bergapten. Strong ovipositional responses were only evoked by mixtures of these allelochemicals, which implied a synergistic effect. The distribution of the three groups of compounds (phenylpropenes, furanocoumarins and C17-polyacetylenes) over the plant families suggested that the combination of the identified oviposition stimulants is distinctive of the umbelliferous host plants (Städler, 1986). Thus the carrot fly appears to be a model case of a specialist insect that uses characteristic protective compounds as a chemical "signature" for host recognition. However, the different antixenotic resistance of two carrot cultivars was not correlated with the respective contents of the six known oviposition stimulants (Städler et al., 1990). This indicated that further yet unidentified factors contribute to the host-plant preferences of the carrot fly. Here is the starting point of my thesis.

Rather than to pursue an "in depth" strategy and to continue searching for the causative factors behind the rather subtle variation in acceptability among carrot cultivars, I decided to broaden the perspective by covering a much wider range of host plants, including both wild and cultivated species. Up to now, research has been strongly biased towards carrot, the economically most important host species. Yet, the majority of umbellifers tested so far in field studies has proved to be hosts, more than a hundred species belonging to several genera, after all (Hardman et al., 1990). Furthermore, a preliminary study revealed that celeriac, another important cultivated host-plant, exhibited a profile of stimulatory compounds quite different from that of carrot (Städler et al., 1990). This let us suspect that the pattern observed in carrot might not necessarily be typical of other umbelliferous host plants.

In spite of the vast amount of literature about the carrot fly, which has accumulated over more than a hundred years (see bibliography compiled by Hardman et al., 1985), no quantitative information was available about some rather fundamental aspects of this insect-plant relationship. So, first I had to establish an oviposition preference hierarchy of the carrot fly for various host-plant species, as a prerequisite for further chemical investigations. With a selection of host species more variable in acceptability than carrot cultivars, I intended to re-evaluate the "ecological" significance of the known oviposition stimulants and to gather evidence for further not yet identified behaviourally active compounds, stimulants and/or deterrents. My thesis concentrates on chemical and non-chemical plant traits crucial for host acceptance, when the fly is in contact to the foliage. For comparative purposes, I also recorded survival and growth of carrot fly larvae on various host species. In contrast, I did not examine host finding, which certainly is another critical phase in the host-selection process under natural conditions.

Outline of the thesis

Part 2 consists of three small chapters that report the results of some preliminary oviposition experiments. **Chapter 2.1** deals with some aspects of the egg-laying behaviour as shown by individual flies and reviews published life-history data (e.g. total egg production, longevity). In contrast, all other assays were performed with cage populations consisting of numerous individuals. The flies for the experiments originated from a laboratory culture reared on carrots. **Chapter 2.2** addresses to the question whether the larval food can induce a preference for the respective host plant in the adult fly. **Chapter 2.3** reports the

results of some methodical modifications that were aimed at improving the "resolution" of the oviposition choice assay by increasing the relative difference in the number of eggs deposited with distinct treatments.

Part 3 provides the basis for the subsequent experiments presented in Part 5. Herein, I determined the acceptability for oviposition and the suitability for larval development of various host-plant species known to differ widely in susceptibility to carrot fly attack in the field. In **chapter 3.1** I present a preference hierarchy of the carrot fly, which was established in oviposition choice assays. In **chapter 3.2** I assessed the suitability of the respective plant species for survival and growth of the larvae. **Chapter 3.3** summarizes and compares the data given in the two preceding chapters. Here, I examine how well the host choice of the females correlates with the performance of the larvae on the corresponding plants. Also, I tried to estimate the contribution of these two parameters to the variation in susceptibility, which was observed among the plant species in the field.

In **part 4** I explore in what way non-chemical plant properties influence the oviposition behaviour of the carrot fly. The filter paper leaves, which were previously used to test the stimulatory activity of host-plant extracts or of pure compounds, were suboptimal with respect to several physical attributes. Consequently, it appeared advisable to design a more adequate surrogate leaf for chemical studies. This purpose could be combined with an investigation into the effects of leaf shape, colour and surface characteristics on oviposition. The findings are depicted in **chapter 4.1**. **Chapter 4.2** reports of the divergent preferences of three fly species for specific leaf shapes. I adopted a comparative approach to ascertain whether the discrimination by female carrot flies among different foliar forms potentially has an adaptive meaning. To this end, carrot flies, cabbage root flies and onion flies were exposed to identical sets of accurate imitations of host and non-host leaves in oviposition choice assays.

Part 5 deals with chemical plant traits affecting host acceptance by the carrot fly. The formerly used dichloromethane surface extracts were not entirely satisfactory, because surrogate leaves treated with them were much less acceptable than real host leaves. That is why, I evaluated several extraction methods for their potential to produce extracts that strongly stimulate oviposition in the carrot fly, as shown in **chapter 5.1**. The most stimulatory extracts were chosen for further analysis, the results of which are presented in **chapter 5.2**. With a selection of twelve host species, I examined to what extent the stimulatory activities of these extracts reflect differences in acceptability among the real plants. These assays could provide evidence for the presence of yet unidentified stimulatory and inhibitory semiochemicals in host leaves. In addition they allowed to estimate the significance of the already known oviposition stimulants for antixenotic resistance across a wider range of host plants. In **chapter 5.3** the latter problem was investigated in detail. A highly stimulatory fraction of the previously assayed extracts was analysed by GC-MS for the six identified oviposition stimulants and for related compounds. So, I could demonstrate the considerable variation among the various host species in the absolute and relative amounts of these semiochemicals contained in the surface waxes of the foliage.

Part 6 includes the general discussion of this thesis as well as a review of all semiochemicals, which are known to exert a behavioural or physiological effect on carrot fly larvae or adults, and their putative role in host-plant resistance. The section is closed by some speculations about the evolution of host ranges in psilid flies and about the potential significance of our knowledge for future agricultural applications.

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Some aspects of the oviposition behaviour of individual carrot flies

Introduction

Studies on antixenosis resistance of plants against pest insects usually concentrate on the behaviour of whole populations. The larger the population of insects, the higher the probability of obtaining results that are representative for the situation observed in the field. Hence oviposition assays with herbivorous insects are often performed with cage populations that consist of relatively large numbers of individuals. The eggs counted in the experiments that are presented in the following chapters usually originated from tens to hundreds of carrot flies. Therefore background information about the oviposition behaviour of individual flies is useful for a better understanding of the data obtained from the whole fly population. Here "life history data" (e.g. adult longevity, egg production) of individual female flies are given and compared to re-analysed raw data from other sources (Körting, 1940; Bohlen, 1967; Schuler, 1982). Furthermore I inspected the allocation of eggs to several available host leaves during a single oviposition bout (i.e. a one-day period) and I tried to assess the importance of early adult experience with host plants on the subsequent oviposition behaviour. Changes in the host-selection behaviour caused by prior egg-laying experience – often referred to as induced oviposition preferences – have been observed in several insect species (Jermy, 1987) including Diptera, e.g. in the apple maggot fly *Rhagoletis pomonella* (Prokopy et al., 1982).

Materials and Methods

The insects were obtained from a newly initiated culture (2.–4. generation after collection of pupae in the field), which was reared on carrots (Städler, 1971). The flies were kept in a controlled environment room (L16:D8; 21 ± 1 °C, 80 ± 5% r.h.). Freshly emerged females were enclosed together with one or two males in a plexiglass cylinder (∅ 15 cm; height 20 cm) containing a small apple seedling as a resting and copulation place. On the second or third day of life, the females were transferred into plexiglass cages (length × width × height: 40 × 30 × 30 cm) with a round opening (∅ ~20 cm) covered by wire screen at the ceiling. Yeast hydrolysate on a small filter paper strip, water and a sugar solution were offered as food. The cages contained five oviposition dishes (Städler, 1971). The four that were equipped with a single host plant leaf were positioned at the corners, while an additional dish with a non-host leaf (cauliflower *Brassica oleracea* convar. *botrytis*) was placed in the middle of the cage. The host leaves (~20 cm long) were cut from plants grown outdoors in seed beds, either from carrot *Daucus carota* ssp. *sativus* cv. "Tip-Top" or from celeriac *Apium graveolens* var. *rapaceum*. Eggs were counted every day except for the three-day period at weekends. Positions of the leaves were rotated after each experimental period. The foliage was replaced by fresh plant material after 4 periods.

I tested the possible effect of pre-exposure to specific host-plant species on subsequent oviposition. Either four celeriac (aaaa) or carrot (dddd) leaves were provided together with the cauliflower leaf (b) for about a week (4 experimental periods) before both leaf types (adad) were presented in a dual choice assay for the remaining life span of the fly. A third set of flies was allowed access to both types of host leaves right from the start.

Several individuals were not included in Table 1 because they died prematurely (abdomen still containing eggs), possibly due to high air humidity inside the plexiglass cages (limited air exchange). However, data of these individuals were used for Table 2 and Figure 1, where lifetime egg production and longevity were not crucial (e.g. the results in Table 2 refer to experimental units lasting one day only).

Results and Discussion

Life history data of individual female flies. According to Bohlen (1967) and Körting (1940), mated females usually start egg-laying on the second or third day of their imaginal life (mean ± s.e.: day 3.0 ± 0.6; range day 1–12; n = 20). In our study, 15 out of 18 individuals began to oviposit on the third day, on the first occasion they were allowed access to host-plant foliage. The flies had a mean age of 14 ± 1 days (range 2–33; $n = 37$), when they

Table 1. "Life history data" for individual carrot fly females kept in cages: mean ± standard error (minimum – maximum); N_1 = number of oviposition bouts (data of several females pooled); N_2 = number of exactly dated time differences between successive oviposition bouts (data of several females pooled); N_3 = number of females.

data source	N_1	number of eggs per oviposition bout	N_2	days between successive oviposition bouts	N_3	number of oviposition bouts	lifetime egg production	longevity of adult flies in days
this study[1]	62	17 ± 2 (1–45)			11	5.2[3] ± 0.6 (3–10)	89 ± 13 (20–154)	18 ± 2 (9–34)
Körting (1940)[2]	53	24 ± 3 (1–81)	41	2.7 ± 0.3 (1–10)	12	4.4 ± 0.6 (1–8)	107 ± 13 (40–167)	23 ± 1 (16–31)
Bohlen (1967)[2]	28	25 ± 2 (2–42)	20	4.0 ± 0.5 (1–8)	8	3.5 ± 0.3 (3–5)	86 ± 10 (58–144)	18 ± 2 (11–25)
[Bohlen (1967)]					20		[99[4]]	
Schuler (1982)[1]	38	24 ± 2 (1–44)	30	2.5 ± 0.2 (1–5)	6	7.0 ± 1.5 (3–12)	159 ± 41 (51–329)	22 ± 4 (12–34)
Total	181	22 ± 1 (1–81)	91	2.9 ± 0.2 (1–10)	37	4.9 ± 0.4 (1–12)	106 ± 9 (20–329)	20 ± 1 (9–34)

[1] nutrition: mixture of cane sugar, yeast hydrolysate, vitamine C and water; once mated females later isolated from males
[2] nutrition: sugar solution; females kept together with a male
[3] minimal values, because some experimental units lasted longer than one day
[4] mean for 20 females kept together in a cage

deposited the last egg batch, and they usually died a few days later (mean 6 ± 1; range 0–23; $n = 37$). As the comparison with published data shows, batches of about 20 eggs are laid in five bouts that follow each other at 3-day intervals on average (Table 1). Generally the findings of the studies cited in Table 1 are in good accordance. Significant differences among the data sources were only found regarding the number of eggs per oviposition bout and the space between successive bouts (Kruskal-Wallis test: $P = 0.03$ and $P = 0.02$, respectively). Similar results – an average of 109 eggs per female – were obtained by McLeod et al. (1985). According to Collier & Finch (1996) female carrot flies lay between one and five batches that consist of about 20–40 eggs (range 1–70). In contrast, Whitcomb (1938) and Van't Sant (1961) reported considerably lower lifetime egg production in insectary studies: 6–35 eggs and 5–22 eggs, respectively. An mean number of only about 17 eggs was likewise observed by Scott (1952) with very shortlived flies (mean adult longevity: 6.5 days; range 2–10 days). Jørgensen & Thygesen (1968) estimated an average of 41 eggs per female in a more extensive study of field-caught flies ($n = 405$).

Some discrepancies among the studies might be attributable to varying size of the insects (depending on the quality of the larval food), nutrition of the adults, environmental conditions (temperature, light quality) and fly density in the experimental cages. Flies provided only with water lived on average less long than flies that were fed a sugar solution (Körting, 1940): 8 days (2–24; $n = 25$) versus 25 days (5–51; $n = 36$). Städler (1971) recorded a mean number of 75.4 eggs per female when offering cane sugar and yeast hydrolysate as compared to only 46.4 eggs per female with a cane sugar diet alone, which emphasizes the role of proteinaceous nutrients for egg formation (Brunel, 1979). Collier & Finch (1996) showed that pre-oviposition period, intervals between batches of eggs and adult longevity all declined with increasing temperatures at which the flies were kept. In the laboratory, longevity and total egg production was also affected by illumination and population density (Naton, 1968; Städler, 1977): more eggs were laid in yellow light than in "day-light" and at lower than at higher fly density (mean number of eggs/female: 57–196 versus 55–109).

Egg fertilisation – possibly depending on the frequency of matings – appears to be another factor influencing total numbers of eggs. Unmated females did not oviposit except in few cases in a single bout just prior to death, even though their abdomens were filled with plenty of eggs (Bohlen, 1967; Brunel, 1979). Females that had copulated only once before being isolated from male contact, laid on average fewer eggs than females kept together with males (Benno Richner, unpublished results): 87 (29–120; $n = 11$) versus 126 (41–183; $n = 6$).

Allocation of eggs to several oviposition dishes. The egg batch of a single day was dispersed mostly over two oviposition devices; only in about a third of the

Table 2. Allocation of eggs to the four (five) available oviposition dishes. Only experimental periods that did not last longer than one day (i.e. only one oviposition "bout" as defined here) and during which at least four eggs were laid, are considered; eggs laid on the dish with the non-host leaf are not included (in parenthesis: number of eggs/period ≥ 1; eggs on the dish with the non-host leaf included)

oviposition dishes with at least 1 egg	*n* (oviposition bouts)		number of eggs per dish mean ± s.e.	
1	15	(20)	11.9 ± 2.6	(9.2 ± 2.2)
2	21	(20)	8.2 ± 1.1	(7.2 ± 1.0)
3	16	(16)	8.2 ± 1.1	(8.7 ± 1.2)
4	3	(6)	7.8 ± 1.3	(6.1 ± 1.0)
(5)		(0)		

cases was it confined to a single dish (Table 2). The egg distribution was not influenced by the number of host species present (1 or 2: 2 carrot and 2 celeriac versus 4 carrot or 4 celeriac leaves; $\chi^2 = 0.4$; df = 3; $P = 0.9$). The mean number of eggs per dish diminished only insignificantly with increasing number of dishes covered with eggs (Kruskal-Wallis test: $P = 0.3$), i.e. the bigger the total egg batch, the more the females tended to distribute the eggs over several oviposition dishes. This behaviour may reduce competition among larvae for food resources and the risk of predation or parasitation. The results also imply that flies intent on egg-laying usually visit several leaves (here considered as equivalents of neighbouring plants) and thus have an opportunity to rate the host quality ("weighing the alternatives"), which is considered a necessary requirement if host-plant preferences in a strictly defined sense are to be established (Miller & Strickler, 1984).

Influence of experience with particular host plants on subsequent oviposition choices. Flies that were exposed to celeriac foliage during the first week of their life, later deposited a slightly higher proportion of their eggs below the celeriac leaves than the other two fly groups, but no analogous effect was observed with flies that were first presented with carrot leaves (Figure 1). There was no detectable influence of pre-exposure on the discrimination between celeriac (a) and carrot (d) leaves in the second life period (Kruskal-Wallis test using discrimination indices {eggs a – eggs d}/{eggs a + eggs d}: $P = 0.99$). In larvae of the tobacco hornworm *Manduca sexta*, the occurrence and strength of induction of feeding preferences correlated inversely with the taxonomic relatedness of the plant species paired in the assays (de Boer & Hanson, 1984). Städler and Hanson (1978) suggested that these diet-induced preferences are based upon the phytochemical differences among the species tested. The two host species compared in our study were similar in acceptability (another celeriac cultivar was less acceptable than carrot in later assays, see chapter 3.1), but they were shown to exhibit distinct profiles of oviposition stimulants (Städler et al., 1990; see also chapter 5.3). Thus the carrot fly should have been able to distinguish between both species by chemosensory means, which appears to be a crucial prerequisite for the detection of induction effects.

Obviously, the sample size in our study was too low for any statistically firm conclusions. Although our data do not rule out the possibility that preference for a particular host plant may be induced by early experience in the carrot fly, they nevertheless suggest that "learning" effects – if such indeed occur – do not profoundly alter pre-existing preferences. This has some relevance regarding the way the preference hierarchies given in chapter 3.1 were established: the same cage populations of insects were successively exposed to standard carrot leaves (*Daucus carota sativus* cv. "Danvers") together with different test leaves. Hence the flies – inhomogeneous in age and previous experience – were continually in contact with the standard carrot cultivar, but only temporarily with the various test species. I tried to reduce the problem of potential bias due to experience by allowing the flies access to both test and standard leaves for a three-day period (weekends) before the start of the actual choice experiment.

period 1:
2./3. - 7./8./9. day
of adult life
(mean duration 6 days,
range 5 – 7 days)

period 2:
8./9./10. day
of adult life – death
(mean duration 6 days,
range 2 – 25 days)

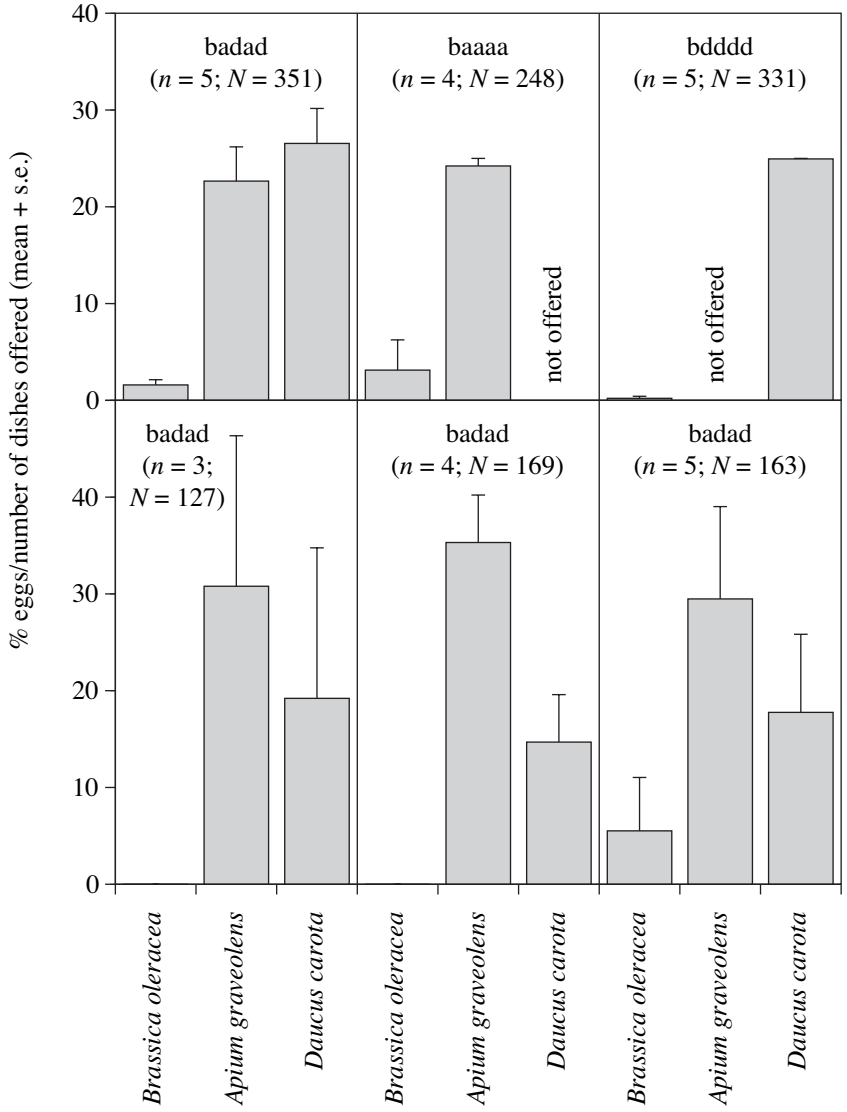

Figure 1. Influence of previous experience with a particular host plant on subsequent oviposition in a choice assay. The treatments are characterized by 5 letters referring to the five leaves presented in the experiments: a = *Apium graveolens* var. *rapaceum*, celeriac (cultivar unknown); b = *Brassica oleracea* convar. *botrytis*, cauliflower; d = *Daucus carota* ssp. *sativus* cv. "Tip-Top", carrot. *n* = number of females; *N* = total number of eggs.

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Host-plant choice of adult carrot flies is not influenced by larval food

Introduction

Host recognition and acceptance in phytophagous insects, though basically genetically determined, may be modified by previous experience with particular host and non-host plants. Such induced preferences are a widespread phenomenon observed during either the larval or the imaginal stage (Jermy, 1987). The effects of induction may be persistent through two moults and one whole larval instar (Jermy et al., 1968). Theoretically, larval experience might also alter the subsequent feeding and oviposition behaviour of the adult insects. Such "larval memory" effects have been postulated by the hypothesis commonly referred to as Hopkins' host-selection principle, which states that adult insects prefer the host plant on which they have accomplished the larval development (Hopkins, 1917). So far, only little experimental evidence has been found in support of this hypothesis (Jermy, 1987).

All the flies for the oviposition assays, the results of which are shown in the following chapters, originated from larvae that fed on carrots. To investigate the possible effect of larval food on the oviposition behaviour, three subsets of flies reared on the roots of different hosts for one generation were presented with leaves of these plant species in dual choice experiments.

Materials and Methods

Before the onset of the experiments, the flies were maintained in culture for about six generations after collecting the pupae in the field. They were reared on carrots following the method described by Städler (1971). The experimental food plants were carrots *Daucus carota* ssp. *sativus* cv. "Tip-Top", parsnips *Pastinaca sativa* ssp. *sativa* cv. "Halblange" and hamburg parsley *Petroselinum crispum* var. *tuberosum* cv. "Berliner". They were grown outside in seed beds and stored after harvest in a cold room (~ 4°C) for 74 to 123 days before inoculation. Sixteen roots each were inserted into boxes (Eternit®; ~ 30 × 30 cm wide, 15 cm high) about half-filled with moist sand. Some conspecific seeds were added to provide seedlings for the newly hatched larvae (Städler, 1971). Carrot fly eggs were rinsed onto the sand 4 to 14 days after embedding of the roots. The boxes were kept in a greenhouse at about 20 ± 2 °C and were regularly watered. Six weeks after the inoculation, the pupae were washed out of the sand. Each subset of flies emerged separately in the cage (70 × 70 × 70 cm) where the oviposition assays were performed.

The leaves for the oviposition experiments were obtained from freshly grown shoots of non-inoculated roots that were kept in the greenhouse in the same manner as the infested roots. The dual choice assays were performed as described in more detail in the chapters 2.3 and 3.1. Four pairs of uncovered oviposition devices each equipped with a single cut leaf (24 cm long) of either species were arranged in an alternating fashion around a non-host plant (apple seedling) in the centre of the cage. Every individual leaf was tested at two positions in each cage (8 individual leaves per species × 2 positions per cage = 16 replicates). The numbers of eggs laid were expressed as percent of total oviposition and discrimination indices were calculated for pairs of neighbouring leaves a and b as follows: (eggs a – eggs b)/(eggs a + eggs b). The data were compared with the Friedman test modified for repetitions within a block (i.e. one experimental unit lasting 1 to 3 days; Conover, 1980).

Table 1. Numbers and weights of pupae and emergence rate of flies obtained by rearing the larvae in pots (n = 12–13) with roots of three different host-plant species (mean ± s.e.)

species	eggs	total root weight	pupae	yield in % of eggs	mean pupal weights	emergence rate in %
Daucus carota	659 ± 141	905 ± 35	114 ± 28	28 ± 5	3.0 ± 0.1	78 ± 4
Pastinaca sativa	630 ± 134	936 ± 37	252 ± 69	44 ± 5	2.3 ± 0.1	87 ± 1
Petroselinum crispum	575 ± 145	334 ± 18	54 ± 8	19 ± 5	1.9 ± 0.1	72 ± 3

Results and Discussion

Comparatively few pupae were gathered from parsley roots, probably because of the low root weight (Table 1). In contrast, high yields of pupae were achieved with parsnips. This may be due to the presence of many lateral roots that are crucial for the survival of first larval instars (Städler, 1971). Carrots supported only moderately large numbers of larvae, but these attained the highest pupal weights. The percentage of flies emerging from the puparia was positively correlated with mean pupal weight per pot ($r = 0.38$; $P = 0.02$; $n = 8$ pots).

While the size of the flies was markedly affected by the larval host, no significant differences regarding the oviposition preferences could be found among the three fly groups (Friedman test using discrimination indices $\{a - b\}/\{a + b\}$: $P > 0.1$ for all three pairs of host species). There was no indication whatever of a preference for leaves of the larval food plant (Figure 1). Generally, the flies did not strongly discriminate among the three host species. Parsley leaves were slightly more and parsnip leaves were slightly less acceptable than carrot leaves, the difference being only significant when the data of all fly groups are combined (Friedman test: $P < 0.025$ and $P < 0.005$ respectively). It could well be that more pronounced differences in the acceptabilities of the hosts are necessary to produce any detectable effects. Hopkins' host-selection principle was shown to operate in the host-selection behaviour of the parasitoid *Nasonia vitripennis* only when a choice between very different dipterous host species was offered, but not with a pair of closely related flies (Smith & Cornell, 1979). This is a rare example of larval conditioning influencing adult ovipositional preference. Most other investigations dealing with this question have failed to provide any evidence for an impact of larval experience on oviposition (see review of Jermy, 1987), e.g. Wiklund (1974) studying swallowtail butterflies *Papilio machaon* and umbelliferous host plants. On the other hand, recently Anderson et al. (1995) reported that larval diet induced a change in host acceptance (rather than in host preference) of the polyphagous Egyptian cotton leaf worm *Spodoptera littoralis*.

I conclude that the preference hierarchies of *Psila rosae* presented in the chapters 3.1 and 3.3 were most likely not biased by the larval food plant (carrot) used for our fly culture.

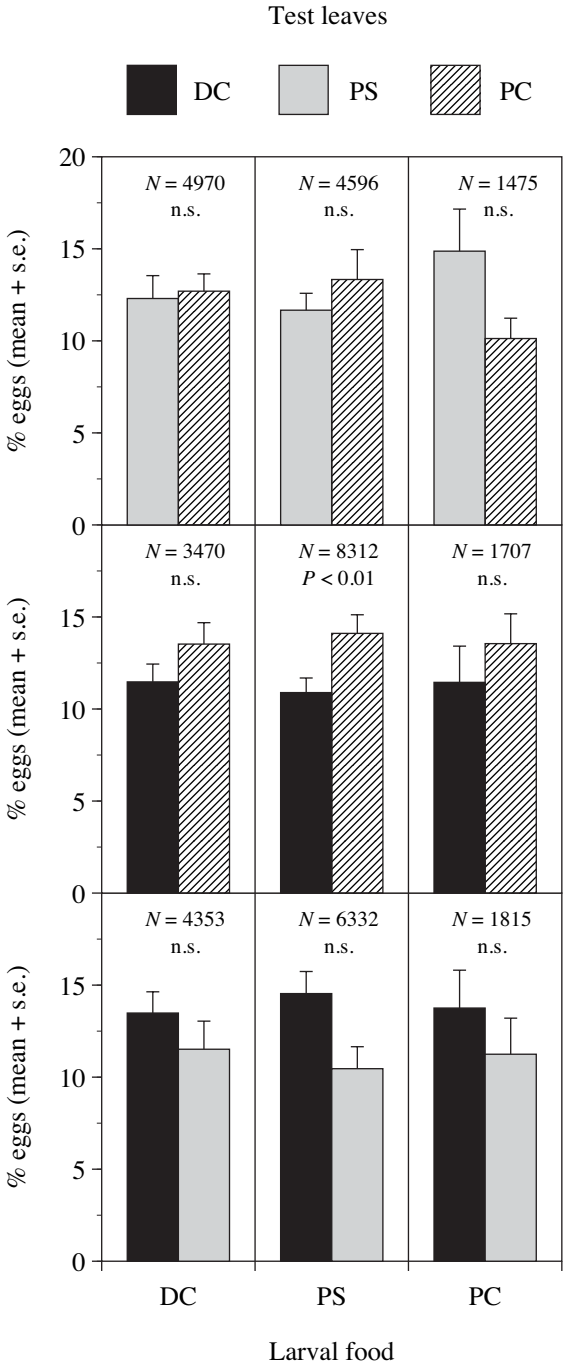

Figure 1. Influence of larval food on the host plant choice of adult flies. The Friedman test was used to compare the numbers of eggs laid below the different leaves. DC = *Daucus carota* ssp. *sativus* cv. "Tip-Top", carrot; PS = *Pastinaca sativa* ssp. *sativa* cv. "Halblange", parsnip; PC = *Petroselinum crispum* var. *tuberosum* cv. "Berliner", Hamburg parsley; N = total number of eggs; n = 16 (only 12 replicates for assay DC versus PC with flies obtained from PC as larval food).

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Short communication

An improved oviposition assay for the carrot fly

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Introduction

Reliable laboratory assays for the study of oviposition behaviour of herbivorous flies are important tools for analysing the cues affecting oviposition. In the case of the carrot fly *Psila rosae* (F.) (Diptera: Psilidae), oviposition assays performed in cages have often been unsatisfactory regarding their ability to discriminate between treatments. The reason for this shortcoming may lie with some particularities in the pre-ovipositional behaviour. After being stimulated for oviposition during exploratory runs over the leaves, female carrot flies proceed down the leaf or stem axis and deposit their eggs in moist crevices in the soil near the base of the host plant (Bohlen, 1967). Yet, when probing with the ovipositor for suitable egg-laying sites, females do not always stay close to the host plant, but can move up to 15 cm away from it (Overbeck, 1978). In contrast, oviposition is more restricted to the immediate proximity of the plant base in some other root-feeding phytophagous flies, e.g. in the cabbage root fly *Delia radicum* (L.) (Zohren, 1968; Freuler & Goy, 1977) and in the onion fly *Delia antiqua* (Meigen) (Havukkala et al., 1992). The relatively loose association of oviposition site with host plant observed in the carrot fly is a limitation in laboratory assays where different treatments are spaced fairly close to each other. Furthermore, complete leaf and stem runs are for carrot flies not the only means of reaching the oviposition substrate. A few females stimulated for oviposition fly off the leaves and land somewhere nearby – occasionally on neighbouring ovipositon dishes – where they start to search the substrate for appropriate oviposition sites (Städler, 1977). During investigations aimed at establishing preference hierarchies of carrot flies for various host-plant species, differences in number of eggs laid around host (Apiaceae) and non-host leaves were found to be much less pronounced than expected. A certain small percentage of the eggs was laid even onto devices that were devoid of leaves. This prompted us to modify the assay so that 'stray' oviposition was reduced by restricting the access of the flies to the egg-laying substrate. To this end we tested several covers put on top of the oviposition dishes to determine whether they increased treatment differences.

Materials and methods

Insects. The flies for the assays were reared in a climate controlled room (21 °C ± 1 °C, 80 ± 5% r.h.) for ≤ 15 generations after pupae were collected in the field (Städler, 1971). Adults continuously emerged in cubic screen cages (0.34 m^3), in which the choice experiments were performed. Even illumination and constant background were achieved by enclosing the cage in a box made of white cardboard. Light was provided by four white (Osram L 20 W/20 S) and three yellow (Philips TLD 18W/16) fluorescent tubes located on top of the cages during 16 h of the day, including two twilight periods with only yellow light (6:00–8:00, 20:00–22:00). The fly population in a cage typically consisted of 100–400 individuals (males and females).

Bioassays. Plastic dishes (∅ 9 cm, height 4 cm) lined with wet foam rubber and covered successively with a black cloth and a polyethylene grid (mesh 1 mm) served as oviposition devices (Städler, 1971). We counted the numbers of eggs laid around standard

and test leaves when using either uncovered dishes or dishes topped by different kinds of covers in no-choice assays (regarding the covers; Table 1). The covers consisted of black plastic pots turned upside down with variable openings of 2 cm ∅, 5 cm × 5 cm and 9.5 cm ∅, through which the flies could gain access to the oviposition substrate. In addition, we tested uncovered dishes against the same set of covers in a choice assay using surrogate leaves sprayed with host-plant extract or solvent, respectively (see Figure 1). In a further no-choice experiment, we separated the oviposition devices from each other (including the 20 cm long leaves) by surrounding them with transparent plexiglass cylinders (∅ 15 cm, height 20 cm) so that the leaves were only accessible from above.

The foliage was obtained from wild plants (e.g. lady fern *Athyrium filix-femina*, creeping buttercup *Ranunculus repens*) and from plants sown in pots (cauliflower *Brassica oleracea* convar. *botrytis*) or in seed beds (the remaining species). Single leaves were cut to the same length (20 cm or 24 cm) and introduced through a narrow central opening in the grid into a small water-filled plastic vessel incorporated in the oviposition dish. For some test plant species (*Daucus capillifolius*, silverweed *Potentilla anserina*) more than one leaf per dish was supplied to partly compensate for differences in leaf mass.

The leaf models were made of green cardboard, covered with a thin layer of paraffin wax (Merck, melting point 42–44 °C) and kept upright by a metal wire perpendicularly protruding from the stem base that was fixed to the dish with two rubber bands (Degen & Städler, 1997). To provide chemical oviposition stimuli, 4 gram leaf equivalents of a host-plant extract were applied to the models with a chromatography sprayer. The extract was prepared by submerging undamaged carrot leaves for 60 s in hexane heated to about 50 °C (T. Degen, G. Poppy & E. Städler, in prep.).

Eight oviposition dishes were arranged in the cages in a circle around an apple seedling (resting and copulation site). The two treatments (test/standard leaves or extract/control) were assigned to the positions in an alternating fashion (extract/control pairs with the same cover were juxtaposed). After the eggs were counted and removed, positions were switched for the next experimental period (block) so that finally each treatment was located once at each position. The number of replicates is given by the number of experimental periods multiplied by the number of repetitions per treatment within a block (four in the dual choice assays, one in the multiple choice assay). Individual real leaves were tested during two consecutive experimental periods with the same cover type before they were replaced, whereas the surrogate leaves were used for all eight replicates. Discrimination indices between two treatments A and B were calculated as follows:

$$\text{discrimination index} = \frac{\text{eggs A} - \text{eggs B}}{\text{eggs A} + \text{eggs B}} \times 100.$$

Hence the index can take values between 0 (no difference between A and B) and +100 (all eggs laid with A) or –100 (all eggs laid with B).

Results

Age of the fly culture and discriminatory behaviour. Insect populations reared in captivity may undergo behavioural changes, because the selective forces in nature and laboratory are not necessarily identical. This phenomenon could have been responsible for the unexpectedly poor discrimination of the ovipositing flies between standard carrot leaves *Daucus carota sativus* cv. 'Danvers' and lady fern leaves *Athyrium filix-femina* (Table 1). Therefore we tested whether flies kept for approximately 12 generations in culture differentiate less between standard carrot leaves and fern leaves *Dryopteris wallichiana* than flies emerging from pupae collected in the field. There was no significant difference between the discrimination indices of both groups: 93.8 ± 1.9 for laboratory flies versus 87.6 ± 7.6 for wild flies (mean ± s.e.; Wilcoxon signed rank test: $P = 0.80$; $n = 16$ leaf pairs; $N = 4448$ eggs). When we repeated this experiment later with the fern *Athyrium filix-femina*, the results were analogous: 83.1 ± 3.6 for the old culture about 15 generations removed from the field versus 80.9 ± 4.7 for the new culture kept for ≤ 3 generations in the laboratory (mean ± s.e.; Wilcoxon signed rank test: $P = 0.96$; $n = 16$ leaf pairs; $N = 7000$). In both assays, covers with a 5 cm × 5 cm opening were placed over the oviposition dishes.

Table 1. Influence of covers, placed over the oviposition dishes, on the difference in numbers of eggs laid below leaves of various plant species (only one cover type was used in an experiment). Results of dual choice experiments using the susceptible carrot cultivar *Daucus carota sativus* cv. 'Danvers' (Hardman et al., 1990) as standard plant. The discrimination index for a pair of juxtaposed standard and test leaves was calculated as follows: (standard – test)/(standard + test) × 100. We used either the Wilcoxon signed rank test (W), the Friedman test (F) or the Mann-Whitney U-test (MW) for the comparison, depending on whether the individual leaves tested with the different methods were identical (W and F) or not (MW). H = host plant; NH = non-host plant; *n* = number of replicates (leaf pairs); *N* = total number of eggs

Test leaves compared to standard carrot leaves in dual choice assays	Discrimination indices (mean ± s.e.)				*P*	test	*n*	*N*
	no cover	cover with opening						
		∅ 9 cm	5 × 5 cm	∅ 2 cm				
Foeniculum vulgare cv. 'Tardo', finocchio (H)	30 ± 12	-	31 ± 18	-	0.79	MW	8	1706
Foeniculum vulgare cv. 'Fino', finocchio (H)	31 ± 9	-	50 ± 5	-	0.09	MW	8	3040
Athyrium filix-femina, lady fern (NH)	32 ± 9	-	40 ± 8	-	0.26	W	8	5411
Pastinaca sativa sativa, cultivated parsnip (H)	32 ± 11	-	56 ± 6	-	0.07	MW	8	4067
Daucus capillifolius (H)	54 ± 8	-	69 ± 7	-	0.17	MW	8	4183
Pimpinella major, greater burnet saxifrage (H)	56 ± 3[1]	63 ± 6	71 ± 5[1]	73 ± 9	0.20	F	8	12715
Brassica oleracea convar. *botrytis*, cauliflower (NH)	74 ± 3	-	85 ± 3	-	0.00	W	16	10954

[1] difference significant at $P = 0.03$ (W)

Barriers between dishes. When we compared the incidence of oviposition around carrot leaves (cv. 'Tip-Top') and non-host leaves *Ranunculus repens* (creeping buttercup), isolation of the oviposition devices by plexiglass cylinders resulted in lower discrimination values than obtained with a set-up lacking these barriers: 75.8 ± 3.7 versus 87.1 ± 1.8 (mean ± s.e.; Wilcoxon signed rank test: $P = 0.03$; $n = 16$ leaf pairs; $N = 12811$ eggs).

Distance between dishes. When we placed only four instead of eight dishes (topped by covers with 5 cm × 5 cm opening) in the cage, thereby increasing the distance between neighbouring leaves from 20 cm to 35 cm, the discrimination indices calculated for pairs of host and non-host leaves (standard carrot cultivar and silverweed *Potentilla anserina*) did not become higher: 72.6 ± 4.4 with four dishes versus 73.7 ± 2.7 with eight dishes (mean ± s.e.; Wilcoxon signed rank test: $P = 0.78$; $n = 8$ leaf pairs; $N = 4436$ eggs).

Covers over dishes. Differences in the number of eggs deposited below test and standard leaves were invariably greater when the oviposition dishes were topped by the cover that had a 5 cm × 5 cm opening, though the effect was only significant in two cases (Table 1). However, the values for covered and uncovered oviposition dishes are significantly different, when they are combined for all test species: 60.8 ± 3.7 versus 48.0 ± 3.6 (mean ± s.e.; Mann-Whitney U-test: $P = 0.007$; $n = 64$ leaf pairs). In the experiment with greater burnet saxifrage *Pimpinella major* the discrimination indices increased with diminishing width of the opening through which the fly reaches the oviposition substrate. Except for the fennel cultivar 'Tardo', all the host plants chosen for the experiment were significantly less acceptable for oviposition than the standard carrot cultivar with or without the use of covers (Friedman test; $P < 0.05$). These species were also shown to be generally less susceptible to carrot fly attack in the field than the standard (Hardman et al., 1990).

A choice experiment with surrogate leaves revealed the impact of the covers on total ovipositon: the narrower the opening in the cover, the lower the absolute numbers of eggs on the dishes (Figure 1). Again discrimination between the treatments was slightly, though not significantly improved when the covers had medium to small openings (ANOVA with arcsine-transformed percentages: cover: $F = 10.0$; df = 3; $P < 0.0001$; chemical treatment: $F = 165.8$; df = 1; $P < 0.0001$; cover type × chemical treatment: $F = 0.6$; df = 3; $P = 0.6$). Oviposition dishes with control leaves topped by the covers with a 5 cm × 5 cm opening received significantly fewer eggs than the corresponding uncovered dishes, while no significant difference was found between these two treatments in the case of the extract-treated leaves.

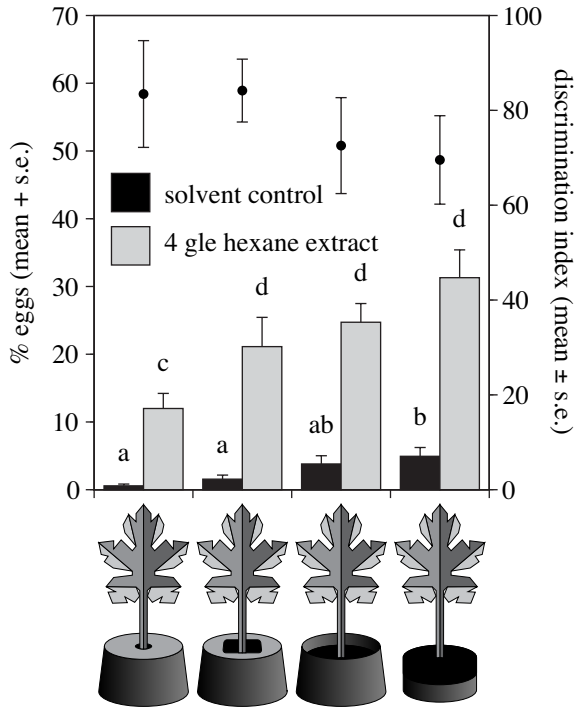

Figure 1. Influence of different covers, placed over the oviposition dishes, on total number of eggs and on discrimination between leaf models treated with host-plant extracts (at a concentration of 4 gram leaf equivalents) and control leaves (treatment effect: $P < 0.0001$; Friedman test). Results of a multiple choice assay. Multiple comparisons were made following the method ('least significant rank sum differences') given in Conover (1980). Means accompanied by the same letter are not significantly different at the 5%-level. Discrimination indices did not differ significantly (cover type: $P = 0.7$; Friedman test). Discrimination index = (extract − control)/(extract + control) × 100. Total number of eggs = 2346; $n = 8$.

Discussion

In previous oviposition experiments, separation of surrogate leaves by plexiglass cylinders resulted in an increased discrimination between host-plant extract and control treatments (Städler, 1977). Here we noted the opposite effect when real host and non-host leaves were placed in plexiglass cylinders. Females that have already been moderately stimulated for oviposition during previous runs over host foliage and accidentally land on a non-host leaf may be prevented from flying off by the cylinders and may subsequently end up laying eggs onto the 'wrong' dish. Yet, using covers that only concealed a segment of the dish improved the resolution of the assay. We assume that females flying off the leaf on which they were stimulated are less likely to gain access to the oviposition substrate belonging to another leaf when covers are present. Flies that landed on a cover were never seen to jump onto the dish just about 1 cm below, even after following the edge of the opening for some time. Furthermore the covers may also stop the flies from walking off the dishes or from reaching them when moving over the cage floor in search of an appropriate oviposition site (moist soil crevices mimicked by the polyethylene grid on the dishes).

Thus the modified bioassay may be slightly biased towards flies that perform complete leaf and stem runs, which is the most frequent, but not exclusive way for the flies to get to the oviposition substrate. During the exploratory runs females gradually adopt a positive geotactic behaviour and when moving downwards are occasionally 'trapped' on a dipping part of a leaflet. Such runs are often terminated by a short flight towards the substrate or the cage floor (Städler, 1977). The percentage of oviposition events occurring after flights is usually low and probably similar for most of the host-plant species. Yet there might be examples of plants (e.g. species with very slippery waxy surfaces and/or with morphological peculiarities such as fennel), on which flights are the common way for the flies to reach the oviposition substrate, rather than stem runs. In these rare cases, covers may lead to apparent differences among plants that are equal in acceptability under field conditions.

The relatively high numbers of eggs deposited below the fern leaves (Table 1) cannot be fully attributed to methodical shortcomings nor to a loss in the discriminative capabilities of the flies in our culture. Since we were no longer confronted with such low discrimination values in later experiments that included the same fern species, this phenomenon must rather be ascribed to variation in leaf quality: the standard leaves may for some unknown reason have been less acceptable than is normally the case, or the fern leaves may have lacked deterrent compounds or – less likely – were the source of some non-specific chemical oviposition stimuli.

Relatively low discrimination between hosts and non-hosts is also a consequence of the simplified experimental design withholding the flies from the full range of stimuli ('Gestalt') originating from plants under natural conditions. Presenting the flies with intact plants (including for example root

volatiles) or with devices equipped with many instead of only single leaves (Guerin & Städler, 1984) may also increase the differences among plants with respect to oviposition (Degen & Städler, in prep.). The former method may prove to be indispensable when screening for the rather small variation in antixenosis resistance among carrot cultivars. However, with morphologically divergent host species it is more difficult to adjust for differences in size among whole plants than when using cut leaves. Furthermore, testing potted plants is much more time-consuming and hence not suitable for the examination of large sets of species.

Since placing dishes further apart from each other in the cage does not yield better results, the use of covers appears to be a convenient way to improve the oviposition assay in limited laboratory space. Covers with a 5 cm × 5 cm opening were chosen for forthcoming experiments as they only moderately restrict access to the oviposition substrate and furthermore provide shady spots, which are favoured oviposition sites (Bohlen, 1967).

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Host-plant susceptibility to the carrot fly, *Psila rosae*. 1. Acceptability of various host species to ovipositing females

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Summary

A preference hierarchy of the oligophagous carrot fly for 30 umbelliferous host-plant species respectively varieties and 6 non-hosts was established. Foliage of the test plants was presented to a laboratory population of the fly in oviposition choice experiments together with leaves of a standard plant, the susceptible carrot cultivar "Danvers". Only two species (*Anthriscus cerefolium, Carum carvi*) proved to be more acceptable than the standard plant, while about half of the species received significantly fewer eggs. Some umbellifers (*Anethum graveolens*, *Pimpinella major*) came close to the low acceptability of non-hosts (non-umbelliferous plants). The results obtained with dual and multiple choice assays were in good agreement. Cut leaves were shown to be representative for whole plants, as a multiple choice assay with potted plants yielded a very similar ranking of the species as the corresponding assay using cut leaves. Variability in the exploratory runs performed by the females on the leaves prior to egg-laying was described and quantified for hosts and non-hosts. Differences in post-alighting pre-ovipositional behaviour of the female flies on the leaves accounted for a major part of the variation recorded in egg-laying.

Introduction

The carrot fly, *Psila rosae* (F.) (Diptera: Psilidae), is one of the most serious insect pests of carrots and other umbelliferous crops (e.g. celeriac, parsnip) in north temperate regions. Extensive field studies substantiated the long held assumption that its host range is restricted to members of the family Apiaceae (Hardman & Ellis, 1982; Hardman et al., 1990). About eighty percent of the umbelliferous species tested so far have been shown to be hosts. The most recent list of host records comprises 121 wild and cultivated species or subspecies, respectively (Ellis, Hardman & Saw, 1992; herein also a review of historical records).

Apart from cultural practices (e.g. choice of sowing and harvest time, crop rotation) the choice of less susceptible cultivars has been advanced as an integrated pest management strategy for carrot fly control (reviews in Dufault & Coaker, 1987; Finch, 1993). Ellis & Hardman (1981) and De Ponti & Freriks (1980) found consistent variation among carrot varieties in terms of damage caused by carrot fly larvae. Several attempts have been made to breed carrot lines with higher levels of resistance (Ellis et al., 1985; Ellis et al., 1991), e.g. by crossing carrot cultivars with *Daucus capillifolius*, a wild species of low susceptibility (Ellis et al., 1993). However, a profound knowledge of the underlying resistance mechanisms is still lacking, but might be crucial for substantial progress in breeding programs. There is some evidence that both antibiosis (Guerin et al., 1981; Maki & Ryan, 1989) and antixenosis, i.e. non-preference by the larvae (Maki & Ryan, 1989) and by the adults (Guerin & Ryan, 1984; Guerin & Städler, 1984), contribute to the partial resistance of carrot varieties against carrot fly.

Semiochemicals isolated from the surface of carrot foliage synergistically stimulate oviposition in the carrot fly (Städler & Buser, 1984). However, the leaf contents of these stimulants (propenylbenzenes, furanocoumarins, polyacetylenes) failed to account

for the relatively small differences in antixenotic resistance detected among carrot cultivars (Visser & de Ponti, 1983; Guerin & Städler, 1984; Städler et al., 1990). We believe that a wider range of host species needs to be included in studies of host selection by the carrot fly to achieve a more comprehensive understanding of the mechanisms underlying the choice process. Bohlen (1967) inferred the acceptability of various umbelliferous and non-umbelliferous plants from observations of the post-alighting pre-ovipositional behaviour of carrot flies without surveying the actual number of eggs laid. Quantitative investigations into the host-plant preferences have been confined so far to carrot cultivars (Guerin & Städler, 1984). In this study, we determined the preference hierarchy of the carrot fly for various host-plant species that were shown to differ largely in susceptibility in the field (Hardman et al., 1990). To this end, oviposition in response to foliage of test and standard plants was recorded in laboratory choice assays. The ranking of species according to their acceptability provides the basis for future investigations aimed at elucidating the mechanisms of host selection (e.g. the role of semiochemicals).

Materials and Methods

Insects. The carrot flies for the oviposition experiments were kept in a permanent laboratory culture reared on carrots (Städler, 1971) for ≤ 17 generations after wild pupae had been collected in a field at the Swiss Federal Research Station in Wädenswil. Adult flies continuously emerged from pupae in the cages in which the choice experiments were carried out. There is no evidence so far that preceding contact to particular host plants profoundly alters subsequent host acceptance in the carrot fly (T. Degen, unpublished). Nonetheless, possible effects of early adult experience were minimised by allowing the flies access to test and standard leaves for three days before the onset of the actual experiment, which typically lasted four days (four consecutive experimental periods).

Plant material. The foliage for the choice experiments was obtained from wild plants or from plants grown outdoors either in seed beds or in pots. Most of the seeds for the sowings were supplied by Horticulture Research International Wellesbourne, Warwick, UK; some extra seed material was acquired from commercial Swiss seed companies (Table 1). Every leaf tested originated from a different individual plant, except in few cases where insufficient leaf material was available (e.g. *Daucus capillifolius*, *Pimpinella major* in 1993). We gathered the second to fourth youngest leaf of the plant, provided that it was suitable in size (in about 80% of the cases). The leaves to be compared in a choice assay were cut to the same length, mostly to 24 cm or 20 cm (range 14 cm to 34 cm). The surface of each leaf was either measured with an area meter LI-3100 (Li-Cor, inc., Lincoln, Nebraska) or calculated from the fresh weight (in the 1992 experiments) with species-specific coefficients taken from linear regressions (without intercept as a simple approximation). Normally each oviposition dish was set up with only one leaf, in a few cases with two to four to compensate at least partly for pronounced differences in leaf area (e.g. Daucus capillifolius). With two annual plants (Aethusa cynapium, Daucus broteri), we used whole shoots because single leaves were too small.

Oviposition choice assays. The experiments were carried out in cubic screen cages (0.34 m^3), which were located in a controlled environment room (21 ± 1 °C, 70–80% r.h., L16:D8). Illumination was the same as chosen for a previous study (Degen & Städler, 1997a). The leaves were introduced into a small vessel containing water, which was incorporated into the oviposition devices. These consisted of plastic dishes (∅ 9 cm, height 4 cm) covered by a moist black cloth and a black polyethylene grid (Städler, 1971). The oviposition dishes were uncovered in the very first experiments, i.e. in the assays with leaves variable in size (Table 3) and some further assays performed in 1992 (species concerned are denoted in Figure 1). Later on we topped the dishes by an inverted black plastic pot allowing the flies access to the oviposition substrate only through a 5 × 5 cm wide opening. This set-up was shown to improve the resolution of the assay by reducing "stray" oviposition (Degen & Städler, 1997b).

Two different experimental approaches were adopted: dual choice assays in which leaves of a single test species were compared to leaves of the susceptible standard carrot cultivar "Danvers" and multiple choice assays that included a non-host, six different umbelliferous test species and the standard

Table 1. List of the plant species tested in the oviposition assays and origin of the plant material. Abbreviations: Api = Apiaceae (Umbelliferae); Asp = Aspleniaceae; Ran = Ranunculacae; Bra = Brassicaceae (Cruciferae); Ros = Rosaceae; Ast = Asteraceae (Compositae); ab = abbreviation of species name; gf = growth form: a = annuals; b = biennials (some of the biennial plants already flowered in the first year); p = perennials; or = origin of seeds: w = Genetic Resources Unit of Horticulture Research International Wellesbourne; g = Geissler Samen (Mioplant); s = Samen Mauser; lb = leaves from plants sown in beds; lp = leaves from plants sown in pots; lw = leaves from wild plants; wp = whole plants sown in pots filled with sand

family	species	ab	gf	or	lb	lp	lw	w
Api	*Aegopodium podagraria* L., ground elder	ap	p				+	
	Aethusa cynapium L., fool's parsley	ae	a	w	+			
	Anethum graveolens L., dill	ag	a	w,s	+	+		+
	Anthriscus cerefolium (L.) Hoffm., garden chervil	ac	a	g,s	+	+		+
	Apium graveolens var. *rapaceum* (A. W. Hill) cv. "Balder", celeriac	ar	b	w	+			
	Carum carvi L., caraway	cc	b	w,s	+	+		+
	Conium maculatum L., hemlock	cm	b	w	+	+		
	Daucus broteri Ten.	br	(a)	w	+			
	Daucus capillifolius Gilli	ca	b	w	+	+		
	Daucus capillifolius × *Daucus carota* ssp. *sativus*	dd	b	w	+			
	Daucus carota L. ssp. *azoricus* Franco	az	(b)	w	+			
	Daucus carota L. ssp. *commutatus* (Paol.) Thell.	co	b	w	+			
	Daucus carota L. ssp. *drepanensis* (Arc.) Heywood	dr	(b)	w	+			
	Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. "Danvers", standard carrot cultivar	da	b	w	+	+		+
	Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. "Sytan", carrot	sy	b	w	+			
	Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. "Tip-Top", carrot	tt	b	s	+			
	Daucus littoralis Sibth. & Sm.	li	(a)	w	+	+		
	Daucus muricatus (L.) L.	mu	(a)	w	+	+		
	Daucus pusillus Michaux	pu	(b)	w	+			
	Foeniculum vulgare Miller, fennel	fv	p	w	+	+		+
	Foeniculum vulgare var. *azoricum* (Miller) Thell., finocchio (HRI Wellesbourne)	fa	p	w	+	+		
	Foeniculum vulgare var. *azoricum* (Miller) Thell.cv. "Fino", finocchio	fi	p	s	+			
	Foeniculum vulgare var. *azoricum* (Miller) Thell. cv. "Tardo", finocchio	ta	p		+			
	Heracleum sphondylium L., hogweed	hs	p				+	
	Levisticum officinale Koch, lovage	lo	p	s	+			
	Pastinaca sativa ssp. *sativa* L. cv. "Halblange", parsnip	sa	b	s	+	+		+
	Pastinaca sativa ssp. *sylvestris* (Miller) Rouy & Camus, wild parsnip	si	b	w	+			
	Petroselinum crispum (Miller) A. W. Hill, parsley	pc	b	w	+			
	Petroselinum crispum var. *tuberosum* Crov. cv. "Berliner", Hamburg parsley	pt	b	s	+			
	Pimpinella major (L.) Huds., greater burnet saxifrage	pm	p	w	+	+		+
	Smyrnium olusatrum L., alexanders	so	b	w		+		
Asp	*Athyrium filix-femina* (L.) Roth, lady fern	af	p				+	
Ran	*Ranunculus repens* L., creeping buttercup	rr	p				+	
Bra	*Brassica oleracea* L. convar. *botrytis*, cauliflower	bo				+		
Ros	*Potentilla anserina* L., silverweed	pa	p				+	
Ast	*Cichorium intybus* L., chicory (Catalogna)	ci		w	+			+
	Tanacetum vulgare L., tansy	tv	p	(s)	+	+		

plant. Eight oviposition dishes were arranged in a circle around a non-host plant (potted apple seedling) in the centre of the cage, which served as a resting and copulation site. In the dual choice situation the positions of the four repetitions for the standard and test leaves were alternated; in the multiple choice situation the eight different treatments were randomly assigned to the positions. The leaves were exposed to the flies – typically 100–400 individuals (males and females) – mostly for one day, rarely for longer intervals (two and three days in about 12% and 1% of the cases, respectively), before the eggs were counted. For the following experimental period, the positions of the dishes were changed in such a way, that finally each treatment was placed once at each position. In the dual choice assays the same leaves were tested during two successive periods on different positions, whereas they were replaced by fresh material after each period in the multiple choice experiments.

For comparison, whole plants grown in pots filled with sand and destined for subsequent antibiosis tests (T. Degen, unpublished) were tested in a multiple choice assay (Table 1; Figure 2). Inevitably there was some variability in size among the plants: the plants were 12–40 cm high and consisted of 4–32 leaves. We placed a fine-meshed plastic screen on the soil around the plants and covered it with wet sand. This sand layer (about 1 cm thick) was removed after each experimental period and the eggs contained in it were collected by floatation with water.

Behavioural observations. The post-alighting preovipositional behaviour of individual flies was observed during the ongoing choice experiments through a small opening in the rear side of the box surrounding the cage. The observations were performed between 16:00 h and 21:00 h, the time of day with high oviposition activity (Städler, 1975). Female flies were distinguished from males according to their behaviour (Städler, 1977). The duration of leaf visits was measured with a stopwatch from the first contact to the final take-off from the leaf. Short flights or hops from leaflet to leaflet were not regarded as departure. Females temporarily occupied with resting and grooming were not included in the analysis. The incidence of typical behavioural elements (e.g. circular runs around the base of the leaf petiole) was noted. We assumed that oviposition occurred when females were persistently probing the egg-laying substrate through the holes in the grid with their ovipositor (see Bohlen, 1967; Overbeck, 1978). After they had spent two minutes displaying this behaviour, the observations were ceased.

Statistics. The numbers of eggs were compared with the Friedman test (multiple choice assays) and with the Friedman test modified for repetitions in a block, i.e. one experimental period in a cage (dual choice assays), and were expressed in the figures and tables as percent of total oviposition. Multiple comparisons among treatments were made following a method based on rank sum differences given by Conover (1980). The repeated exposure of the treatments (usually for four periods lasting one day) to the same cage population may be considered only as a minor statistical problem, since female carrot flies take on average 3–4 days between two successive oviposition bouts (Körting, 1940; Bohlen, 1967). Hence only a part of the individuals contributed eggs to more than one replicate. Also the majority of the plants were tested in two independent experiments (see Figure 1).

Results

Host plant preferences. Only three out of the 35 different species, subspecies or cultivars tested in dual choice assays elicited more egg-laying than the susceptible standard carrot cultivar "Danvers": *Anthriscus cerefolium*, *Carum carvi* and the hybrid *Daucus capillifolius* × *Daucus carota* (Figure 1). Several plants received almost the same numbers of eggs as the standard, including the partially resistant carrot cultivar "Sytan". With the exception of *Daucus broteri* and *Daucus capillifolius*, none of the *Daucus* species, subspecies or cultivars differed drastically from the standard carrot cultivar. About half of the host plant species was less acceptable than the standard plant and only one umbelliferous species, *Anethum graveolens*, was ranked lower than the least rejected non-umbelliferous plant.

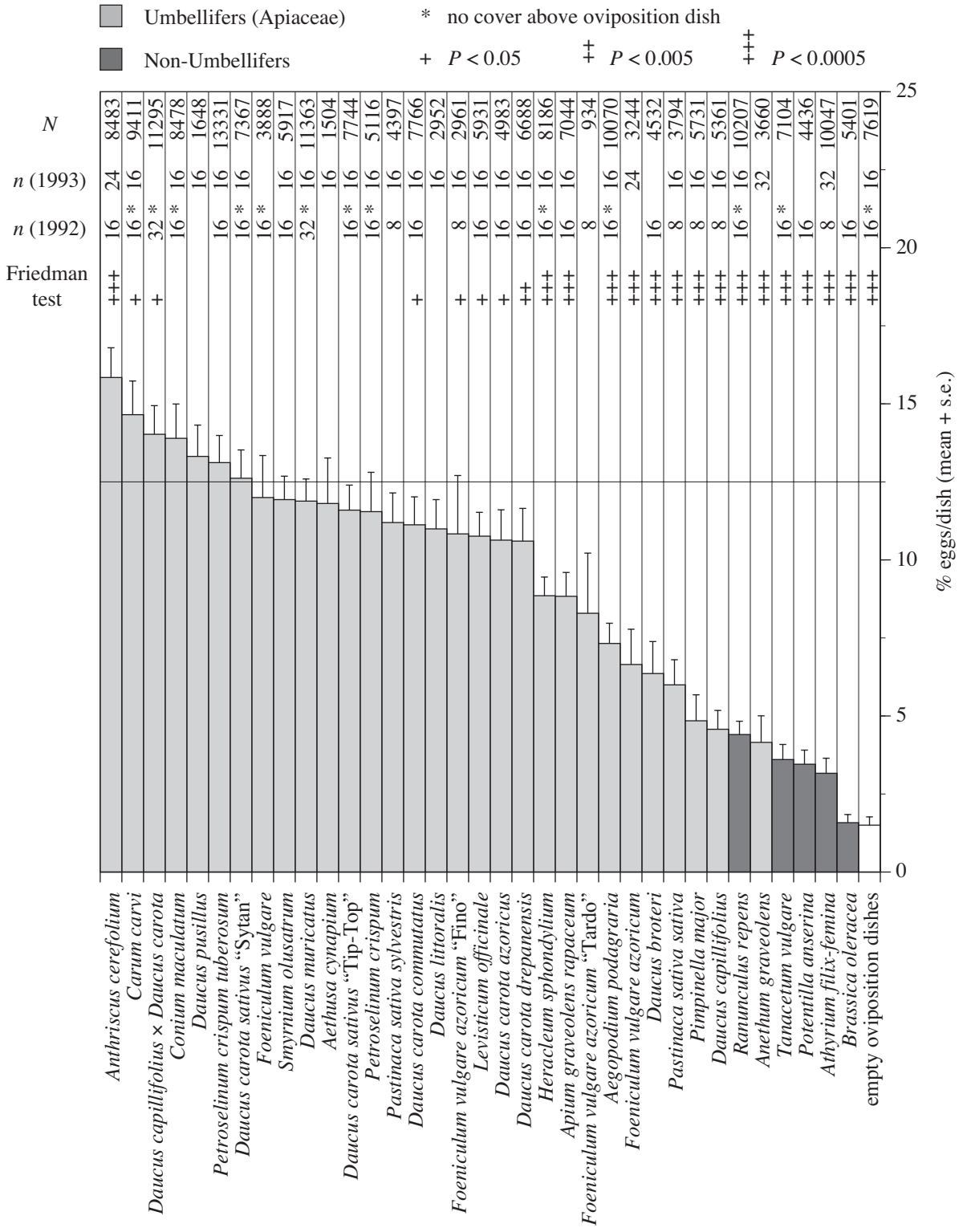

Figure 1. Oviposition preference hierarchy of carrot flies as established by dual choice assays. Foliage of the test plant species was compared to foliage of the standard plant *Daucus carota sativus* cv. "Danvers". The area above the columns corresponds to the complementary percentage of eggs deposited around standard leaves. Since four test leaves and four standard leaves were included in each experimental unit, the percentages add up to 25% instead of 100%. *n* = number of replicates; *N* = total number of eggs.

Table 2. Influence of age of standard plants *Daucus carota sativus* "Danvers" on the number of eggs deposited around cut leaves. Results of dual choice experiments. *n* = number of replicates; *N* = total number of eggs

assay	sowing date	plant age (days after sowing)	% eggs/dish (mean ± s.e.)	*P* (Friedman test)
1	28 April 1992	125–127	9.3 ± 1.4	< 0.005 (*n* = 16; *N* = 2424)
	3 July 1992	59–61	15.7 ± 1.4	
2	1 April 1993	102–104	10.0 ± 1.4	NS (*n* = 16; *N* = 2135)
	19 May 1993	54–56	15.0 ± 1.5	
3	19 May 1993	103–105	11.2 ± 1.6	NS (*n* = 16; *N* = 1467)
	17 July 1993	44–46	13.8 ± 1.7	

Most of the plants were tested in two independent dual choice assays conducted in 1992 and 1993, respectively (see Figure 1). The ovipositional data (% eggs/dish) obtained in both years were quite consistent ($r = 0.81$; $P < 0.0001$; $n = 24$). There were only three major discrepancies: the non-host *Athyrium filix-femina* was more acceptable in 1992 than in 1993 (7.5 ± 1.1% vs. 2.1 ± 0.3%), while the opposite was true for *Foeniculum vulgare* var. *azoricum* cv. "Fino" (6.4 ± 1.0% vs. 13.1 ± 2.6%) and *Petroselinum crispum* var. *tuberosum* (10.7 ± 1.2% vs. 15.6 ± 1.0%). Variability in acceptability of test and standard plants depending on age and/or season may have been responsible for these deviations.

Carrot plants – particularly when older – tended to be infested by the fungal disease *Alternaria porri* f. *dauci* that causes the formation of black spots all over the leaf. Leaves with 100–200 spots stimulated considerably less oviposition than unspotted leaves (≤ 5–10 spots): 6.7 ± 0.8% eggs per dish versus 18.3 ± 1.0% eggs per dish (mean ± s.e.; Friedman test: $P < 0.0005$; n = 16; N = 2844 eggs). For the comparative assays, leaves with no or only few spots were chosen. In order to provide standard plants that were as healthy as possible, we sowed two and three batches of carrots in 1992 and 1993, respectively. Hence the test species were not all tested against the same set of standard plants as far as sowing date and plant age were concerned. When we compared the standard plants of different ages, invariably the younger plants were preferred to the older plants, though the difference was only significant on one occasion (Table 2). These differences in acceptability may have been due to changes directly associated with plant maturation and/or due to seasonally variable growth conditions (e.g. with respect to temperature and irradiation).

The age of the plants tested ranged from 45 to 194 days (from sowing to leaf harvest). In the dual choice assays, oviposition around test plants (mean % eggs with test plant/mean % eggs with standard plant) was negatively correlated ($r = –0.32$; $P = 0.04$; $n = 44$) with the ratio between test plant age and standard plant age, which varied from minimally 0.9 to maximally 2.4 (mean = 1.4; mode = 1.0). This is further evidence that plant age affected oviposition of the carrot flies, with younger plants tending to be more acceptable than older plants.

To verify if single leaves are indeed representative of whole plants, i.e. that they supply essentially the same information to the flies, we compared oviposition around whole plants grown in pots with oviposition around cut leaves of the corresponding plant species in multiple choice assays (Figure 2). The preference hierarchies obtained with the two approaches were very similar. Since size is more difficult to control with whole plants than with single leaves, the higher variability in size and hence in conspicuousness among whole plants may have accounted for some of the deviations between the results for cut foliage and for intact plants. In particular, the potted *Anethum graveolens* plants probably attracted few flies to alight, as they were least apparent of all the tested plants (narrow upright shoots with relatively small leaves). We tested two additional sets of leaves originating from eight plant species each including the standard and a non-umbelliferous plant. The results of these multiple choice experiments agreed closely with the data for the dual choice experiments (Figure 3).

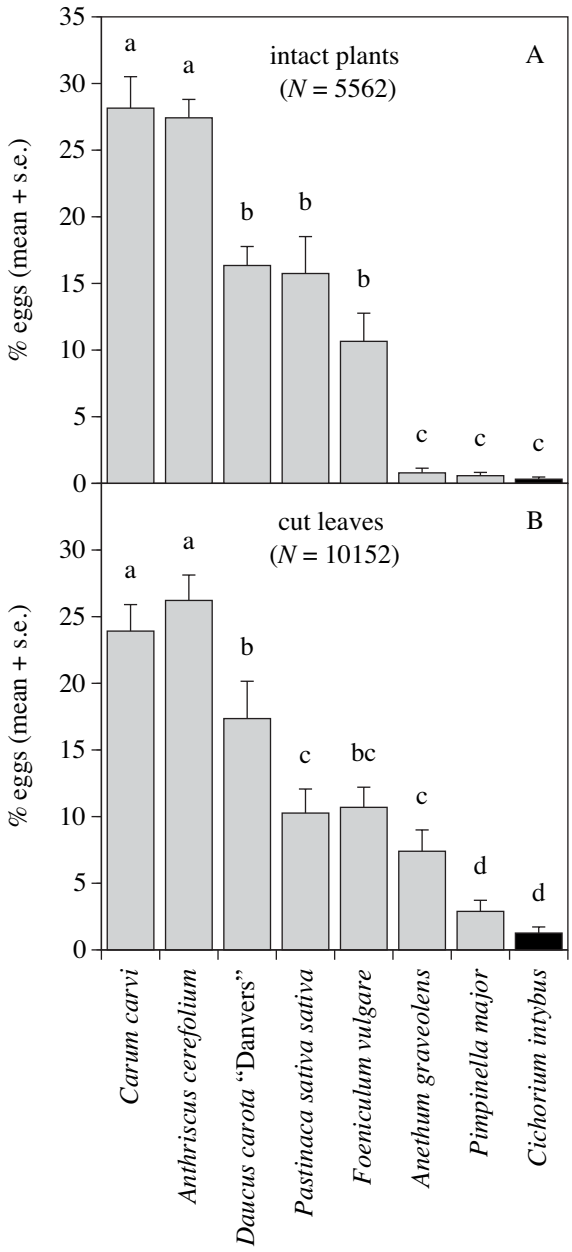

Figure 2. Oviposition around whole plants in pots (A) as compared to oviposition around cut leaves from plants grown in the field or in pots (B). Eight plant species including a non-umbellifer (black column) were tested simultaneously in the cages (multiple choice assays). Oviposition was significantly influenced by the plant species in both cases at $P < 0.0001$ (Friedman test). Significant differences at $P < 0.05$ between the individual species are indicated by different letters. The data obtained with the two methods are highly correlated ($r = 0.96$; $P = 0.0002$).

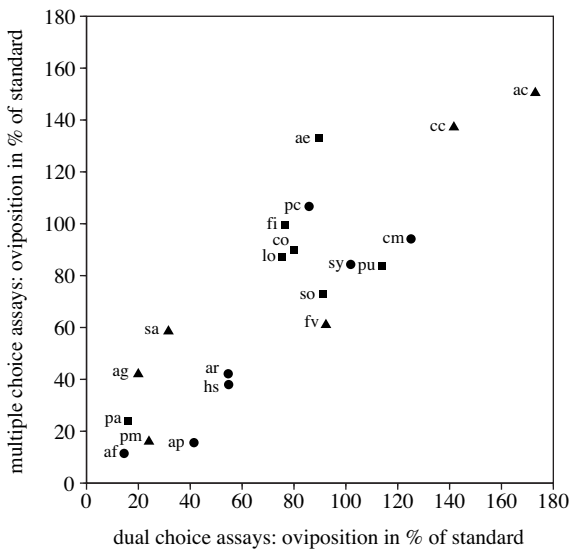

Figure 3. Correlation between oviposition preference hierarchies established by two different experimental approaches: dual comparisons between test and standard plant and multiple comparisons among eight species including the standard plant ($r = 0.86$; $P < 0.0001$; $n = 20$). The abbreviations of the plant names are explained in Table 1. Plants with identical symbols were tested in the same multiple choice assay. % oviposition of standard = (mean % eggs laid per dish with test leaves/mean % eggs laid per dish with standard leaves) × 100.

Influence of leaf size and morphological leaf properties on oviposition. Although the length of carrot leaves was varied in dual choice oviposition experiments by up to a threefold difference, a significant effect of leaf size was detected in only one instance (Table 3; assay 1). On the other hand, when leaf size was identical, but the oviposition dishes were raised to 10 cm above the ground, they received fewer eggs than dishes standing on the cage floor (Table 3; assay 6). No overall influence of leaf size (mean area of test leaves in percent of mean area of standard leaves) on oviposition (mean number of eggs around test leaves in percent of mean number of eggs around standard leaves) was detectable in the dual choice assays ($r = -0.10$; $n = 61$; $P = 0.44$). Also at the level of singular leaves, there was at best a weak positive relationship between the relative leaf size and the relative number of eggs with most of the plant species.

With very few exceptions, umbelliferous plants are characterized by possessing either pinnately or

Table 3. Influence of leaf size on oviposition. Dual choice experiments with carrot leaves (*Daucus carota* cv. "Tip-Top") differing in length and/or total height of leaf top above the cage floor

assay	length (cm)	mean area (cm^2)	leaf top above cage floor (cm)	% eggs/dish (mean ± s.e.)	*P* (Friedman test)
1[a]	8	20	12	8.4 ± 1.4	< 0.005 (n = 32; N = 1052)
	20	76	24	16.6 ± 2.0	
2	10	26	14	12.5 ± 0.6	NS (n = 64; N = 32132)
	20	64	24	12.5 ± 0.7	
3	10	25	14	12.7 ± 1.3	NS (n = 16; N = 7406)
	30	114	34	12.3 ± 1.4	
4	20	69	24	12.6 ± 1.5	NS (n = 16; N = 7535)
	30	159	34	12.4 ± 0.9	
5	10	27	24[b]	11.8 ± 1.2	NS (n = 16; N = 4674)
	20	60	24	13.2 ± 1.0	
6	20	54	24	16.4 ± 1.1	< 0.0005 (n = 16; N = 12546)
	20	59	34[b]	8.6 ± 0.7	

[a] carried out in a 50 × 50 × 50 cm screen cage
[b] oviposition dishes elevated 10 cm above the cage floor

ternately compound leaves. The degree of "dissection" varies widely among the species and is reflected in the number of "first-order leaflets" perpendicular to the leaf axis, which ranged from 2 to 16. Both in dual and multiple choice assays, oviposition on the test plant species (in percent of standard) was correlated with the relative number of leaflets (mean number for test plants in percent of mean number for the standard plant): $r = 0.28$; $n = 54$; $P = 0.04$ and $r = 0.48$; $n = 18$; $P = 0.05$, respectively.

Post-alighting pre-ovipositional behaviour. The objective of the observations was to scrutinize whether differential oviposition actually resulted from differential behaviour displayed by the females upon direct contact with foliage. After landing on a host-plant leaf, females ready to lay eggs performed exploratory runs, sometimes intermitted by short flights from leaflet to leaflet (or away from and back to the leaf). About a third of the observed individuals proceeded from leaf to stem runs, which were terminated in more than half the cases by a "circular run" (180–360°) around the stem base before stepping onto the oviposition substrate (Figure 4). There were some variations from this typical pattern of behaviour: a minority of the individuals reached the accompanying or a neighbouring dish by flight. For instance, the waxy surface of fennel leaves was obviously slippery and made regular stem runs difficult for the flies. A few runs leading to oviposition were also accomplished on non-host plants. We summarized the various run types in three main categories (see Table 4). Apart from regular leaf and stem runs, "complete leaf runs with oviposition" included also females that did not perform stem runs, but flew directly from the leaf to the oviposition site. "Complete runs without oviposition" comprised all other cases that were intermediate between runs with oviposition and interrupted runs and hence were difficult to assign to either group, such as females that were presumably stimulated for oviposition, but landed on the cage floor inappropriate for oviposition, or flies that visited the oviposition substrate without laying eggs. Females highly stimulated for oviposition showed rather quick, non-interrupted exploratory runs.

During exploratory runs females often performed up-and-down motions of the abdomen. This behaviour was more frequently observed with complete runs (with or without ensuing oviposition) than with

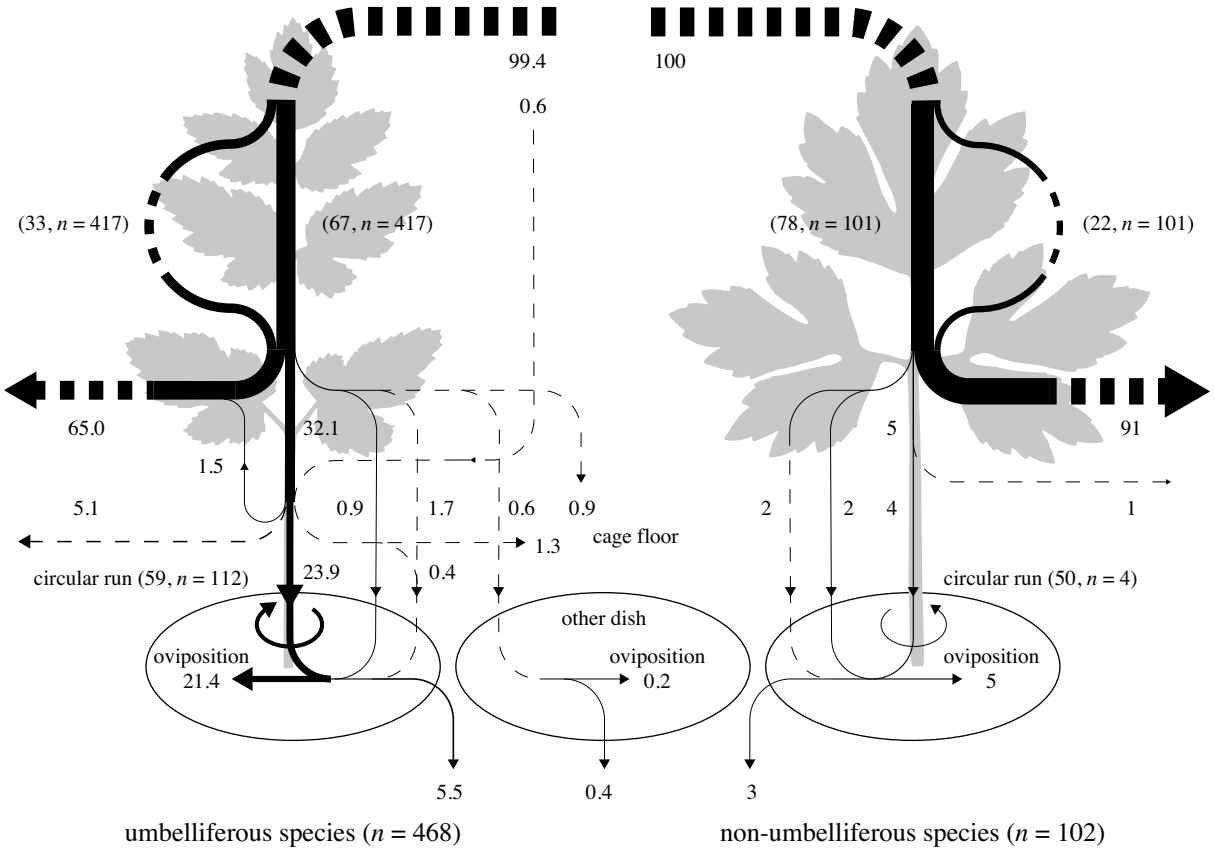

Figure 4. Pre-ovipositional behaviour of carrot flies on host and non-host foliage. The thickness of the lines corresponds to the proportion of females (also given as percentages) that showed the respective behaviour. The 20 umbelliferous (host) and 5 non-umbelliferous (non-host) plants covered are listed in Table 4. solid lines = run; dashed lines = flight.

interrupted runs (99% of runs versus 79% of runs; $N = 452$; missing values = 118; $\chi^2 = 23.0$, $P < 0.0001$) and more frequently on umbelliferous than on non-umbelliferous leaves (87% versus 73%; $\chi^2 = 9.1$, $P = 0.003$). The ovipositor that is usually folded and hidden below the abdomen was extended in 97% of complete runs, but in only 42% of interrupted runs ($N = 245$; missing values = 325; $\chi^2 = 53.3$, $P < 0.0001$). Extension of the ovipositor was also shown more often on umbelliferous than on non-umbelliferous leaves (61% against 29% of runs; $\chi^2 = 15.0$, $P = 0.0001$). Tapping with the proboscis was recorded in 100% of complete and in 87% of interrupted runs ($N = 216$; missing values = 354; $\chi^2 = 5.3$, $P = 0.02$). There was no difference between umbelliferous and non-umbelliferous foliage regarding the incidence of proboscis contact with the leaf surface (90% versus 91%; $\chi^2 = 0.0$, $P > 0.99$). These frequencies probably constitute maximal values, as it is easier to ascertain the occurrence than the absence of these behaviours, which is also reflected in the high number of missing values, i.e. observations with no data available on the behaviour concerned (ovipositor extension, proboscis contact).

Although the data are based only on a low number of observations for a particular plant species (Table 4), there was a strong correlation between the relative numbers of eggs deposited and the relative frequency of leaf runs resulting in oviposition (Figure 5). This implies that post-alighting preferences account for a major part of the variation in egg-laying (adjusted $r^2 = 0.54$). The strongest deviation from the expected value was observed with *Daucus capillifolius*, a species with presumably low apparency (comparatively small leaf area; narrow, hair-like leaflets), indicating indirectly that landing frequency may also have substantially contributed in this special case to the low numbers of eggs laid.

Table 4. Duration of three categories of leaf runs on different plant species. The species are arranged according to the decreasing percentage of runs with oviposition. Non-hosts (non-umbellifers) are written in bold letters

run type	complete run with oviposition		complete run without oviposition		interrupted run		
plant species	*n*	duration (s) mean ± s.e.	*n*	duration (s) mean ± s.e.	*n*	duration (s) mean ± s.e.	
Conium maculatum	8	26 ± 4	1	22	5	18 ± 6	
Daucus carota cv. "Danvers"	47	34 ± 3	13	34 ± 3	72	27 ± 3	
Petroselinum crispum	6	52 ± 14	2	50 ± 2	12	25 ± 6	
Carum carvi	5	25 ± 6			14	16 ± 2	
Smyrnium olusatrum	1	29			3	15 ± 6	
Daucus capillifolius	4	28 ± 8			12	19 ± 6	
D. capillifolius x *D. carota*	4	52 ± 17	4	62 ± 16	10	23 ± 6	
Daucus carota cv. "Tip-Top"	3	28 ± 2	2	31 ± 1	9	41 ± 15	
Foeniculum vulgare	3	35 ± 8	2	10 ± 6	9	46 ± 10	
Daucus carota cv. "Sytan"	5	48 ± 21			23	29 ± 4	
Petroselinum crispum tuberosum	3	37 ± 3			14	23 ± 6	
Daucus muricatus	2	40 ± 14	2	57 ± 26	10	22 ± 8	
Levisticum officinale	3	23 ± 2	3	18 ± 7	16	29 ± 13	
Athyrium filix-femina	3	58 ± 19	1	12	24	22 ± 5	
Daucus broteri	2	22 ± 16	3	40 ± 6	15	22 ± 4	
Ranunculus repens	2	40 ± 5			21	12 ± 2	
Heracleum sphondylium	2	42 ± 2	3	38 ± 7	21	24 ± 4	
Apium graveolens	1	13	1	3	15	9 ± 2	
Foeniculum vulgare cv. "Fino"	1	54	2	36 ± 21	14	20 ± 3	
Pastinaca sativa sativa	1	25			18	17 ± 3	
Tanacetum vulgare			1	6	13	19 ± 3	
Brassica oleracea			1	27	16	10 ± 2	
Aegopodium podagraria					18	18 ± 3	
Pimpinella major					19	11 ± 2	
Potentilla anserina					20	20 ± 4	
umbellifers (Apiaceae)	101	35 ± 2	38	36 ± 3	329	23 ± 1	$P < 0.0001$[2]
non-umbellifers	5	51 ± 12	3	15 ± 6	94	17 ± 2	$P = 0.01$[2]
		$P = 0.09$[1]		$P = 0.06$[1]		$P = 0.003$[1]	

[1] Mann-Whitney U test
[2] Kruskal-Wallis test

The time spent on a single exploratory run ranged from 2 to 202 s. The females remained on average longer on umbelliferous (27 ± 1 s, mean ± s.e.) than on non-umbelliferous leaves (19 ± 2 s). Since the duration of runs varied also with the run types – complete runs usually and plausibly lasted longer than interrupted runs – this difference among host and non-host plants may partly be attributed to differences in the frequency of these run types (Table 4). Nevertheless, the mean duration of interrupted runs was significantly shorter on non-umbelliferous than on umbelliferous leaves, too. Yet, there was some overlap among non-host and host species and interspecific variation was quite pronounced within both plant groups. The mean length of interrupted runs for a particular species seemed to be indicative of the acceptability, as it was positively correlated with the number of eggs deposited (in percent of standard; $r = 0.48$; $n = 25$; $P = 0.01$). The duration of runs may depend on the chemical and physical properties of the leaf surface as well as on leaf morphology. There was a significant correlation

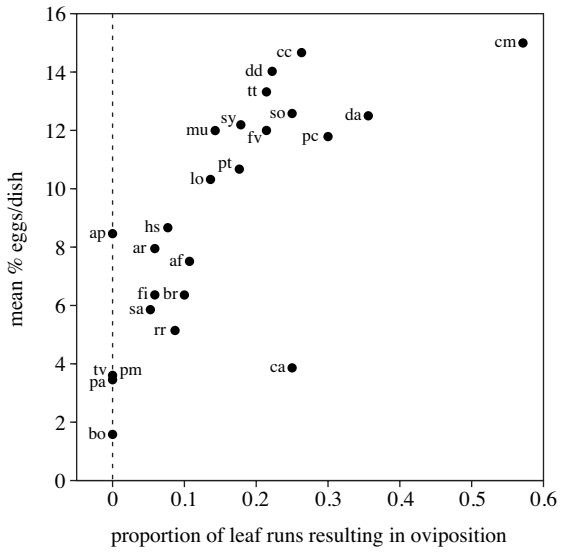

Figure 5. Correlation between the frequency of leaf runs completed by oviposition for the different plant species and the number of eggs deposited in the dual choice experiments ($r = 0.75$; $P < 0.0001$; $n = 25$). The full names corresponding to the abbreviations are given in Table 1. For the standard plant mean percent eggs per dish was set to 12.5.

between the mean number of first order leaflets along the main axis of the leaf and the mean duration of leaf runs ($r = 0.47$; $n = 24$; $P = 0.02$). The flies may have experienced more difficulty in finding the way to the soil on higher order compound leaves, which is also reflected in a higher frequency of intermittent short flights from leaflet to leaflet on these plants. Other factors may include physical properties of the leaf surface such as slipperiness of the cuticle or hairiness. For instance, the flies often failed to cling to the waxy cuticle of cauliflower leaves upon and after landing.

Discussion

Preference hierarchy. The differences among host-plant species in susceptibility in the field were greater by almost two orders of magnitude than the corresponding differences detected in our laboratory oviposition assays (Hardman et al., 1990). This may be explained in part by the fact that the present study focused on host acceptance after the flies were in contact with the leaf surface thereby by-passing some steps in the normal sequence of host finding and selection processes in a natural environment. Susceptibility in the field as measured by the numbers of flies produced by a plant is also affected by factors that we eliminated or at least tried to keep constant in our experiments, i.e. suitability for larval development (antibiosis resistance) and apparency (related to plant size and morphology), respectively.

Though there are similarities between the ranking of species according to susceptibility in the field (Hardman et al., 1990) and the oviposition preference hierarchy established in the laboratory, the relationship is not very close. Plants that supported high numbers of flies in the field (e.g. *Aethusa cynapium*, *Daucus muricatus*) invariably received comparatively high percentages of eggs in our assays. On the other hand, several species with low or only moderate susceptibility in the field were highly acceptable to the egg-laying flies, e.g. *Smyrnium olusatrum*, *Daucus pusillus* and *Carum carvi*. *Daucus capillifolius* and *Pimpinella major* were ranked virtually as non-hosts. However, these umbelliferous plants – both highly resistant in the field – might still be preferred to the non-umbellifers upon direct comparison in dual choice assay. The same applies to *Anethum graveolens*, which is a proven host-plant (Hardman & Ellis, 1982) contrarily to Bohlen's (1967) conclusion based on observations of caged flies. We can confirm here its status as a very low-ranking host.

In earlier choice assays performed with a comparable experimental design, a preference hierarchy was determined that was essentially in good accordance with the findings reported here (E. Städler, unpubl.). A notable deviation was found with celeriac, *Apium graveolens* var. *rapaceum* (cv. "Volltreffer"), that was as acceptable as carrot, *Daucus carota sativus* (cv. "Nandor"), whilst in our study the foliage of cv. "Balder" elicited less egg-laying than leaves of the standard carrot cultivar "Danvers". This discrepancy may be due to varietal differences in acceptability. On the other hand, we were not able to confirm the earlier reported preference for the susceptible carrot cultivar "Danvers" as compared to the partially resistant cultivar "Sytan" (Guerin & Städler, 1984; Städler et al., 1990). Very generally, there was no pronounced intraspecific variation in acceptability within the species *Daucus carota*, i.e. among cultivars and wild subspecies.

Methodical aspects. Our bioassay clearly had rather a low "resolution" as shown by the fact that a certain percentage of the eggs was found even on dishes devoid of leaves (for a detailed discussion of this issue see Degen & Städler, 1997b). However, this may not be just a cage artefact, as carrot flies were also observed to lay eggs around non-umbelliferous plants that grew in the vicinity of carrots in the field (Baker et al., 1942). The relatively loose association of oviposition site with the host plant may reflect the strong need of carrot flies to search for humid crevices in the soil suitable for egg-laying (Overbeck, 1978) and the ability of the larvae to find host-plant roots over some distance (Jones & Coaker, 1980).

Assays using cut leaves yielded a preference hierarchy very similar to the one obtained with intact plants, but the differences among the plants were more pronounced with the latter method. In a field study, access of the flies to the foliage alone was insufficient to reproduce differential egg-laying found with two carrot cultivars, it was necessary to expose the root tops as well, an effect that was tentatively assigned to attractants and/or oviposition stimulants in root scent (Maki & Ryan, 1989). When screening for the rather subtle variation in antixenotic resistance among carrot cultivars in breeding programs, choice and no-choice experiments with intact plants under conditions as natural as possible are likely to be indispensable, e.g. oviposition assays with intact plants grown in pots. Alternatively, oviposition can also be directly recorded in the field either with an egg collection method (Guerin & Ryan, 1984) or with egg traps (Freuler & Fischer, 1983; Maki & Ryan, 1989). However, these methods are very time consuming and hence not suitable for the examination of large sets of different test plants.

Multiple and dual choice experiments gave essentially the same results. Dual choice assays are advantageous from a statistical point of view, but they may be preferable only when a real standard is available, e.g. a surrogate leaf treated with a host-plant extract stable in stimulatory activity over long time periods. On the other hand, more species may be screened within a certain limited time when performing multiple comparisons. Moreover, results are directly comparable among test plants comprised in the same experiment and variability in simultaneously tested plants due to environmental factors or age is easier to control. Since the number of eggs laid around foliage of a particular species depends on the quality of the other treatments present in the assay, it might be helpful to include also a non-host standard plant as a negative control so that the outcomes of different multiple choice assays can be better compared.

In the field, the number of eggs laid by carrot flies increases with the size of the carrot foliage (e.g. Petherbridge & Wright, 1943). We were not able to detect such a correlation between leaf size and oviposition in our laboratory assays. This could be a cage artefact due to our experimental set-up. If we assume that landing frequency is proportional to leaf size, our results imply that fewer flies completed leaf and stem runs on big than on small leaves. Flies stimulated to oviposit may experience more difficulties in finding the way to the oviposition dish when running over big leaves and may be more prone to fly off in search of an oviposition site. However, this explanation does not apply to the situation with leaves of identical size, but different total height above the cage floor: the flies deposited fewer eggs on elevated oviposition dishes. On several occasions sexually displaying males were observed to disrupt the exploratory run of a nearby female, when alighting on a leaf. The probability of such encounters – also depending on fly density in the cages – might be higher in the upper half of the cage and consequently disturbance by males more intense on longer than on shorter leaves. In conclusion, the overall outcome of our study was not influenced by variation in leaf size, but such an effect cannot be ruled out in exceptional cases (e.g. *Daucus capillifolius*). To control size, along with foliage height, more accurately, it may be desirable to keep total leaf area constant by adjusting weight according to specific regression coefficients.

Behavioural observations. We did not count the number of flies alighting on the foliage of the various plant species. However, differences in landing frequency are supposed to be minor as the test leaves were quite similar in apparency to the standard leaves with only few exceptions, e.g. *Daucus capillifolius*. The observations suggest that most of the differences in oviposition arose because of differential acceptance upon direct contact of the flies with the leaf surface. The exploratory runs lasted on average longer on host than on non-host leaves in accordance with earlier findings (Bohlen, 1967; Städler, 1977; Luisier, 1989). This suggests

that rejection of non-hosts can be achieved more rapidly than acceptance of hosts. The percentage of leaf runs followed by oviposition was comparatively low, e.g. on carrot foliage 18–36% in our study as opposed to 48% and 85% in previous investigations. This low proportion of runs leading to egg-laying implies that the flies usually encountered several leaves before egg-laying ensued. Hence they probably had an opportunity to "weigh the alternatives" and so it may be justified to use the terms "host preference" and "host selection" along with "acceptance" in this context (for definitions see Miller & Strickler, 1984). It is also conceivable that "cumulative" stimulation on several leaves was necessary for the females to reach a threshold and to be ready to oviposit. Females in a state of high stimulation might have then occasionally also conducted complete runs with oviposition on non-host leaves.

During exploratory runs the flies can gain manifold sensory information about chemical and physical plant characteristics. The frequently performed circular runs around the stem base have also been described for the cabbage root fly *Delia radicum*, a species with similar pre-oviposition behaviour (Städler & Schöni, 1990). It was suggested that the circling of the stem might be a means of assessing available resources, i.e. plant size (Roessingh & Städler, 1990). Almost all females showed vertical movements of the abdomen and extended their ovipositor during exploratory runs with subsequent oviposition. Therefore we cannot rule out that the trichoid sensilla located on the ovipositor (Behan & Ryan, 1977) play a role in the perception of host-specific compounds. However, this behaviour may be rather an effect than a cause of the stimulation. Ablation experiments and electrophysiological recordings indicated that tarsal D-hairs are involved in host recognition (Städler, 1977; Städler, 1982). The oviposition stimulants previously identified (Städler & Buser, 1984) are perceived by olfactory sensilla on the antennae, while contact chemoreceptors on the tarsi and on the proboscis seemingly are not sensitive to these compounds (Städler & Roessingh, 1991).

Plant cues influencing host selection. Host-plants with leaves consisting of many leaflets (e.g. *Daucus carota*, *Conium maculatum*, *Carum carvi*) tended to receive more eggs than host plants with only moderately dissected compound leaves (e.g. *Apium graveolens*, *Heracleum sphondylium*, *Aegopodium podagraria*). Since several chemical and physical leaf characteristics may interact in a complex way, this correlation among relative number of leaflets and relative acceptability does not necessarily imply any causal relationship, but it suggests that factors other than leaf chemistry may also affect host selection. Indeed, studies using paper surrogate leaves revealed the influence of leaf shape: pinnate leaves received more eggs than non-pinnate leaves (Städler, 1977; Degen & Städler, 1996). Leaf morphology might explain the lower ranking of the cauliflower as compared to the other non-hosts, which were mostly distinguished by compound leaves resembling umbelliferous foliage. Surface characteristics may also have played a role in this context: extensively crystallized epicuticular waxes (e.g. wax bloom in *Brassica oleracea*) can impede attachment and locomotion of insects (Eigenbrode et al., 1996). Nevertheless, non-chemical leaf traits lack the specificity that is necessary to explain the restricted host range of the carrot fly and therefore we conclude that semiochemicals (e.g. stimulants, deterrents) are more likely to be the key to the understanding of the host selection process. The preference hierarchy established in this study may serve as a basis for forthcoming research on chemical aspects in this insect-plant relationship.

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Host-plant susceptibility to the carrot fly, *Psila rosae*. 2. Suitability of various host species for larval development

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Summary

Several apiaceous and two asteraceous species were tested for their suitability to support larval development of the carrot fly. Plants grown in pots or transplanted from seed beds into pots, were inoculated with a specific number of eggs. Pupae and non-pupated larvae were collected 6–7 weeks after inoculation. Both the number and weights of pupae produced varied widely among the species. Cultivated carrots *Daucus carota sativus* often gave rise to only moderate numbers of pupae, but these invariably attained the highest weights. *Pimpinella major* was the only apiaceous plant tested that did not yield any carrot flies. The two asteraceous plants *Cichorium intybus* and *Tanacetum vulgare* failed to support larval development. Total carrot fly biomass produced per plant was influenced by both the host species and the root weight. Emergence rates of adult flies were positively correlated with pupal weights. Small individuals tended to have a longer total developmental time from egg to adult fly.

Introduction

Given the vast amount of literature (see bibliography compiled by Hardman et al., 1985) published on the carrot fly, *Psila rosae* (F.) (Diptera: Psilidae), it is surprising that certain basic aspects of the relationship of this insect with its host plants are still unknown. On the side of the host plants, research has so far concentrated mainly on cultivated carrots, reflecting the economic importance of losses due to carrot fly attack in this crop, whereas wild and other cultivated host species have received much less attention. However, while the host range of the carrot fly seems to be restricted to the Apiaceae (= Umbelliferae), the majority of species within this plant family may be considered potential host plants: up to now 107 umbelliferous species have been reported as hosts, while only 26 species have failed to support any carrot flies, when tested under field conditions (Ellis et al., 1992). However, susceptibility to attack varied largely among the diverse hosts as indicated by the number of flies emerging from root and soil samples of plants that were exposed to a high natural field population of carrot flies (Hardman et al., 1990). Various wild *Daucus* species and subspecies closely related to cultivated carrot were screened in the same way to identify potential sources of resistance (Hardman & Ellis, 1990). Subsequent attempts to introduce resistance genes into carrot cultivars by crossing them with *Daucus capillifolius,* a wild species of low susceptibility, resulted in some breeding lines with increased resistance (Ellis et al., 1993). However, these breeding efforts have not been based on an evaluation of the underlying resistance mechanisms. Several factors were previously shown to contribute to differences in susceptibility among carrot cultivars: antixenotic resistance both to ovipositing flies (Guerin & Ryan, 1984; Guerin & Städler, 1984) and to larvae (Maki & Ryan, 1989) as well as antibiotic resistance (Guerin et al., 1981; Maki & Ryan, 1989). This study focuses on the latter mode of resistance, i.e. root-mediated effects, as manifested in a wider range of hosts. No quantitative assessment of the relative suitability of various host-plant species to

support larval development has been carried out so far. Here we present data on the performance of carrot fly larvae on several apiaceous genera including different *Daucus* genotypes and on two asteraceous species. We recorded the numbers, individual weights, emergence rates and total developmental times of carrot flies produced on potted plants that were inoculated with a specific number of eggs. This study was intended to complement the results of investigations into the relative acceptabilities of hosts to adult carrot flies, which were conducted with an identical set of plant species (Degen et al., in prep.).

Materials and Methods

Insects. The eggs for inoculation were obtained from a laboratory culture of carrot flies reared on carrots (Städler, 1971a) for ≤ 16 generations after wild pupae had been collected from an infested field in Wädenswil, Switzerland.

Plants. Single plants in clay pots (∅ top 13 cm; ∅ bottom 8 cm; height 16 cm; volume c. 1.2 l) were tested for their ability to support larval development of the carrot fly. Two methods were used to raise the plants. Some plants were grown in pots either from pricked seedlings or from seedling directly germinated in the pots (plants grown in pots). Roots of plants grown outdoors in seed beds or roots of wild plants (*Heracleum sphondylium*, *Aegopodium podagraria*) were dug up and transplanted into the pots (transplanted plants). Most of the seed for the sowings were supplied by Horticulture Research International Wellesbourne, Warwick, UK; some other seed material was acquired from a commercial Swiss seed company (see Appendix with a list of the English plant names). Seed of certain wild species did not germinate at all (e.g. *Heracleum sphondylium*) or only at a very low rate (e.g. *Pimpinella major*, *Daucus capillifolius*), so that these species could not be tested in all the experiments. The susceptible carrot cultivar "Danvers" was chosen as a standard plant and was included in all test series. This cultivar has also been used as a standard by Hardman et al. (1990) in their field study on the host range of the carrot fly. A summary of the different experimental parameters is given in Table 1.

Plants grown in pots. After the seedlings had established in the greenhouse, the pots were transferred into a seed bed outdoors that was covered with an insect net (mesh 1.3 mm) to protect the plants from natural infestation by carrot flies. The plants were moved back into the greenhouse or the climate chamber respectively for inoculation in autumn, on average five months after they had been sown (range: 2–7 months). The biennial plants as well as a few perennial plants in the 1994 experiment (7 out of 12 *Pimpinella major* and 5 out of 10 *Levisticum officinale*) that were tested in the second year had overwintered outdoors in the seed bed.

Transplanted plants. When dug out of the soil (2–5 months after sowing), the roots to be transplanted were inevitably damaged to some degree, especially the branched roots from larger plants (e.g. *Foeniculum vulgare*). In the case of *Aegopodium podagraria*, a clonal plant producing long shoots, we used several pieces (14–40) of subterranean runners, which could not be attributed to an individual plant. Before the roots were embedded in the pots filled with sand, the remaining soil was removed, the leaves were trimmed down and root weight was recorded. To allow for the development of fresh side roots and foliage, the plants were kept in the greenhouse for 2–10 weeks before inoculation (1992: 15–75 d; 1993a: 18–27 d; 1993b: 40–58 d). This marked difference in time elapsing between embedding and inoculation may have contributed to variation in numbers of pupae produced per plant species, but we were not able to detect a clear-cut effect. We failed to completely sort out roots already naturally infested by the carrot fly larvae as evidenced by a few adult flies emerging from the potted plants in the greenhouse (e.g. *Apium graveolens* in 1992). However, the number of eggs deposited in addition to the inoculated eggs was probably negligible, because stray flies were caught with yellow sticky traps within the greenhouse compartment.

Substrate. The mixture of compost, peat and sand in equal proportions used initially (experiments with plants grown in pots in 1992) did not prove to be suitable, as it was difficult to spot the pupae within the floating organic matter. Therefore we used sand for the remaining experiments and supplied the plants weekly with soluble fertilizer (Hauert Flory 9 or Flory 2). Nevertheless, it was not known if the plants were adequately supplied with nutrients as growth was usually rather slow and somewhat irregular. In the 1994 experiment performed in the

Table 1. Some important parameters of the antibiosis experiments
method: tp = plants transplanted from seed-beds into pots; gp = plants directly grown in pots
substrate: so = soil, mixture of compost, peat and sand; sa = sand; ls = mixture of loam particles and sand; f9 = soluble fertilizer Hauert Flory 9 (15% N, 10% P, 22% K, 3.6% Mg, 0.12% Fe, 0.03% B, 0.05% Mn, 0.002% Cu, 0.005% Mo, 0.01% Zn); f2 = soluble fertilizer Hauert Flory 2 (15% N, 5% P, 25% K, 2% Mg, 0.02% B, 0.05% Mn, 0.04% Cu, 0.01% Zn)
location: gh = greenhouse; cc = climate controlled room

method	substrate	location	date (month)	particularities
tp	sa	gh	1992 (Dec–Feb 93)	humidifier used in one greenhouse compartment
tp	sa (+f9)	gh	1993a (Jul–Sep)	
tp	sa (+f9)	gh	1993b (Nov–Jan 94)	
tp	so	gh	1992 (Jul–Oct)	biennial plants in second year (2y); grown from roots overwintered in a cold room; pots kept outdoors after transplantation of roots; inoculation with 20 or 60 eggs
gp	so	gh	1992 (Jul–Nov)	standard plant not included in all accessions, seedlings pricked into the pots after germination; inoculation with 20 or 60 eggs
gp	sa (+f9)	gh	1993a (Aug–Nov)	seedlings germinated in Petri dishes, pricked; beneficials (*Aphidius matricariae*, *Aphidoletis aphidimyza*, *Phytoseiulus persimilis*); irrigation with system "Tropf-blumat®"
gp	sa (+f9)	gh	1993b (Oct–Dec)	seedlings germinated in Petri dishes, pricked; beneficials (*Phytoseiulus persimilis*)
gp	sa (+f9,2)	gh	1994 (Jun–Jul)	biennial plants in second year (2y); seedlings germinated (1993) in Petri dishes, pricked
gp	sa (+f2)	gh	1994 (Aug–Oct)	seedling germinated in the pots, surplus seedlings removed
gp	sa (+f2)	cc	1994 (Oct–Dec)	seedling germinated in the pots, surplus seedlings removed; beneficials (*Amblyseius cucumeris*, *Phytoseiulus persimilis*, *Aphidius matricariae*)
gp	ls (+f2)	cc	1994 (Oct–Dec)	only standard carrot cultivar "Danvers"
tp	sa (+f2)	cc	1994 (Oct–Dec)	only standard carrot cultivar "Danvers"

climate chamber, we included standard carrot plants for comparison raised in a mixture of sand and fine-grained loamy particles, which were obtained by rinsing soil samples with water until all floating litter was removed.

Inoculation. Freshly laid eggs (one day old) were collected with a small paint brush, transferred to small pieces of wet black cloth and counted under a binocular microscope. Each plant was inoculated with 60 eggs, which were rinsed with water into the pots. In the experiments carried out in 1992 with plants grown in pots, half of the plants received only 20 eggs. The pots were regularly watered, because carrot fly eggs and larvae are very susceptible to desiccation, i.e. mortality is negatively correlated with humidity (Overbeck, 1978). An irrigation system consisting of small hose pipes ("Tropf-Blumat") did not notably improve yield of pupae and thus was only utilized once (experiment 1993a with carrots grown in pots).

Location of the inoculated plants. The inoculated plants were kept in small greenhouse compartments. This allowed partial control of air temperature (approximately 21 ± 2 °C; min. 16 °C; max. 28 °C; cooling in summer with a ventilator, heating in autumn and winter with an oven), but not of air humidity (approximately 70–90%; min. 50%; max. 100%). Therefore the temperature within the pots containing a moist substrate could not be accurately kept constant, because it depended on the air humidity (cooling due to evaporation). The numbers of pupae produced per plant (i.e. larval mortality) were quite variable even for the same host species. As this was probably in part due to changing abiotic conditions in the greenhouse, we conducted an additional experiment in a climate controlled room (20–21°C in the pots; 90 ± 5% r.h.) in 1994. In this case, illumination was provided by four sodium-vapour lamps (Philips SON-T Plus 400W; approximately 7000 lux 50 cm above the floor). Several plant species (e.g. *Daucus pusillus*, *Daucus muricatus*, *Anethum graveolens*) did not support stress due to high air humidity

and/or due to carrot fly attack, occasionally occurring infestation by other insects (e.g. aphids, thrips) or fungal pathogens (e.g. mildew, *Botrytis cinerea*) both in the greenhouse and the climate chamber. To avoid any noxious effect on the carrot fly larvae, no insecticides or fungicides were used against these pests, but in some cases we tried to control the aphids, thrips and spider mites by releasing natural enemies (Table 1).

Washing out of pupae and non-pupated larvae. Six to seven weeks after inoculation (range 41–49 d; 42 d in 40% of the cases), pupae and non-pupated larvae were washed out of the pots using a washing apparatus and collected on a fine-meshed sieve. Floating or submerged puparia and larvae – unless dead – were picked up with forceps and weighed the same day to an accuracy of ± 0.1 mg on an electronic balance (Mettler AE 260 DeltaRange®).

Root weight was recorded whenever possible; in rare cases of totally rotten plants that were not ignored for the data analysis, we used the mean root weight attained by the species in the particular experiment. With the transplanted plants, the mean of the two measurements – before embedding and after washing out – was used in the analysis (Figure 1A; Table 3). Root weight was lower on the second measurement in most cases. This decrease may be partly due to shrinking of the roots, partly due to the fact, that soil particles adhering to the roots were removed with washing. However, variation in weight loss had no obvious effect on the ranking of the species according to the numbers of pupae produced per plant.

Carrot fly damage was assessed for some of the cultivated carrots, *Daucus carota sativus*: the estimated proportion of the surface that was occupied by mines was multiplied by total surface area, which we calculated from the length of the tap root and from its diameter at the top and at the root tip assuming an ideal conical shape. Furthermore, in the 1993a experiments, carrots were cut into slices of about 1 cm thickness to determine the mean number of internal mines running in an axial direction along the central cylinder.

Roots that were entirely rotten and did not give rise to pupae or non-pupated larvae were excluded from the analysis. Likewise, we discarded totally or partially rotten plants producing flies, when decomposition was obviously caused by pathogens (e.g. fungi, bacteria) rather than by carrot fly attack, but most probably substantially affected larval survival and development. Clearly, cause and effect were often difficult to separate in such cases and hence the selection was somewhat arbitrary.

Emergence. For determination of hatching date and rate, the pupae were stored individually in compartments of ELISA-plates covered by transparent perforated plastic foil, which prevented the flies from escaping. In a climate controlled room (21 ± 1 °C, 70 ± 5% r.h.), the plates were put in plastic boxes (33 × 22 × 9 cm, lid with two openings covered with screen, ∅ 5 cm) above a water layer to provide high air humidity (~ 100%). Emergence of the flies was recorded daily during the week, but not regularly at the weekends. Thus, the hatching date was known to the exact day in 72.4% of the individuals, to an accuracy of +1 day, –1 day and ± 1 day in 6.1, 6.7 and 14.8% of the cases, respectively. Since we ceased to regularly monitor emergence when no flies hatched anymore for a longer period, the precise hatching date of a few "overlaying" insects was not known. The last control was carried out on average 128 days after inoculation (range: 88–238 days).

Weights of pupae obtained from plants naturally infested in the field. For comparative purposes, we recorded pupal weights of wild carrot flies that had developed on various plants species grown in seed beds. Pupae were washed out of samples of soil that surrounded the roots of attacked plants. In addition, pupae were collected from infested carrots and celeriacs, which were inserted into boxes (Eternit®; ~ 30 × 30 cm wide, 15 cm high) filled with moist sand and which were kept at 20 °C and 90% r.h. in a climate controlled room until pupation. Since different plant species were only spaced 30–40 cm from each other in the seed beds and since larvae were shown to move up to 60 cm along and between carrot rows (Jones & Coaker, 1980), allocation of the larvae to the host plants was only possible with some reservations.

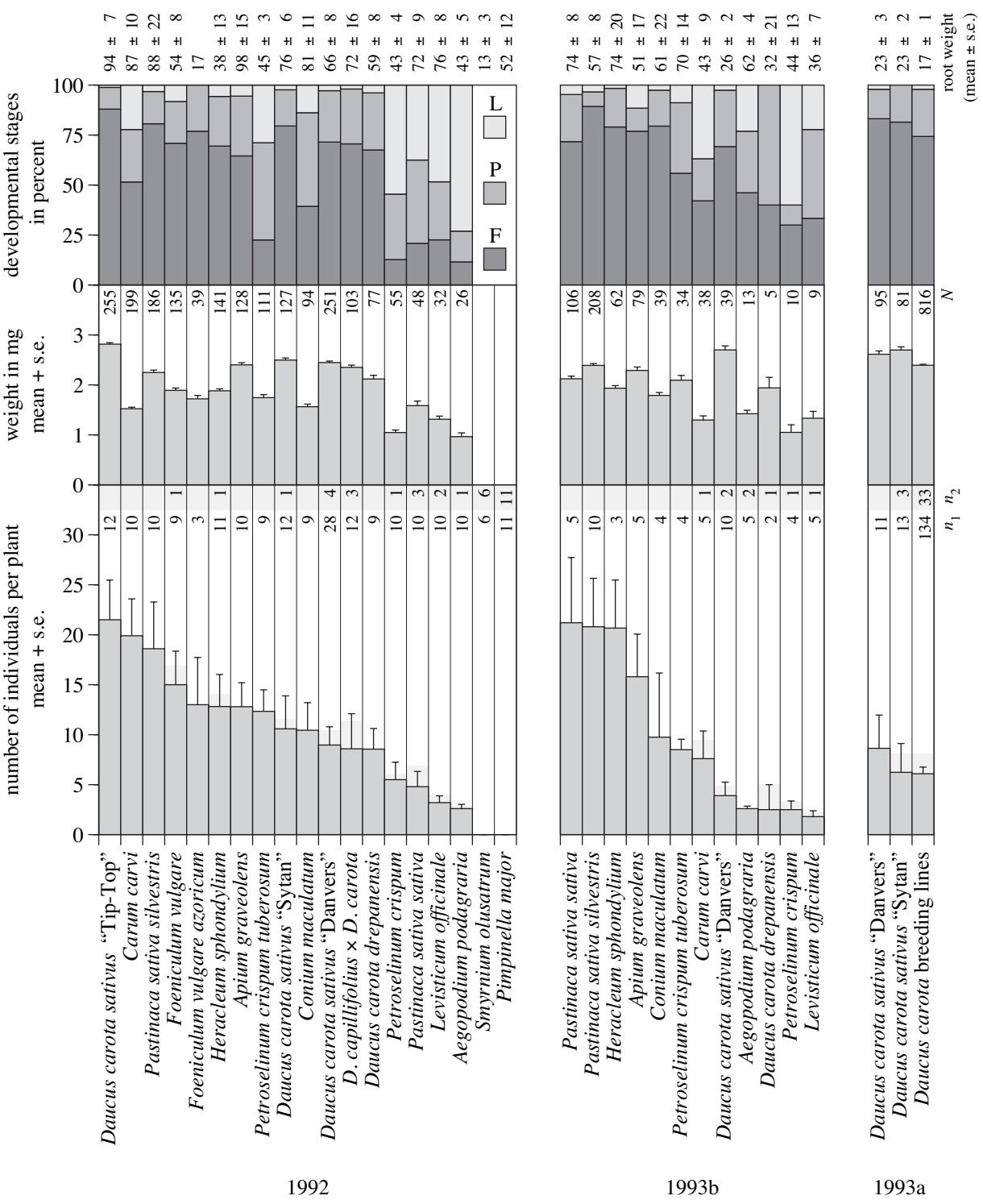

Figure 1. (A) Emergence rate, mean weight and mean number of carrot fly individuals produced per plant. Results of experiments performed with transplanted plants. Development stages reached: L = larvae, non-pupated; P = pupae, no emergence of adult flies; F = adult flies; N = number of carrot fly individuals; n_1 = number of potted plants; n_2 = number of potted plants yielding no pupae or larvae; Grey areas above the columns refer to the mean number of individuals only for plants that produced at least one individual.

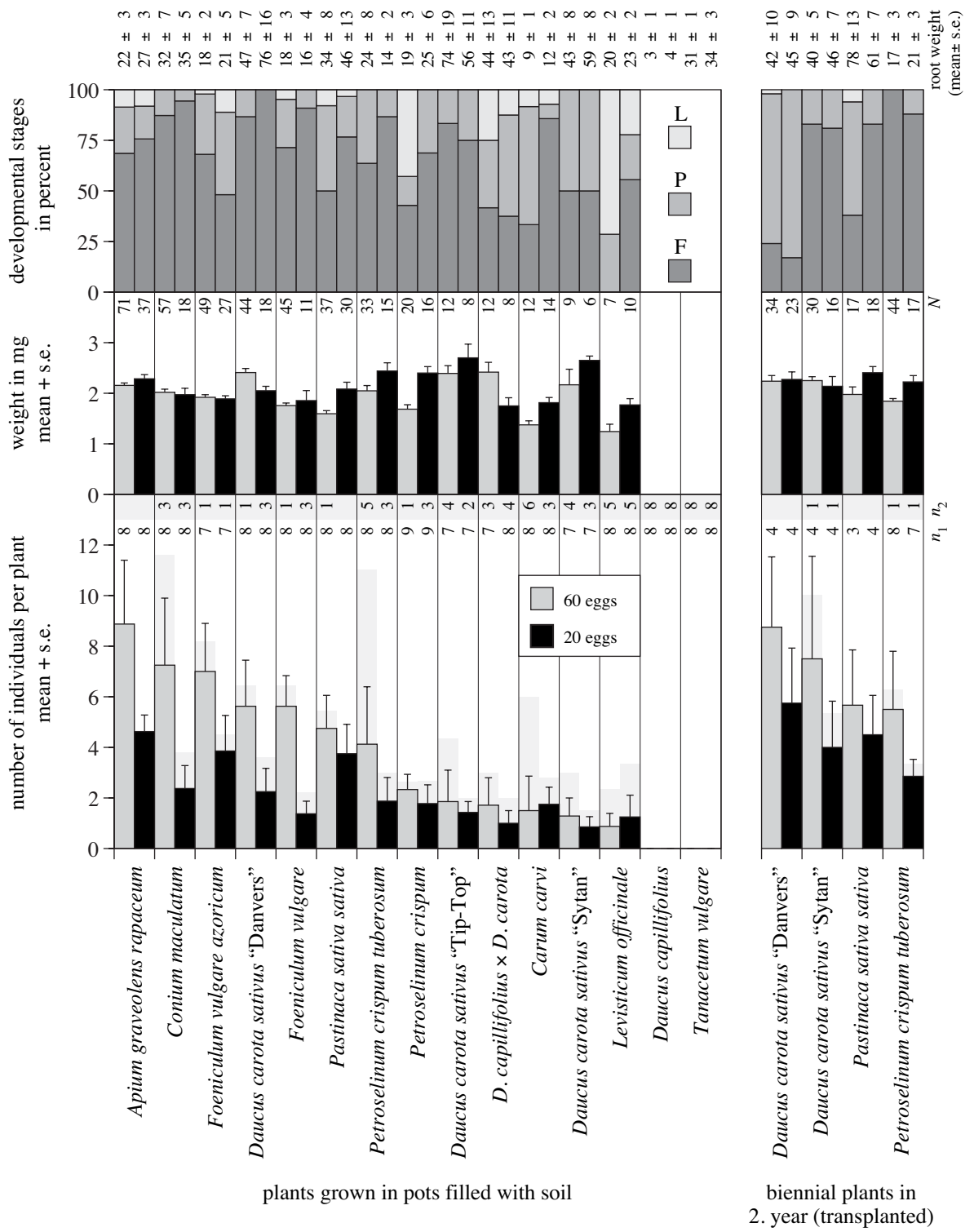

Figure 1. (continued; B). Emergence rate, mean weight and mean number of carrot fly individuals produced per plant. Results of experiments performed with plants grown in pots filled with a mixture of compost, peat and sand (gp-so-gh-1992) and of experiments with biennial plants in the second year grown from transplanted roots, which had overwintered in a cold room (tp-so-gh-1992-2y). The plants were inoculated with 20 or 60 eggs respectively.

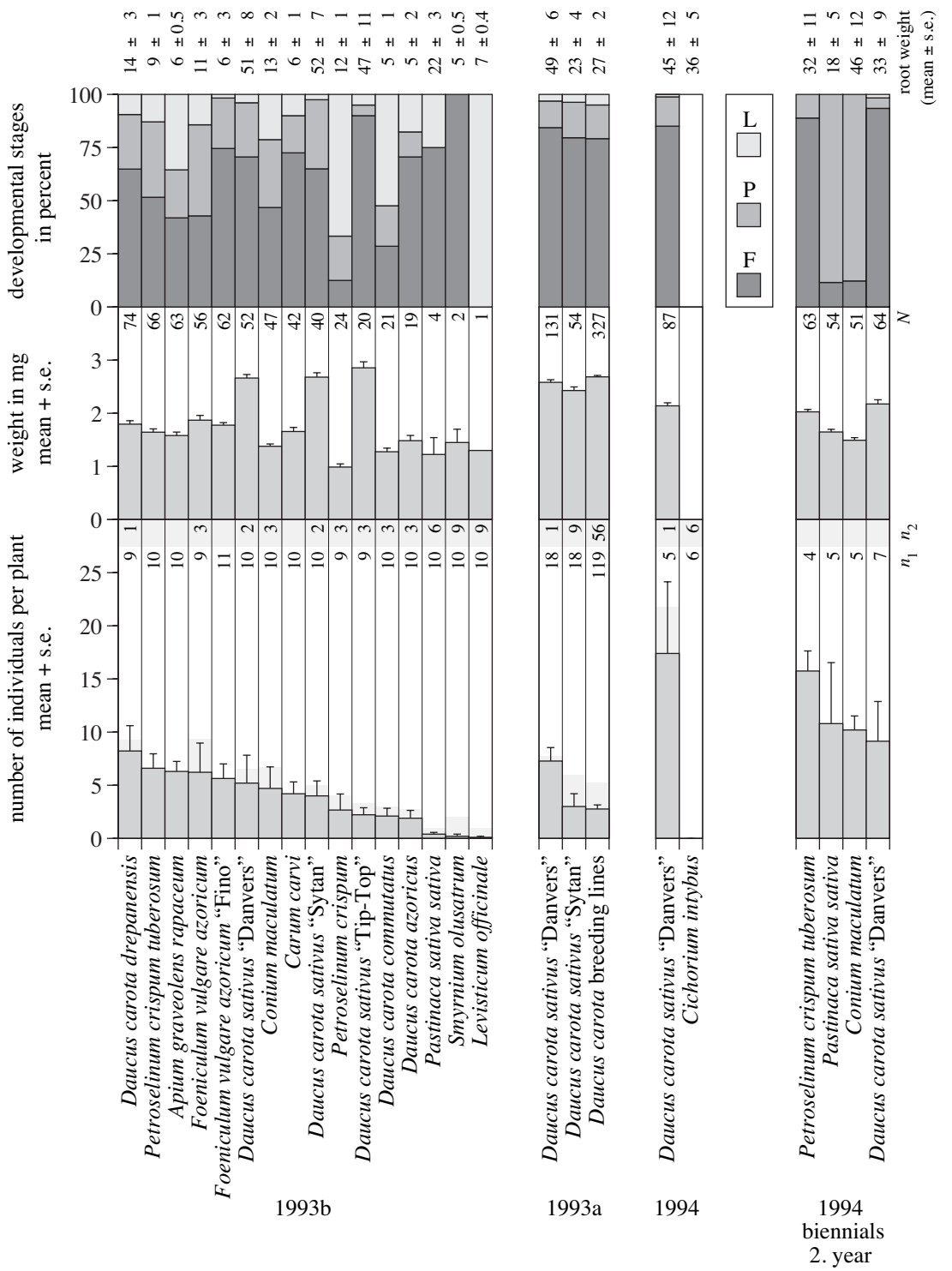

Figure 1. (continued; C). Emergence rate, mean weight and mean number of carrot fly individuals produced per plant. Results of experiments performed with plants grown in pots filled with sand and kept in the greenhouse after inoculation.

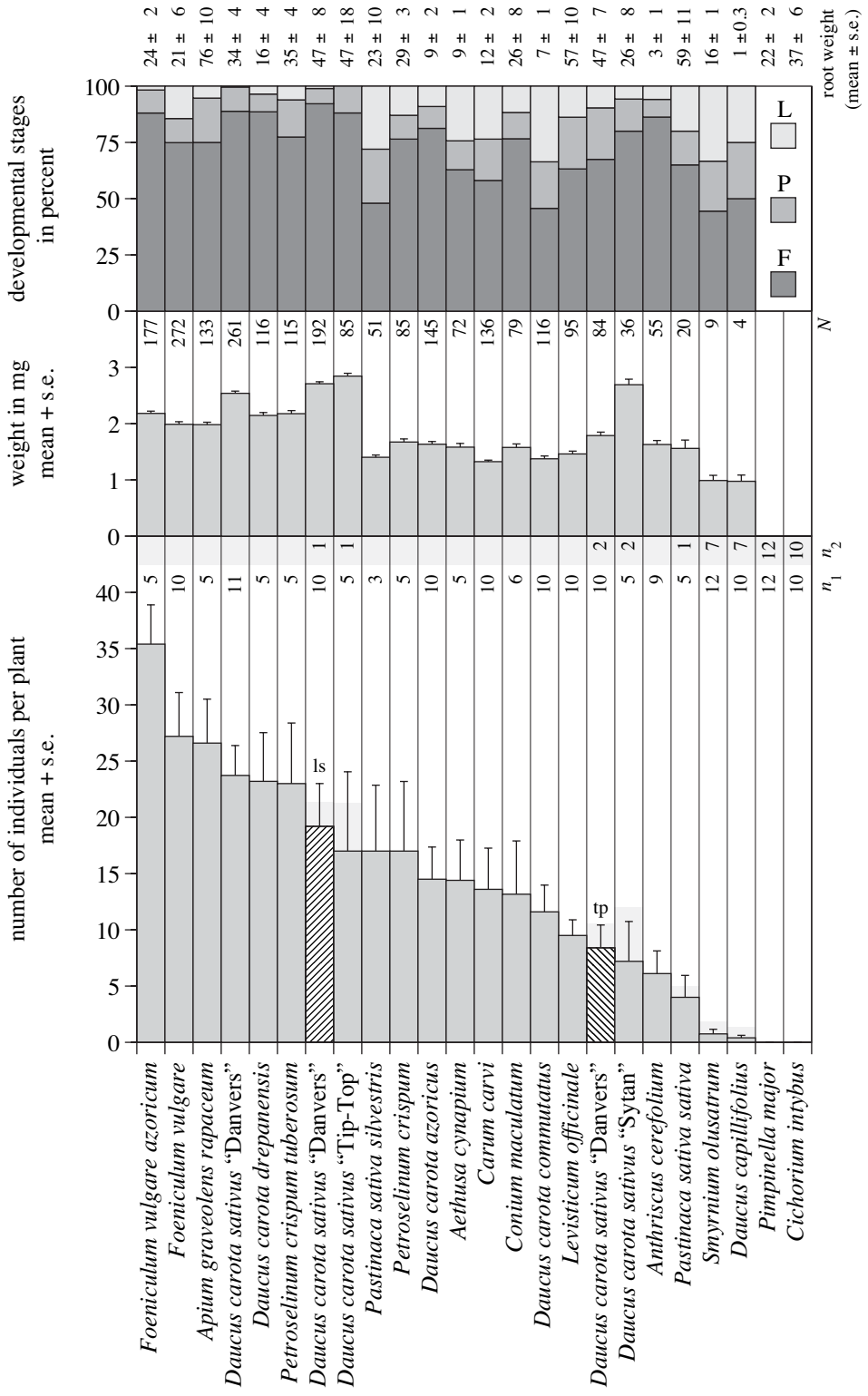

Figure 1. (continued; D). Emergence rate, mean weight and mean number of carrot fly individuals produced per plant. Results of experiments performed with plants grown in pots filled with sand and kept in a controlled environment room after inoculation (gp-sa-cc-1994).

Results

Number of individual carrot flies produced per plant. For a specific plant species, the mean rate of larval development from egg to pupa or non-pupated third instar larva at the time of harvest was frequently quite variable both within and among the experiments (Figure 1A–D). For the standard carrot cultivar "Danvers", which was included in all experiments, it ranged from 6.5% (transplanted plants 1993b; Figure 1A) to 39.5% (experiment performed in climate chamber; Figure 1D). The total carrot fly biomass (log-transformed) produced per standard plant differed significantly among the experiments conducted with plants grown in pots filled with sand (ANOVA: df = 3, $F = 6.4$; $P = 0.001$), but not among the experiments with transplanted plants (ANOVA: df = 3, $F = 0.4$; $P = 0.73$), whether differences in root weight were taken into account or not. When tested simultaneously in the 1994 experiment performed in the climate controlled room, significantly fewer carrot flies were collected from transplanted carrots than from carrots grown in pots filled with sand (ANOVA: df = 1, $F = 16.6$; $P = 0.001$). The substrate, sand or a mixture of loam and sand, did not influence total fly biomass produced on standard carrots grown in the pots (ANOVA: df = 1, $F = 1.1$; $P = 0.31$). Since otherwise the two methods were not applied concurrently, the results for transplanted plants and plants grown in pots are not directly comparable. The highest yields of carrot flies were obtained in the experiment performed in the climate controlled room – with a maximum of 54 individuals (90%) supported by a fennel plant *Foeniculum vulgare* – followed by the two experiments conducted with transplanted plants (1992 and 1993b). Only low numbers of pupae were attained in the experiments with plants grown in pots 1992 and 1993b, along with a high frequency of plants that did not support any carrot flies even for hosts suitable for larval development. Yet, when zero values were eliminated, the arrangement of species according to augmenting mean numbers of pupae per plant was not drastically altered with only few exceptions (*Petroselinum crispum tuberosum*, *Carum carvi*; Figure 1B). The proportion of pupae developing from eggs was generally higher, when the plants were inoculated with 20 eggs instead of with 60 eggs.

As the absolute yields of carrot fly individuals, the ranking of species was somewhat variable among the experiments (Figure 1A–D). While some of the species consistently supported comparatively high (e.g. *Foeniculum vulgare* var. *azoricum*, *Apium graveolens*) or low numbers of flies (e.g. *Levisticum officinale*, *Smyrnium olusatrum*) throughout the different experiments, others varied greatly in the relative quantity of flies produced depending on the method used. This was most obvious in the case of *Pastinaca sativa sativa*, which was normally a poor host, but achieved the highest rank on one occasion (transplanted plants 1993b; Figure 1A). Except for *Pimpinella major*, all umbelliferous plants tested were shown to be capable of supporting larval development to some degree, including some plant species that had to be discarded from analysis because of untimely rotting due to either carrot fly and/or pathogen attack (*Anethum graveolens*, *Daucus pusillus*, *Daucus muricatus*). As long as they were not senescing, the four biennial species tested in their second year allowed larval development. Yet, these findings cannot quantitatively be compared with the results obtained with the respective first-year plants, as the tests were carried out separately. No pupae were obtained from the two composites (Asteraceae), *Cichorium intybus* and *Tanacetum vulgare*.

Weights of pupae and non-pupated larvae. The mean weights attained by the carrot fly larvae were highly variable depending on the host-plant species, but remained more constant throughout the different experiments than did the mean numbers of flies produced per plant (Figure 1A–D). Variation in pupal weights was also detectable among the plants belonging to the same species within an experiment: pupal weights differed significantly among the plants in 80% of the cases with ≥ 5 replicates per species ($n = 77$). For the standard carrot cultivar "Danvers", no significant differences in mean pupal weights per plant were found among the experiments (ANOVA: df = 11, $F = 1.8$; $P = 0.06$) and between the two methods used (ANOVA: df = 1, $F = 1.5$; $P = 0.22$). Species giving rise to only low numbers of flies tended also to produce small-sized individuals. However, this relationship was not very strong and only significant in two out of the six experiments comprising more than ten test species: transplanted plants 1992 ($r = 0.54$; $n = 17$; Fisher's r to z P-value = 0.02; Figure 1A) and plants grown in pots 1994 ($r = 0.54$; $n = 22$; Fisher's r to z P-value = 0.01; Figure 1D). The highest weights were invariably recorded from

Table 2. Mean weight of pupae originating from potted plants (antibiosis experiments) and from plants grown in the field. Field/boxes refers to roots infested in the field and subsequently transferred to boxes filled with sand where larvae underwent metamorphosis. Only weights of pupae from which carrot flies emerged were included for the comparison to avoid any bias, as pupae parasitized by *Dacnusa gracilis* – approximately 20% of the field population – weighed, on average, less than unparasitized pupae. Means accompanied by different letters are significantly different at the 5%-level (Tukey-Kramer post-hoc test; ANOVA: $F = 144$; df = 13; $P < 0.0001$)

species	origin	pupation	washed out	pupal weights in mg			
				mean ± s.e.	min. – max.	*N*	
Daucus carota "Danvers"	pots			2.51 ± 0.02	0.9 – 4.0	1083	d
	field	autumn[1]	15.3.93	2.69 ± 0.06	1.9 – 4.2	76	de
	field/boxes	winter[3]	20.1.93	4.43 ± 0.13	2.0 – 5.9	40	h
Apium graveolens	pots			2.25 ± 0.02	1.0 – 3.5	342	c
	field	autumn[1]	21.12.92	2.79 ± 0.06	2.2 – 3.8	49	ef
	field/boxes	winter[3]	21.1.93	3.36 ± 0.08	1.9 – 4.9	58	fg
Pastinaca sativa sativa	pots			2.08 ± 0.04	0.9 – 3.6	182	b
	field	autumn/winter[2]	31.3.94	2.98 ± 0.07	1.2 – 4.2	63	ef
Petroselinum crispum	pots			1.85 ± 0.05	0.9 – 3.2	96	a
	field	autumn/winter[2]	30.3.94	3.02 ± 0.10	1.8 – 4.8	46	efg
Carum carvi	pots			1.64 ± 0.02	1.0 – 3.4	232	a
	field	autumn/winter[2]	10.3.94	2.76 ± 0.16	2.0 – 3.6	10	def
Levisticum officinale	pots			1.59 ± 0.05	0.9 – 2.6	67	a
	field	autumn/winter[2]	31.3.94	3.57 ± 0.23	2.7 – 5.2	10	g

[1] pupation in autumn, as roots were lifted from the fields on 1.12.92 (carrots) and 11.12.92 (celeriac)
[2] pupation in autumn or winter, as roots were left in the soil
[3] infested roots embedded in boxes filled with sand on 22.12.92

individuals grown on cultivated carrot, *Daucus carota sativus*, which normally produced only moderate numbers of flies. A mean weight below 2 mg was only once registered with cultivated carrots (transplanted plants in climate chamber experiment 1994), whilst mean weights above 2 mg were reached regularly or occasionally with only few other species or subspecies: *Daucus carota drepanensis* (2×), *Apium graveolens* (4×), *Conium maculatum* (1×), *Pastinaca sativa sylvestris* (2×) and *sativa* (3×), *Petroselinum crispum crispum* (1×) and *tuberosum* (6×), *Foeniculum vulgare azoricum* (1×).

Mean pupal weights of emerged flies in our experiments were lower than the corresponding mean weights of pupae originating from the field in all six host species examined (Table 2). However, it must be remembered that the latter were collected mostly in winter. This makes it difficult to compare the experimental and field data, because the average larval and pupal weight was shown to increase during the winter (Burn & Coaker, 1981), which results in a size difference between autumn and spring formed puparia (Wright et al., 1947). Variation in the weights of pupae of field origin attributable to the host species was not very pronounced, but again, the highest mean weight was achieved by flies that had developed on carrots.

Influence of root size. Analyses of covariance were carried out to assess the effect of the plant species on the carrot fly biomass produced by the plants when accounting for differences among the plants in root weight. While pupal weights followed a normal distribution (see values given in Figure 2), this was not the case for the numbers of individuals, even after a logarithmic transformation. For the ANCOVA we used log-transformed total carrot fly biomasses per plant, which were approximately normally

Table 3. Effect of plant species and plant size on the carrot fly biomass produced per plant. Results of analyses of covariance (ANCOVA) with log (fly biomass produced per plant in mg + 1) as dependent variable, plants species as independent variable and log (root weight in g + 1) as covariate

experiment	effect: plant species			effect: root weight			summary of fit		interaction
	df	*F*-ratio	*P*	df	*F*-ratio	*P*	r^2	(adjusted)	
tp-sa-gh-1992	18	7.8	< 0.0001	1	33.9	< 0.0001	0.55	0.50	(lost dfs)
tp-sa-gh-1993a	2	1.4	0.26	1	16.1	0.0001	0.11	0.09	n.s.
tp-sa-gh-1993b	11	3.5	0.001	1	16.5	0.0002	0.61	0.51	n.s.
tp-so-gh-1992-2y-20 eggs	3	0.2	0.89	1	0.2	0.63	0.09	–0.17	n.s.
tp-so-gh-1992-2y-60 eggs	3	0.2	0.92	1	0.7	0.41	0.11	–0.14	n.s.
gp-so-gh-1992-20 eggs	14	3.8	< 0.0001	1	4.0	0.05	0.38	0.29	s.
gp-so-gh-1992-60 eggs	14	5.2	< 0.0001	1	14.7	0.0002	0.48	0.40	n.s.
gp-sa-gh-1993a	2	4.4	0.01	1	22.1	< 0.0001	0.22	0.20	n.s.
gp-sa-gh-1993b	15	6.2	< 0.0001	1	7.0	0.009	0.42	0.36	s.
gp-sa-gh-2y	3	1.5	0.26	1	5.0	0.04	0.38	0.22	n.s.
gp-sa-gh-1994	1	57.0	0.0001	1	25.6	0.001	0.92	0.90	(s.)
gp-sa-cc-1994*	23	19.1	< 0.0001	1	12.1	0.0007	0.75	0.71	s.

* including the transplanted standard carrots (tp-sa-cc-1994) and the standard carrots grown in pots containing a mixture of loam particles and sand (gp-ls-cc-1994)

distributed, when plants that did not give rise to any carrot flies were ignored. Nevertheless, for the analysis these zero values were included, since they were not only due to inadequate environmental conditions in the greenhouse (e.g. low air humidity), but also due to biotic factors, i.e. unsuitability of the plants. In the majority of the experiments, both plant species and root weight affected the carrot fly biomass produced (Table 3). With the experiment performed in the climate controlled room, these two effects explained a major part of the variation in carrot fly biomass, which suggests that the influence of abiotic factors was less important than with the other experiments. When the zero values were excluded from the ANCOVA, the same result was obtained except for a few additional cases, where either plant species (gp-so-gh-1992-20 eggs; gp-sa-gh-1993a) or root weight (gp-so-gh-1992-60 eggs; gp-sa-gh-1993b) had no significant effect. Only in few experiments (tp-sa-gh-1993b; tp-so-gh-1992-2y-60 eggs; gp-so-gh-1992-20 eggs; gp-sa-cc-1994), was there a significant relationship between mean pupal weight per plant (dependent variable) and log-transformed root weight (covariate) in an ANCOVA with plant species as independent variable.

Pupation and emergence of adult flies. We pooled all available data for Figure 2 and Table 4, including individuals originating from plants that had to be eliminated from the previous analyses. Unsuitability of a host plant according to the low numbers and weights of carrot fly individuals was often associated with high percentages of non-pupated third instar larvae and low emergence rates (Figure 1A–D). About eight percent of the larvae did not succeed in pupating prior to being washed out of the pots. Some of these larvae were distinguished by rust-coloured stripes at the segmental margins, which were probably identical to the reddish pigmented areas consisting of cuticular denticles, which have been described by Ashby and Wright (1946) as an unusual trait. This peculiarity was regularly observed with larvae grown on caraway, but occasionally also with larvae of other origin. The non-pupated larvae on average weighed less than the pupae (Figure 2; Table 1). Only a small part of them managed to pupate later on, i.e. when stored in the ELISA-plates, and just one single fly emerged from these pupae. Flies derived from plant species with high percentages of non-pupated larvae tended to have longer developmental times, but this relationship was usually week and only significant in one experiment (gp-so-gh-1992-20 eggs). Thus a high percentage of non-pupated larvae may not only be due to delayed development, but also due to inability to pupate.

Considerably fewer flies emerged from non-floating pupae than from floating pupae. Most of these “heavy and dense” puparia shrunk later on and

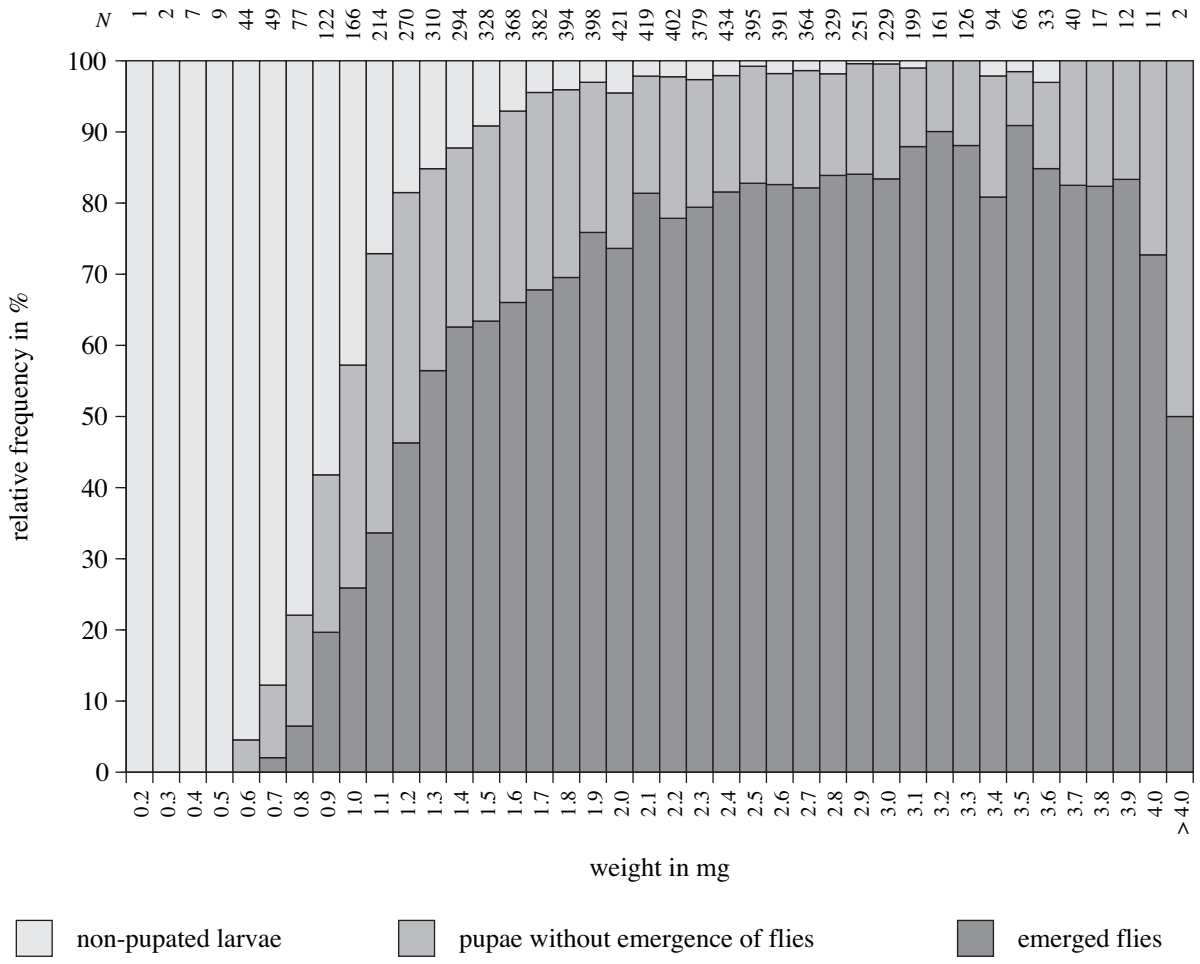

Figure 2. Relative frequency of development stages reached by carrot fly individuals with respect to their weight. *N* = number of individuals.

hence were probably injured. Pupal weight was highly correlated with hatching success. Individuals of small size were more prone to fail in emerging, to be stuck within the puparial wall upon hatching or to fail in fully unfolding their wings after eclosion (Table 4). Pupae that failed to produce flies were of two types. In the vast majority of cases, the flies inside the puparia were darkly pigmented, implying that they had completed development, but were not capable of breaking open the operculum. The very few cases of light, unpigmented pupae may have indicated individuals with a strongly delayed development ("overlayers", see below).

Total developmental time. Development from egg to emergence of adult flies lasted on average 56 ± 5.6 days (SD; *N* = 5954), ranged from 43 to 166 days and followed a normal distribution slightly skewed to the right. Ninety percent of the individuals hatched before the 61. day, 99% before the 73. day after oviposition. Ten individuals (0.2%) took longer than 100 days to accomplish development. Since the interval between inoculation and last control for emergence was somewhat variable (minimum: 88 days), such "overlaying" flies (total developmental time > 88 days) were excluded from the analysis to avoid any bias. In 6 out of 11 experiments, host species had a significant effect on developmental times (means per plant), but the range between the extremes (means per species) of an experiment rarely surpassed ten days. As with pupal weight, there was also some variation in the development time of individual flies that could be attributed to variation among the plants belonging to the same species

Table 4. Development stage reached, hatching success and weight of individual larvae and pupae, respectively. The categories differed significantly in mean weight (ANOVA: $F = 373.7$; df = 5; $P < 0.0001$). Means accompanied by different letters differ significantly at the 5%-level (Tukey-Kramer post-hoc test)

categories	washed out as (in %)				*N*	weight in mg	correlation between
	larva	non-floating pupa	floating pupa	total		mean ± s.e	weight and frequency (Spearman rank correlation)
	N = 739	*N* = 461	*N* = 7018	*N* = 8210			
larvae, not pupated	93.0			8.3	683	1.25 ± 0.02 a	ρ = –0.95 (*P* < 0.0001)
shrunk pupae	2.7	56.1	3.7	6.5 (7.1[1])	534	2.01 ± 0.03 d	ρ = –0.42 (*P* = 0.01)[1]
pupae without emergence of flies	4.2	30.2	11.5	11.9 (13.0[1])	979	1.96 ± 0.02 cd	ρ = –0.81 (*P* < 0.0001)[1]
flies stuck in puparium upon hatching		2.2	2.6	2.3 (2.5[1])	192	1.69 ± 0.04 b	ρ = –0.71 (*P* < 0.0001)[1]
flies failing to unfold fully (e.g. wings)		0.2	1.2	1.1 (1.2[1])	87	1.80 ± 0.08 bc	ρ = –0.53 (*P* = 0.001)[1]
fully unfolded flies	0.1	11.3	81.0	69.9 (76.2[1])	5735	2.27 ± 0.01 e	ρ = 0.69 (*P* < 0.0001)[1]

[1] frequency in % of pupae

(effect significant in 38 out of 60 cases with ≥ 5 replicates). Emergence tended to be delayed with smaller individuals (Figure 3). This negative relationship between pupal weight and total development time was not very strong, but significant in all except for three experiments (tp-so-gh-1992-2y 20 eggs and 60 eggs, gp-sa-gh-1994).

Carrot fly damage to the tap root. The tap root was inspected macroscopically for carrot fly damage. No root damage was detectable with the two asteraceous plants *Tanacetum vulgare* and *Cichorium intybus*. Both of them had formed many side roots, but a prominent tap root was only present in the latter. Umbelliferous plants supporting only few or no carrot flies showed little or no obvious damage: with *Pimpinella major* usually no signs of damage were discovered except for superficial "nibbles" in rare cases; with roots of *Smyrnium olusatrum* and *Levisticum officinale* only "shaft mines" occurred (single tunnel opening to the surface by a circular hole), but not other types of mines ("sinuous", "sub-epidermal" or "open" mines) as could be found with most of the other umbellifers (for a classification of carrot fly damage see Ellis et al., 1978). Furthermore, damage was relatively restricted in *Aethusa cynapium*, *Carum carvi* and *Petroselinum crispum* (but not var. *tuberosum*), i.e. it never extended to more than 20% of the tap root surface. However, damage to the tap root was not essential for the production of high numbers of pupae as exemplified by the fennel plant

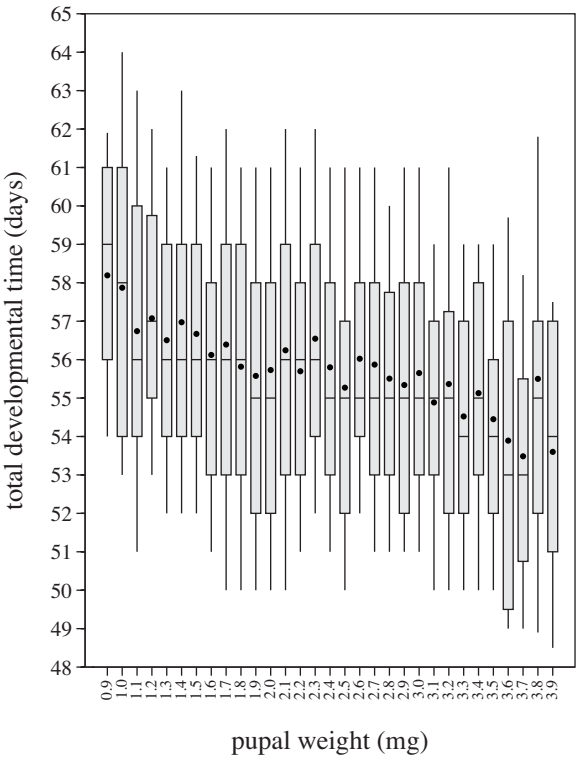

Figure 3. Total development time from egg to emergence of adult flies in relation to pupal weight. The box plots show 10-, 25-, 50-, 75- and 90-percentiles as well as means (black dots). Weight categories with less than 10 individuals and flies with development times longer than 88 days were excluded from analysis.

Table 5. Relationship among root damage (damaged surface area, number of internal mines) and carrot fly biomass produced with transplanted carrots and carrots grown in pots (experiment 1993a). Regression lines were significantly different between the methods (ANCOVA with biomass as dependent variable, method as independent variable and damage parameters as covariates). Carrots supporting no flies and having no sign of damage were excluded from the analysis

method	regression line	R^2 (adjusted)	*P*-value	*N*
transplanted	log(biomass in mg + 1) = 0.40 + 0.91 × log(damaged surface area in cm^2 + 1)	0.60	< 0.0001	94
grown in pots	log(biomass in mg + 1) = 0.10 + 1.00 × log(damaged surface area in cm^2 + 1)	0.71	< 0.0001	100
	ANCOVA effects: method: $F = 27.4$; $P < 0.0001$			
	log(damaged surface area in cm^2 + 1): $F = 355.0$; $P < 0.0001$			
transplanted	log(biomass in mg + 1) = 0.49 + 0.91 × log(mean number of internal mines/slice + 1)	0.53	< 0.0001	91
grown in pots	log(biomass in mg + 1) = 0.71 + 0.84 × log(mean number of internal mines/slice + 1)	0.27	< 0.0001	100
	ANCOVA effects: method: $F = 12.4$; $P = 0.0005$			
	log(mean number of internal mines/slice + 1): $F = 119.1$; $P < 0.0001$			

that gave rise to 54 pupae but showed almost no damage to its tap root (≤ 1% of the surface). This plant had developed a high number of strong side roots, on which the larvae could feed.

In the 1993a experiments with carrot cultivars, the relationship between carrot fly biomass and root damage was different depending on the method chosen: with transplanted carrots the larvae caused comparatively less damage on the surface, but produced considerably more internal mines running axially along the border between the cortex and the central cylinder, than with carrots grown in pots (Table 5). With carrots grown in pots, no difference in the carrot fly biomass per damaged surface area was found between the susceptible cultivar "Danvers" ($n = 31$) and the partially resistant cultivar "Sytan" ($n = 14$) according to an analysis of covariance with log-transformed biomass as dependent variable, cultivar as independent variable ($F = 1.5$; $P = 0.21$) and log-transformed damaged surface area as covariate ($F = 166.7$; $P < 0.0001$).

Discussion

The experiments described in this paper concentrated on the final result of larval development from egg hatch to pupation, i.e. the numbers and weights of pupae as well as adult emergence. Therefore we cannot provide precise information about the impact of the various host plants on survival and growth rates of different larval stages. The components of fitness which were measured, i.e. survival to pupal stage, individual weights, emergence rates and total development time, were all more or less strongly interrelated, suggesting that they were altogether affected by some common causative principles (i.e. nutrients, allelochemicals). Nevertheless, the ranking of plant species was somewhat variable depending on the fitness parameter considered, e.g. the largest pupae were generally collected from carrot, but often only in moderate numbers.

Survival of larvae to pupal stage. According to Overbeck (1978) and various other authors (see review of Dufault & Coaker, 1987), two phases of infestation can be distinguished on carrots: first-instar larvae feed on the fibrous side roots; second and third instars predominantly mine the tap root. While even with carrots feeding on the enlarged main root does not seem to be obligatory for accomplishing larval development (Jones, 1979), the availability of small side roots is believed to be crucial for the survival of first instar larvae (Overbeck, 1978). Since damage was only rarely detectable in roots of plants that did not give rise to pupae (e.g. in some *Daucus capillifolius* plants) and since only very few dead third instar larvae were collected upon washing out the pots, mortality was presumably highest in the first larval instar in these experiments. At this stage, competition among the larvae for side-root invasion may have been involved as a critical factor affecting survival. It is mainly here that size effects may have come into play, as side-root density is expected to be correlated with root mass (see also Overbeck, 1978). The rates of pupation could not be determined precisely because some larvae with delayed development might have pupated later on unless removed from the soil.

Pupal weight. In this study, pupal weight may be regarded as a better measure of host-plant suitability than survival of the larvae because it was less influenced by root weight. There were dramatic differences in the size of the pupae produced among the host species. However, it is questionable whether such strong variation exists in the field. The mean weights of pupae collected from different plant species in the wild were less variable. Yet, these data are not conclusive and only comparable to a certain extent, because larvae grown under the conditions prevailing in winter attain considerably higher weights (Burn & Coaker, 1981). Van't Sant (1961) also reported appreciable variation in pupal length among carrot fly populations from different regions in the Netherlands. The very low weights of some pupae as well as the morphological peculiarities of some larvae in our study may be attributable to unfavourable nutritional conditions. Some of the test plants possibly were not adequately provided with nutrients, depending on their specific requirements. Therefore food quality of the respective plant species may be notably higher under field conditions. The impact of plant nutrition (e.g. nitrogen supply) on the growth rate of carrot fly larvae requires further research.

Survival from pupal to imaginal stage. There was a strong positive relationship between emergence rates of adult flies and pupal weights. Flies successfully hatched from a high percentage of the large pupae raised on carrot roots, in accordance with the values reported in earlier studies: over 90% (Bohlen, 1967), 75–80% (Naton, 1971) and 69–92% (Städler, 1971a). It is conceivable that small pupae are more susceptible to water loss because of their higher surface to volume ratio. However, pupae were stored at approximately 100% relative air humidity and therefore we assume that additional fitness-related factors must have been operating. Inability to hatch was probably a much more frequent cause of mortality than failure to undergo metamorphosis, as in most pupae that did not produce adult flies, dark-pigmented imagines were shimmering through the puparial wall. The strong variability in pupal weight should also have a profound impact on fecundity of female carrot flies. For instance, Zohren (1968) found a positive correlation between body length and the number of ovarioles and eggs contained in the abdomen of cabbage root flies, *Delia radicum*.

Total developmental time. Full development from egg to adult emergence lasted on average 56 days at about 21 °C in this study including several host species, as compared to 59.3 days (at 20 °C), 57.8 days (at 22 °C) and 60 days (at 21.5 °C) respectively in three other investigations using carrots as food plants (Stevenson, 1981; McLeod et al., 1985; Collier & Finch, 1996). Apart from abiotic factors (temperature, humidity) food quality also contributes to variation in the duration of larval development. As in this study, a negative relationship between larval development time and pupal weight was found in the multivoltine butterfly *Pieris rapae*; by contrast, heavier pupae had a longer larval development time in the closely related bivoltine *P. napi oleracea* (van der Reijden & Chew, 1992). These two species are presumed to maximise different components of fitness, i.e. fast maturation is more critical for *P. rapae*. The latter may also hold true for the carrot fly. In north temperate regions, there are usually only two generations per year (Dufault & Coaker, 1987). Yet, faster growing individuals may achieve an additional third generation under favourable climatic conditions.

Ranking of species. The majority of the apiaceous plants examined in this study may be considered suitable hosts, as all species gave rise to at least a few flies, except for *Pimpinella major*. The ranking of species according to the numbers of flies produced in our study was similar to the ranking found in earlier experiments: *Apium graveolens* and *Foeniculum vulgare* var. *azoricum* proved highly suitable; *Levisticum officinale* and *Petroselinum crispum* supported only low to moderate numbers of flies, whilst only one single pupa could be collected from several inoculated *Pimpinella major* plants (E. Städler, unpublished). Wiesmann (1942) succeeded in infesting plants of *Anethum graveolens*, *Anthriscus (cerefolium?), Heracleum sphondylium* and *Pimpinella (major?)* in glasshouse experiments, but provided no quantitative data. The partially resistant carrot cultivar "Sytan" produced smaller numbers of flies than the susceptible standard cultivar "Danvers" in all but one experiments, whilst no analogous varietal differences were observed with respect to pupal weights. This implies that root resistance in carrots might operate mainly via differential survival and invasion of first instar larvae, but less so via variable growth and survival after prolonged feeding. Cultivar differences may have been influenced by root weight in

some cases, but are unlikely to be explained by this factor alone. The mean number of flies harvested from roots of the three wild *Daucus carota* subspecies (*azoricus*, *commutatus, drepanensis*) lay roughly within the range observed for cultivated carrots, for pupal weights though this was not the case. It is quite doubtful that these plants represent promising genotypes for breeding carrot cultivars less susceptible to carrot fly attack (see also Hardman & Ellis, 1990). The very low yields in pupae noted for *Daucus capillifolius* plants, which are highly resistant in the field (Ellis et al., 1993), seems to be largely attributable to the exceptionally small roots. Though, our assays did not allow to judge the potential of resistance factors other than root size in this species.

The accordance among our data on suitability and the field data on susceptibility (Hardman & Ellis, 1982; Hardman et al., 1990) is not strong. The most notable agreement is the consistently low ranking of both *Pimpinella major* and *Smyrnium olusatrum*, which we could unequivocally identify as major sources of (antibiotic) resistance within the host-plant family. Otherwise, the hierarchy among the host-plant species with respect to suitability – somewhat variable among the experiments – must be considered as only preliminary, since important fitness components, above all the number of flies produced, have not been adjusted for root size effects in this study.

Van't Sant (1961) reported that larvae completed their development on *Cichorium intybus* (Asteraceae) after adult flies had been caged on plants. Also in preceding experiments (E. Städler, unpublished), small numbers of larvae had grown to pupation on *Ruta graveolens* (Rutaceae) and *Tanacetum vulgare*, but not on *Achillea macrophylla*, another asteraceous plant. We were not able to confirm these results, as both non-umbelliferous plants included in our study, *Cichorium intybus* and *Tanacetum vulgare*, yielded no pupae. For a more complete view, further investigations are needed to identify potentially suitable plants outside the Apiaceae and to see if plants families with similar allelochemistry (e.g. Rutaceae, Araliaceae, Asteraceae) are more liable to support larval development than chemically more distinct families.

Methodical aspects. Both carrot fly eggs and larvae – especially the first instar – are very susceptible to high temperatures and desiccation (e.g. Overbeck, 1978). Although the climate in the greenhouse generally did not reach the lethal threshold, transient deviations from the optimal conditions for embryonic and larval development may have been responsible for the pronounced intraspecific variability in numbers of pupae and complete failures in otherwise very suitable hosts. In the controlled environment room, comparatively high yields of pupae were achieved and biotic factors (plant species, root size) accounted for a higher percentage of the variation in fly biomass produced than in the greenhouse assays. This emphasizes the necessity to control temperature and humidity accurately in this kind of experiments.

Another undesirable outcome was the almost ubiquitous influence of root size on carrot fly biomass produced. Even though in the field, foliage vigour and root weight likewise play a role in differential susceptibility of carrot cultivars (Ellis et al., 1978), it is preferable to have effects of plant size eliminated, as they obscure the differences in antibiotic resistance among the plants. In this study, size effects were probably accentuated by the relatively large numbers of eggs placed around the plants, which exceeded the egg densities commonly found around host plants in nature (Guerin & Ryan, 1984). Still, this may have had only limited consequences for the assessment of comparative suitability of the species. Inoculating carrots with augmenting numbers of eggs definitely reduces the relative yield in pupae, but the absolute yield appears to rise asymptotically up to a certain level (Overbeck, 1978). Also when increasing numbers of eggs were added to carrots in our laboratory culture, we noted no decline in the total yield of pupae, at least up to a quantity of 8 eggs per gram root (T. Degen, unpublished). Surplus larvae presumably died at an early stage so that carrot fly attack was rarely severe enough to destroy the plants. Yet, "dose-response" relationships could differ among the plant species. In any case, the data for the very small plants (e.g. *Daucus capillifolius*; several species in the experiment 1993b, Figure 1C) are somewhat dubious and must be cautiously interpreted. With carrots as a food plant, the mean proportion of eggs reaching the pupal stage was 40% maximum in our study, as compared to 40–60% maximum with different mass-rearing methods (Naton, 1971; Städler, 1971a; McLeod et al., 1985) and to maximally 10% in field trials (Overbeck, 1978; Maki & Ryan, 1989). Here we must also

consider that on average about 20% of the eggs are sterile as estimated by Overbeck (1978).

There were no clear-cut differences in the yield and weight of the pupae between transplanted plants and plants grown in pots. Nevertheless the former method may be less convenient as judged by the atypical nature of the damage in carrots (high proportion of mines running along the central cylinder) and by the weight loss of the transplanted roots. Also stress due to lesions inevitably formed upon transplantation might elicit changes in secondary metabolism, which in turn could affect suitability for larval development. Several plant species, notably the annuals (e.g. *Anethum graveolens*, *Anthriscus cerefolium*), but also some biennials or perennials (e.g. *Daucus capillifolius*, finocchio *Foeniculum vulgare* var. *azoricum*) did not survive transplanting. The comparatively high susceptibility of the transplanted plants to rotting was one of the major drawbacks of this method. The method using plants grown in the pots could be improved by employing a substrate optimized both for collection of pupae and plant growth. Pure sand supplied with fertilizer seems to be appropriate for raising carrots, as the admixture of loam particles did not lead to higher yields of pupae. However, this may not be valid as well for other host plants.

Plant factors influencing suitability. Few studies have investigated the mechanisms underlying root-mediated resistance to carrot fly attack. Under natural conditions, larvae are inclined to move around in the soil and thereby can infest more than one plant (Overbeck, 1978; Jones & Coaker, 1980). Although we did not allow the larvae to choose different plants, we cannot rule out the possibility that antixenosis, i.e. plant characteristics resulting in negative behavioural reactions (non-preference) or avoidance by the larvae, was involved in our experiments. In practice it may often be difficult to distinguish between deterrent effects prior to feeding and post-ingestive effects due to toxic compounds or unfavourable nutrient balance (see Bernays & Chapman, 1987). Notwithstanding, it seems justified to assume that antibiosis, i.e. the physiological impact of the food on growth and survival of the larvae, was most effective as a resistance factor in this study. No difference in tolerance, i.e. in the extent of damage caused per carrot fly biomass produced, was detected between the carrot cultivars "Danvers" and "Sytan".

Carrot fly larvae find host roots by migrating towards sources of carbon dioxide (Städler, 1971b) and specific volatiles (Jones & Coaker, 1977): mono- and sesquiterpenes (e.g. bornyl acetate, α– and β–ionone), methyleugenol and polyacetylenes (falcarinol, falcarindiol) have been identified as attractants from carrot roots (Jones & Coaker, 1977; Ryan & Guerin, 1982; Maki et al., 1989), while another volatile constituent *trans*-2-nonenal, acts as repellent and has also insecticidal properties (Guerin & Ryan, 1980). In contrast, no chemical signals (feeding stimulants; antifeedants) are yet known that are perceived upon direct contact with the roots. Post-ingestive suitability, i.e. the adequacy of the selected food to sustain growth, survival and reproduction, is dependent on availability of nutrients – particularly nitrogen content – and on quality and quantity of allelochemicals (Scriber, 1984). Virtually nothing is known about this aspect of biology in the interaction of the carrot fly with its host plants. Cole (1985) found a positive correlation between susceptibility of various carrot cultivars and the respective contents in chlorogenic acid. Any causative relationship among the above mentioned semiochemicals and host-plant resistance to the carrot fly remains to be unequivocally determined, though. This report provides an experimental basis for the identification of chemical factors determining host-plant suitability and for the screening of successions in breeding programs targeted at antibiosis resistance.

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Appendix. List of the plant species tested in the inoculation assays and origin of the plant material. All species belong to the Apiaceae (Umbelliferae) except for *Cichorium* and *Tanacetum* (Asteraceae). Origin of seed: w = Genetic Resources Unit of Horticulture Research International Wellesbourne, Warwick, UK; s = Samen Mauser; n = Nunhems Zaden (The Netherlands)

plants species	origin of seed
Aegopodium podagraria L., ground elder	
Aethusa cynapium L., fool's parsley	w
Anethum graveolens L., dill	s
Anthriscus cerefolium (L.) Hoffm., garden chervil	s
Apium graveolens var. *rapaceum* (A. W. Hill) cv. 'Balder', celeriac	w
Carum carvi L., caraway	w,s
Conium maculatum L., hemlock	w
Daucus capillifolius Gilli	w
Daucus capillifolius × *Daucus carota* ssp. *sativus*	w
Daucus carota L. ssp. *azoricus* Franco	w
Daucus carota L. ssp. *commutatus* (Paol.) Thell.	w
Daucus carota L. ssp. *drepanensis* (Arc.) Heywood	w
Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. 'Danvers ', standard carrot cultivar	w
Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. 'Sytan', carrot	w
Daucus carota L. ssp. *sativus* (Hoffm.) Arc. cv. 'Tip-Top', carrot	s
Daucus carota L. ssp. *sativus*, carrot breeding lines	n
Daucus muricatus (L.) L.	w
Daucus pusillus Michaux	w
Foeniculum vulgare Miller, fennel	w
Foeniculum vulgare var. *azoricum* (Miller) Thell., finocchio (HRI Wellesbourne)	w
Foeniculum vulgare var. *azoricum* (Miller) Thell. cv. 'Fino', finocchio	s
Foeniculum vulgare var. *azoricum* (Miller) Thell. cv. 'Tardo', finocchio	
Heracleum sphondylium L., hogweed	
Levisticum officinale Koch, lovage	w,s
Pastinaca sativa ssp. *sativa* L. cv. 'Halblange', parsnip	s
Pastinaca sativa ssp. *sylvestris* (Miller) Rouy & Camus, wild parsnip	w
Petroselinum crispum (Miller) A. W. Hill, parsley	w
Petroselinum crispum var. *tuberosum* Crov. cv. 'Berliner', Hamburg parsley	s
Pimpinella major (L.) Huds., greater burnet saxifrage	w
Smyrnium olusatrum L., alexanders	w
Cichorium intybus L., chicory (Catalogna)	w
Tanacetum vulgare L., tansy	s

Host-plant susceptibility to the carrot fly, *Psila rosae*. 3. The role of oviposition preferences and larval performance

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Summary

The acceptability of various plant species to ovipositing carrot flies was only weakly, but significantly correlated with the host's suitability for larval development. Both adult host-plant preferences and larval performance as determined in laboratory experiments explained a part of the variation in susceptibility among the various test plants observed in the field. Across the whole set of plant species examined, antixenosis contributed more substantially to resistance than antibiosis, while the reverse seemed to be true for carrot cultivars.

Introduction

As with many other phytophagous insects, host-plant choice by adult carrot flies is critical for the survival of the offspring. After females have evaluated the acceptability of a host plant during an exploratory run over the foliage, they deposit eggs in crevices in the earth surrounding the plant. Newly hatched larvae penetrate the soil and search for roots of plants suitable for consumption. Since their capacity to move within the soil is limited (Overbeck, 1978), they have little opportunity to choose among different plants. Investigations of oviposition preferences and larval performance have focused so far on carrot, *Daucus carota*, the host on which damage is economically most important. Both antibiosis (Guerin et al., 1981; Maki & Ryan, 1989) and antixenosis, i.e. non-preference by larvae (Maki & Ryan, 1989) and by adults (Guerin & Ryan, 1984; Guerin & Städler, 1984), were shown to contribute to partial resistance of carrot cultivars. The host range of the carrot fly comprises a wide variety of further genera and species belonging to the family Apiaceae, the umbellifers (Ellis et al., 1992). A preference hierarchy of ovipositing carrot flies for various host plants has been established recently in laboratory choice assays (Degen et al., in prep.-a). Likewise, several aspects of host-plant suitability (larval survival until pupation, pupal weight and adult emergence rate) have been assessed with the same set of plant species (Degen et al., in prep.-b). In this paper we compare the two components of resistance, host-plant acceptability to adult flies (i.e. antixenotic resistance) and suitability for larval development (i.e. antibiotic resistance), and attempt to estimate their relative impact on susceptibility to carrot fly attack as observed in the field. The data presented here are based on raw data already published or to be published separately (Hardman & Ellis, 1982; Hardman et al., 1990; Degen et al., in prep.-a; Degen et al., in prep.-b), but are adapted in such a way as to permit a comparison of the parameters in question.

Materials and Methods

Insects. The flies for the oviposition assays as well as the eggs used for the inoculations were obtained from laboratory cultures originating from pupae collected in Wädenswil, Switzerland, and reared on carrots (Städler, 1971) for maximally 17 generations. Susceptibility to carrot fly attack in the field was assessed at HRI Wellesbourne, UK, where a high, relatively uniform resident population of carrot fly had been built up and maintained over a period of 45 years.

Table 1. Relative acceptability of various plant species to ovipositing carrot flies. abbr. = abbreviation of species name; *n*(dual) = number of dual choice assays; *n*(multiple) = number of dual choice assays

family	plant species	abbr.	acceptability index mean ± s.e.	*n* (dual)	*n* (multiple)
Apiaceae	*Anthriscus cerefolium* (L.) Hoffm., garden chervil[2]	ac	182.8 ± 27.9	3	1
Apiaceae	*Carum carvi* L., caraway[1,2]	cc	140.4 ± 1.3	2	1
Apiaceae	*Daucus capillifolius* × *Daucus carota* ssp. *sativus*[1]	dd	127.8	1	
Apiaceae	*Petroselinum crispum* var. *tuberosum* Crov. cv. "Berliner", Hamburg parsley [2]	pt	119.7 ± 45.2	2	
Apiaceae	*Conium maculatum* L., hemlock[1]	cm	116.3 ± 17.1	2	1
Apiaceae	*Aethusa cynapium* L., fool's parsley[1]	ae	111.2 ± 21.7	1	1
Apiaceae	*Daucus carota* L. ssp. *sativus* (Hoffm.) Arc. cv. "Danvers", standard carrot cv.[1]	da	100.0		
Apiaceae	*Daucus pusillus* Michaux[1]	pu	98.9 ± 15.1	1	1
Apiaceae	*Daucus carota* L. ssp. *sativus* (Hoffm.) Arc. cv. "Sytan", carrot[1]	sy	96.2 ± 7.2	2	1
Apiaceae	*Petroselinum crispum* (Miller) A. W. Hill, parsley[1]	pc	92.8 ± 7.2	2	1
Apiaceae	*Daucus muricatus* (L.) L.[1]	mu	90.7 ± 3.5	3	
Apiaceae	*Daucus carota* L. ssp. *sativus* (Hoffm.) Arc. cv. "Tip-Top", carrot[2]	tt	89.6 ± 24.4	2	
Apiaceae	*Smyrnium olusatrum* L., alexanders[1]	so	85.4 ± 8.4	2	1
Apiaceae	*Daucus carota* L. ssp. *commutatus* (Paol.) Thell.[1]	co	83.7 ± 6.1	2	1
Apiaceae	*Pastinaca sativa* ssp. *sylvestris* (Miller) Rouy & Camus, wild parsnip[1]	si	82.9 ± 4.8	2	
Apiaceae	*Levisticum officinale* Koch, lovage[1,2]	lo	79.6 ± 5.0	2	1
Apiaceae	*Daucus littoralis* Sibth. & Sm.[1]	li	78.5	1	
Apiaceae	*Foeniculum vulgare* Miller, fennel[1]	fv	77.0 ± 15.3	1	1
Apiaceae	*Daucus carota* L. ssp. *azoricus* Franco[1]	az	74.0 ± 1.8	2	
Apiaceae	*Daucus carota* L. ssp. *drepanensis* (Arc.) Heywood[1]	dr	73.6 ± 1.8	2	
Apiaceae	*Foeniculum vulgare* var. *azoricum* (Miller) Thell., finocchio[4]	fa	63.7 ± 14.0	5	1
Apiaceae	*Apium graveolens* var. *rapaceum* (A. W. Hill) cv. "Balder", celeriac[1]	ar	50.8 ± 6.5	2	1
Apiaceae	*Heracleum sphondylium* L., hogweed[3]	hs	49.2 ± 5.7	2	1
Apiaceae	*Pastinaca sativa* ssp. *sativa* L. cv. "Halblange", parsnip[2]	sa	40.6 ± 9.3	2	1
Apiaceae	*Daucus broteri* Ten.[1]	br	34.1	1	
Apiaceae	*Aegopodium podagraria* L., ground elder[3]	ap	33.2 ± 10.3	2	1
Apiaceae	*Anethum graveolens* L., dill[2]	ag	27.5 ± 7.6	2	1
Ranunculaceae	*Ranunculus repens* L., creeping buttercup[3]	rr	21.5 ± 4.4	2	
Apiaceae	*Daucus capillifolius* Gilli[1]	ca	21.4 ± 3.2	2	
Aspleniaceae	*Athyrium filix-femina* (L.) Roth, lady fern[3]	af	21.2 ± 10.9	2	1
Apiaceae	*Pimpinella major* (L.) Huds., greater burnet saxifrage[1]	pm	20.5 ± 3.7	2	1
Rosaceae	*Potentilla anserina* L., silverweed[3]	pa	20.1 ± 4.0	1	1
Asteraceae	*Tanacetum vulgare* L., tansy[2]	tv	16.8	1	
Asteraceae	*Cichorium intybus* L., chicory (Catalogna)[1]	ci	7.3		1
Brassicaceae	*Brassica oleracea* L. convar. *botrytis*, cauliflower	bo	6.8	1	

[1] seeds obtained from Genetic Resources Unit of Horticulture Research International Wellesbourne
[2] seeds obtained from Swiss seed producer (Samen Mauser)
[3] wild plants collected at Wädenswil, Switzerland
[4] three different cultivars: "Fino", "Tardo" and cultivar used in field studies Wellesbourne

Plants. Seeds for the sowings at Wädenswil were mainly provided by the Genetic Resources Unit of Horticulture Research International Wellesbourne, Warwick, UK. However, in some cases, the seed sources were not the same for the field experiments as they were for the oviposition and inoculation experiments (Table 1). In all experiments, the susceptible carrot cultivar "Danvers" was included as a standard plant with which the values for the test species were compared.

Table 2. Some important parameters of the inoculation experiments. The significance of the effect of plant species and of root weight on the carrot fly biomass produced per plant was assessed in an ANCOVA with log (fly biomass produced per plant in mg + 1) as dependent variable, plants species as independent variable and log (root weight in g + 1) as covariate. LSM = Least square means (mean carrot fly biomass adjusted for root size effects) calculated from regression lines with either a common slope or separate slopes.
method: tp = plants transplanted from seed beds into pots; gp = plants directly grown in pots
substrate: so = soil, mixture of compost, peat and sand; sa = sand; ls = mixture of loam particles and sand; f = soluble fertilizer Hauert Flory 9 or Flory 2
location: gh = greenhouse; cc = climate controlled room

experiment	method	substrate	location	eggs/plant	date (month)	*P*(species)	*P*(root weight)	r^2 (adjusted)	LSM
I	tp	sa	gh	60	1992 (Dec-Feb93)	< 0.0001	< 0.0001	0.55 (0.50)	common slope
II	tp	sa (+ f)	gh	60	1993a (Jul-Sep)	0.26	0.0001	0.11 (0.09)	common slope
III	tp	sa (+ f)	gh	60	1993b (Nov-Jan94)	0.001	0.0002	0.61 (0.51)	common slope
IV	gp	so	gh	20	1992 (Jul-Nov)	< 0.0001	0.05	0.38 (0.29)	common slope
V	gp	so	gh	60	1992 (Jul-Nov)	< 0.0001	0.0002	0.48 (0.40)	common slope
VI	gp	sa (+ f)	gh	60	1993a (Aug-Nov)	0.01	< 0.0001	0.22 (0.20)	common slope
VII	gp	sa (+ f)	gh	60	1993b (Oct-Dec)	< 0.0001	0.009	0.42 (0.36)	common slope
VIII	gp	sa (+ f)	gh	60	1994 (Aug-Oct)	0.0001	0.001	0.92 (0.90)	separate slopes
IX	gp	sa (+ f)	cc	60	1994 (Oct-Dec)	< 0.0001	0.0007	0.75 (0.71)	separate slopes

Oviposition choice assays. The experimental set-up has been described in detail by Degen & Städler (1997) and Degen et al. (in prep.-a). Invariably, eight oviposition dishes (Städler, 1971) equipped with cut leaves of identical length were presented to cage populations typically consisting of 100–400 male and female carrot flies. Ovipositional responses to foliage of test and standard plants were recorded in dual choice assays and/or multiple choice assays, in which seven test species together with the standard were simultaneously exposed to the flies. Both experimental approaches – dual and multiple choice – were shown to yield very similar results (Degen et al., in prep.-a). The egg counts were expressed as the percentage of total oviposition per experimental period (mostly one day). Each multiple choice experiment comprised 8 replicates per plant species (corresponding to eight experimental periods), each dual choice assay 8–32 replicates (2–8 periods). The relative acceptability of a test plant was calculated for each independent experiment (i.e. carried out in different months or years; 1–6 per species, see Table 1) as follows:

$$\text{acceptability index} = \frac{\text{mean \% eggs laid around leaves of the test species}}{\text{mean \% eggs laid around leaves of the standard species}} \times 100$$

Inoculation experiments. Important parameters of the experiments are listed in Table 2. A more detailed account of the methodology is given in Degen et al. (in prep.-b). Several plant species did not survive transplanting (e.g. *Anethum graveolens*, *Anthriscus cerefolium*, *Daucus capillifolius*) or the stress due to high air humidity, pathogen infestation or – possibly – carrot fly attack (e.g. *Anethum graveolens*, *Daucus pusillus*, *Daucus muricatus*). Totally or partly rotten plants were discarded from analysis, when it was obvious that decomposition was not caused by carrot fly larvae. Therefore we could not provide data on host-plant suitability for certain species, which were tested in oviposition assays. Also, only two non-umbelliferous species (*Cichorium intybus*, *Tanacetum vulgare*) were included.

Six to seven weeks after inoculation, pupae and non-pupated third instar larvae were collected and weighed. Root size was variable both within and among the species tested, which invariably affected the total carrot fly biomass produced (Table 2): survival rates were, on average, higher with larger plants. For comparative purposes, it is desirable to adjust values for these size effects. Thus, for the calculation of suitability indices, we used least square means predicted from an ANCOVA with log(carrot fly biomass in mg + 1) as a dependent variable, plant species as an independent variable and log(root weight in g + 1) as a covariate. The logarithmic transformation reduced heteroscedasticity in the data and accounted for the (theoretically) non-linear relationship between fly biomass and root weight. Many plants did not support any pupae or larvae, which meant that the distribution of the log-transformed

biomasses diverged somewhat from normal. Notwithstanding, we retained these zero values because they were not only due to abiotic factors, i.e. unfavourable environmental conditions, but also due to actual unsuitability of the plants in question (e.g. *Pimpinella major*). Besides, exclusion of zero values did not lead to greatly different results. When there was a significant plant species × root weight interaction, least square means were calculated from regression lines with separate slopes; otherwise they were derived from lines with a common slope (Table 2). The relative suitability of a test plant was calculated for each independent experiment (1–7 per species, see Table 3) as follows:

$$\text{suitability index} = \frac{\text{least square mean log(fly biomass + 1) produced per test plant}}{\text{least square mean log(fly biomass + 1) produced per standard plant}} \times 100$$

Field experiments. Potted plants transferred to seed beds or plants directly sown in the field were exposed to a consistently high population of carrot flies at Wellesbourne (UK), followed by caging of infested plots and trapping of emerged adults in yellow water dishes (Hardman et al., 1990). The susceptibility indices previously published (Hardman et al., 1990) had been based on the highest mean number of flies produced per plant in any of the experimental periods (1–8 depending on the species). Accordingly these values, which are given for comparison in Table 4, originated from only one field trial and were dependent on the size of the carrot fly population at that time. To eliminate the influence of variation in the levels of infestation among different periods, the data for test plants were related to the data for standard plants for each independent experimental season as shown below. Since variation in the field data was by one to two orders of magnitude higher than in the laboratory assays, we subjected these ratios to a logarithmic transformation. First generation attack (emergence August to December) and second generation attack (emergence January to July in the following year) were considered separately for each year (1980–1989). The relative susceptibility of a test plant was calculated as follows:

$$\text{susceptibility index} = \log\left[\left(\frac{\text{mean numbers of flies produced per test plant}}{\text{mean numbers of flies produced per standard plant}} \times 100\right) + 1\right]$$

Results

Oviposition preferences. The relative acceptabilities of 28 umbelliferous plants (species, subspecies and cultivars) and of six non-umbelliferous species are listed in Table 1. A clear-cut distinction between hosts and non-hosts based on the results of laboratory assays is not feasible, as a small percentage of eggs has also been found on oviposition dishes fitted with non-host leaves or even on dishes devoid of leaves (Degen & Städler, 1997; Degen et al., in prep.-a). All non-umbelliferous species must be regarded non-hosts, as none of them has unambiguously proved to be a host plant in the field (Ellis et al., 1992), with the possible exception of *Cichorium intybus* (Van't Sant, 1961). There was some overlap in the acceptability indices of umbellifers and non-umbellifers: *Pimpinella major* and *Daucus capillifolius* were ranked within the non-hosts. However, it is conceivable that such low-ranking umbelliferous plants would still elicit more egg-laying than non-umbellifers when directly compared in dual choice assays. Leaf area, which was variable to some extent among the species, had no obvious effect on the overall outcome of the experiments, i.e. the preference hierarchy. A notable exception may have been *Daucus capillifolius*, which was presumably less apparent with its thin "hair-like" leaflets than the standard carrot leaves and therefore may have attracted fewer flies to alight. Otherwise, egg-laying mainly reflected differential host-acceptance by the female flies upon contact with the foliage (Degen et al., in prep.-a).

Larval performance. The results of the inoculation experiments are summarized in Table 3. In the greenhouse assays (I–VIII), plant species and root size typically accounted for less than 50% of the variation in the carrot fly biomass as compared to about 70% in experiment IX run in the controlled environment room (Table 2). Interfering abiotic factors (e.g. changing air humidity) played a more important role in the greenhouse, where yields of pupae were generally lower. The climate chamber assays appeared to provide more reliable data, and so these are presented separately in Table 3 along with the average suitability indices over all experiments.

Total biomass produced per plant depends on both the number and weight of the pupae. These two fitness components, i.e. survival to pupal stage (or third larval instar) and growth rate, were interrelated,

Table 3. Relative suitability of various plant species for larval development of the carrot fly. Along with average values over all experiments, suitability indices are given separately for experiment IX. *N* = total number of carrot fly individuals produced (pupae and non-pupated third instar larvae)

plant species	suitability index			number of plants tested per experiment (*n*)										*N*	mean individual
	mean ± s.e.		IX	I	II	III	IV	V	VI	VII	VIII	IX	total		weight in mg
Apium graveolens rapaceum	164.9	± 25.1	90.0	10		5	8	8		10		5	46	511	2.13
Foeniculum vulgare azoricum	160.4	± 14.4	107.6	3			7	7		20		5	42	410	1.98
Heracleum sphondylium	136.8	± 9.6		11		3							14	203	1.90
Petroselinum crispum tuberosum	127.9	± 15.8	115.6	9		4	8	8		10		5	44	374	1.95
Foeniculum vulgare	125.7	± 10.8	100.4	9			8	8				10	35	463	1.93
Daucus carota drepanensis	114.9	± 21.9	102.3	9		2				9		5	25	272	2.04
Pastinaca sativa sylvestris	110.7	± 16.5	77.7	10		10						3	23	445	2.22
Carum carvi	109.1	± 14.6	86.9	10		5	8	8		10		10	51	441	1.46
Daucus carota commutatus	101.7	± 7.7	93.9							10		10	20	137	1.36
Daucus carota azoricus	100.6	± 12.1	88.5							10		10	20	164	1.62
Daucus carota sativus "Danvers"	100.0		100.0	28	11	10	8	8	18	10	5	11	109	979	2.49
Conium maculatum	99.1	± 8.8	64.3	9		4	8	8		10		6	45	336	1.67
Aethusa cynapium	98.2		98.2									5	5	72	1.58
Daucus carota sativus "Tip-Top"	89.0	± 23.6	104.0	12			7	7		9		5	40	386	2.81
Anthriscus cerefolium	86.2		86.2									9	9	55	1.63
Pastinaca sativa sativa	82.4	± 28.4	21.2	10		5	8	8		10		5	46	246	1.87
Petroselinum crispum	81.6	± 12.7	70.2	10		4	9	9		9		5	46	211	1.46
Daucus capillifolius	(70.0	± 14.0)	51.2				8	8				10	26	4	0.98
Daucus carota sativus "Sytan"	66.1	± 10.7	53.2	12	13		7	7	18	10		5	72	353	2.57
D. capillifolius × *D. carota sativus*	65.3	± 16.2		12			8	7					27	123	2.32
Aegopodium podagraria	53.2	± 6.0		10		5							15	39	1.12
Levisticum officinale	45.7	± 6.8	40.7	10		5	8	8		10		10	51	154	1.43
Smyrnium olusatrum	36.6	± 12.9	12.5	6						10		12	28	11	1.07
Pimpinella major	4.8	± 4.8	0.0	11								12	23	0	
Cichorium intybus	0.0	± 0.0	0.0								6	10	16	0	
Tanacetum vulgare	–15.3	± 3.7					8	8					16	0	

but not closely. For instance, cultivated carrot usually supported only moderate numbers of carrot fly individuals, yet these invariably attained the highest mean weight (Table 3). Root weight mainly influenced the number of surviving larvae, while their growth was affected to a lesser degree.

Correcting for root size effects by means of regression lines with a common slope led to indices that positively or negatively deviated from zero for plants that did not give rise to any carrot flies (e.g. *Pimpinella major*, *Tanacetum vulgare*). In particular, the values adjusted in this way for *Daucus capillifolius*, a plant species characterized by rather small roots, were questionable and hence are given only with reservation in Table 3.

Susceptibility in the field. The ranking of species according to their susceptibility index is shown in Table 4. The values for some species (e.g. *Carum carvi, Foeniculum vulgare azoricum*) are based on relative scarce data material and consequently must be considered with caution.

Relationships among the parameters. The acceptability index was only significantly correlated with the suitability index obtained in experiment IX, but not with the average suitability index for all experiments (Figure 1), regardless of whether *Daucus capillifolius* was included or not. A notable mismatch between oviposition preferences and larval performance concerned the fairly acceptable, but quite unsuitable *Smyrnium olusatrum*. The opposite situation

Table 4. Relative susceptibility of various plant species to carrot fly attack in the field (Wellesbourne, UK). n = number of experiments (n_0 = number of experiments without any production of flies); max. mean = highest mean number of flies produced per plant in any experimental season; N_{plants} = total number of plants tested; N_{flies} = total number of flies produced

plant species	susceptibility index mean ± s.e.	n	(n_0)	max. mean	N_{plants}	N_{flies}
Anthriscus cerefolium	2.84 ± 0.95	2		2.03	60	80
Daucus carota azoricus	2.75	1		4.83	30	145
Conium maculatum	2.41 ± 0.26	4		3.50	120	213
Daucus muricatus	2.31 ± 0.68	3		17.13	374	574
Pastinaca sativa sylvestris	2.25 ± 0.06	4		1.88	116	153
Daucus carota drepanensis	2.21 ± 0.20	2		1.03	60	38
Aethusa cynapium	2.16 ± 0.27	4		16.43	248	993
Daucus carota sativus "Danvers"	2.00	22		12.97	1026	2693
Daucus carota sativus "Sytan"	1.95 ± 0.83	2		0.20	60	9
Levisticum officinale	1.86 ± 0.43	3		0.93	90	50
Petroselinum crispum	1.75 ± 0.18	2		5.33	60	298
Petroselinum crispum tuberosum	1.66	1		2.87	30	86
Apium graveolens rapaceum	1.60	1		2.47	30	74
Daucus carota commutatus	1.44 ± 0.34	4		5.53	476	596
Foeniculum vulgare	1.40 ± 0.61	4	(1)	3.07	120	132
Heracleum sphondylium	1.20 ± 0.10	5		0.87	125	51
Anethum graveolens	1.05 ± 0.60	4	(1)	3.32	115	133
Carum carvi	0.95	1		0.50	30	15
Aegopodium podagraria	0.59 ± 0.32	4	(1)	1.37	120	51
Foeniculum vulgare azoricum	0.56	1		0.17	30	5
Daucus littoralis	0.52 ± 0.52	4	(3)	0.08	121	6
Daucus capillifolius	0.47 ± 0.47	4	(3)	0.20	115	6
Daucus pusillus	0.34 ± 0.23	8	(5)	0.27	410	17
Pimpinella major	0.17 ± 0.17	4	(3)	0.03	117	1
Smyrnium olusatrum	0.12 ± 0.12	6	(5)	0.17	180	5
Cichorium intybus	0.00 ± 0.00	3	(3)	0.00	90	0
Daucus broteri	0.00 ± 0.00	2	(2)	0.00	40	0

was found with *Apium graveolens*, for example, a highly suitable host plant that is not favoured by ovipositing carrot flies.

There was a weak positive relationship between suitability and susceptibility to carrot fly attack in the field, which became tighter, when some of the less reliable field data (based on results of < 3 trials) were omitted (Figure 1). Essentially there were two different clusters of species: *Pimpinella major*, *Smyrnium olusatrum* and the composite *Cichorium intybus* were unsuitable as hosts and highly resistant in the field, whereas the remaining species supported moderate to high numbers of carrot fly larvae and were fairly susceptible. Highly acceptable hosts tended to be more severely infested in the field than non-preferred hosts, but here again the correlation was not strong (Figure 1). With the restricted set of species – exclusive of plants tested in the field only once or twice – there was a good agreement among oviposition preferences and susceptibility, except for three major outliers: *Smyrnium olusatrum*, *Daucus littoralis* and *Daucus pusillus*.

Both components of resistance – antixenosis and antibiosis – together accounted for a higher percentage of the variation in susceptibility of the plants than each factor alone, but the contribution of antixenosis ("non-preference") seemed to be greater than that of antibiosis, as suggested by the invariably higher standard coefficients obtained by multiple regression (Table 5).

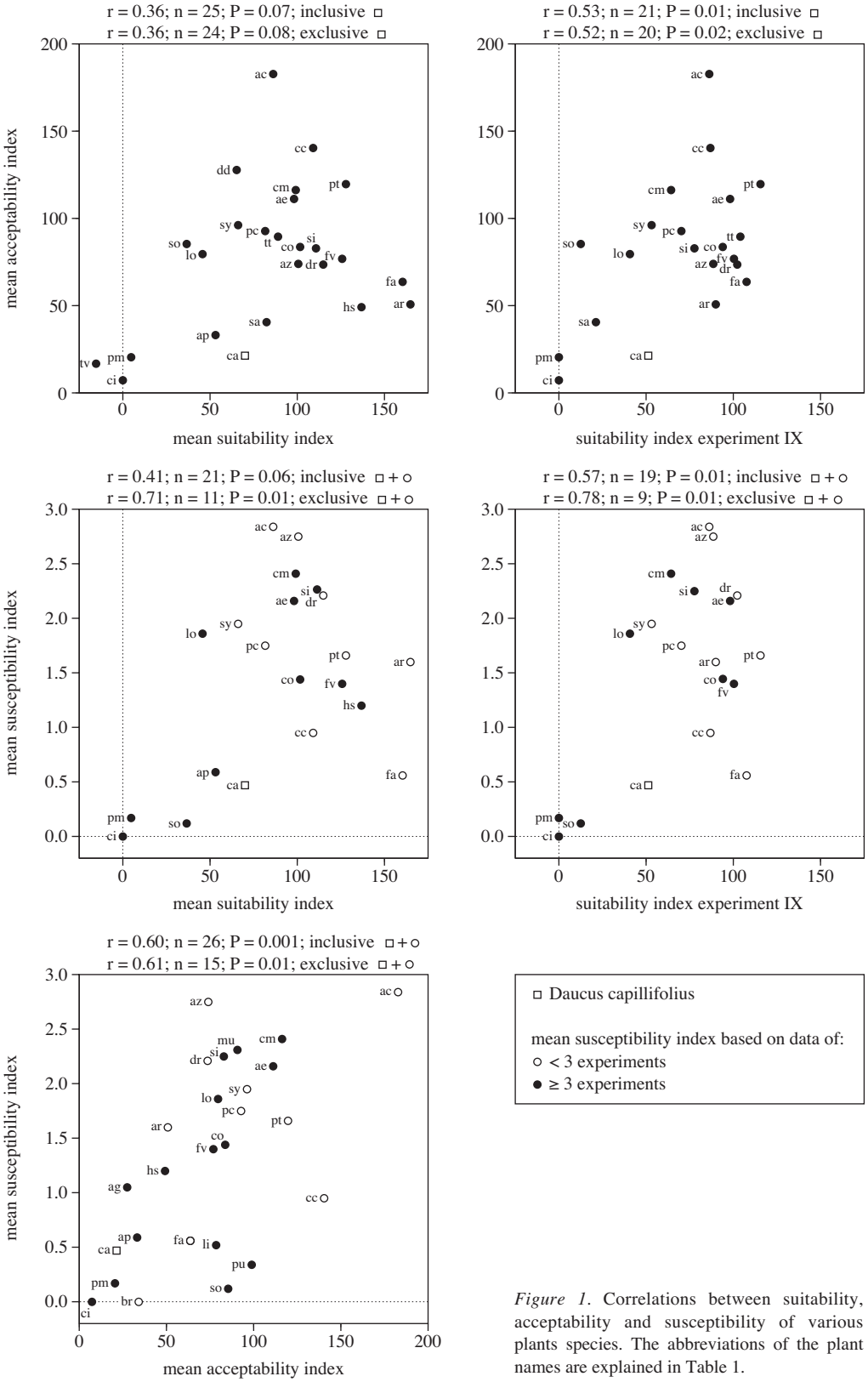

Figure 1. Correlations between suitability, acceptability and susceptibility of various plants species. The abbreviations of the plant names are explained in Table 1.

Table 5. Relative importance of adult host acceptance and larval performance for differential susceptibility of plants to carrot fly attack as indicated by a multiple regression from mean acceptability index and mean suitability index (independent variables) on mean susceptibility index (dependent variable). Exclusive = without *Daucus capillifolius* and species whose mean susceptibility index is based on data of < 3 experiments

selection	independent variables	coefficient	std. coeff.	*t*-value	*P*-value	r^2 (adjust.)	df (total)	*P*-value
all species	mean suitability	0.005	0.24	1.3	0.21	0.46 (0.40)	20	0.004
	mean acceptability	0.012	0.57	3.1	0.006			
	intercept	0.092	0.09	0.2	0.82			
	suitability experiment IX	0.009	0.35	1.6	0.11	0.48 (0.41)	18	0.006
	mean acceptability	0.009	0.45	2.1	0.05			
	intercept	0.100	0.10	0.2	0.81			
exclusive	suitability	0.007	0.37	1.6	0.15	0.72 (0.65)	10	0.006
	mean acceptability	0.014	0.57	2.5	0.04			
	intercept	–0.255	–0.26	–0.7	0.51			
	suitability experiment IX	0.010	0.45	1.5	0.19	0.72 (0.63)	8	0.02
	mean acceptability	0.012	0.47	1.5	0.17			
	intercept	–0.169	–0.17	–0.4	0.73			

Discussion

Conclusions about the adequacy of ovipositional "decisions" of adult insects with respect to larval survival and growth depend on the scope of plant species considered. At the grossest scale, one expects host-selection behaviour to be correlated with offspring performance (Jaenike, 1990). For the carrot fly, this means that accepting umbellifers and rejecting non-umbellifers for oviposition in the majority of cases provides the larvae with suitable hosts. The range of host plants on which larvae are capable of developing is generally broader than the range of hosts acceptable for egg-laying females (Thompson, 1988), as is the case in another umbellifer specialist, the swallowtail butterfly, *Papilio machaon* (Wiklund, 1975). It is not known whether this notion holds true for the carrot fly. First, our laboratory assay does not allow a straightforward discrimination between host and non-host, as all oviposition dishes received at least a small proportion of eggs. Second, to identify non-host plants potentially supporting development of carrot fly larvae, further non-umbelliferous would have to be inoculated with eggs. The available evidence suggests that at least some species belonging to the plant families chemically related to the Apiaceae, e.g. *Ruta graveolens* (Rutaceae) and *Cichorium intybus* (Asteraceae), may give rise to carrot flies (Van't Sant, 1961; Hardman & Ellis, 1982; E. Städler, unpubl.). However, we failed to confirm the latter result and are inclined to believe that the vast majority of non-umbelliferous plants will eventually prove unsuitable as hosts.

In narrowing down the perspective to more restricted sets of host plants differing more subtly in their characteristics, we find that females of some species rank hosts for oviposition in the same order, on average, as their suitability for offspring development, but many such preference-performance correlations are rather weak (see Thompson, 1988; Jaenike, 1990; Fox & Lalonde, 1993). There are several conceivable reasons why this also applies to the carrot fly and its umbelliferous hosts. The possibility of detecting adaptive oviposition behaviour can be diminished by the way the experiments are designed. For example, we did not use the same individual plants for the oviposition assays and for inoculation. According to Jaenike (1990) insects appear to be more apt at identifying the best individual plants within a host species than at identifying the host species that is, on average, most suitable. Also, a close correspondence between host suitability and acceptance may only be manifest at the level of individual insects, while being obscured in analyses based upon population averages (Thompson, 1988). There may have been further inaccuracies in measuring the two parameters. Though the preference ranking established with cut foliage was shown to be

reasonably representative for the ranking of whole plants (Degen et al., in prep.-a), root volatiles, which were excluded in our assays, may also be involved as cues in the host-selection process (Maki & Ryan, 1989). In the inoculation experiments, the growing conditions (i.e. pure sand with fertilizer) were perhaps suboptimal for certain species, while fairly appropriate for others (e.g. carrots), possibly contributing to differences in pupal weights not observed in such a pronounced way in nature. Moreover it is preferable to avoid any influence of plant size rather than to be forced to correct the data for such effects afterwards.

It has also been argued that discrepancies between host suitability and host acceptance can be due to imperfect discriminatory abilities of the female insects, i.e. suitable and unsuitable hosts are indistinguishable (Courtney & Kibota, 1990; Fox & Lalonde, 1993). This argument may specifically apply to the carrot fly because the larvae feed on the subterranean parts of the plant, which are not readily accessible to the flies for examination. Hence a "correct" host identification requires a correlation among the leaf contents of host-associated semiochemicals (oviposition stimulants, deterrents) and the concentration of compounds in the roots that influence host acceptance and performance in the larvae. Polyacetylenes characteristic of umbelliferous plants are potential candidates for such a function, as they have been identified both as oviposition stimulants (Städler & Buser, 1984) and attractants for larvae (Maki et al., 1989). By contrast, it is hard to imagine any such foliar correlates for physical root properties possibly affecting larval survival (e.g. side root density, toughness of the rhizoderm).

Adaptations in host acceptance (e.g. avoidance of unsuitable hosts) and performance can only evolve, when interactions between the insects and the plants in question actually take place. Here factors such as geographical distribution, abundance and apparency of the host plants come into play. Some of the species included in this study cannot be encountered in nature by Swiss carrot fly populations (e.g. *Smyrnium olusatrum*) or by any other existing population at all (e.g. *Daucus capillifolius*, *Daucus pusillus*). So we may speculate for example, whether *Smyrnium olusatrum*, which was willingly accepted in our oviposition assays, is rather rejected by flies inhabiting areas where this highly resistant and unsuitable plant species is readily available and widespread. Nothing is known about genetic variation in oviposition preferences and performance both within and among carrot fly populations except that resistance of various carrot cultivars was found to be consistent throughout several European countries (Ellis & Hardman, 1981). There is no reason to doubt that host selection behaviour varies genetically to some extent within and among carrot fly populations. Still, the overall preference hierarchy may be evolutionarily rather conservative among allopatric insect populations that feed on different host-plants in nature ("local monophagy"), as has been shown for the oligophagous western anise swallowtail butterfly *Papilio zelicaon* (Thompson, 1993).

In the context of this study, the term "host-plant suitability" refers mainly to post-ingestive physiological effects on larval survival and growth (antibiosis). However, behavioural effects (antixenosis) such as deterrence prior to feeding cannot be entirely excluded, even though the larvae were not allowed to choose among different plants in the inoculation experiments. Adult oviposition preferences and larval performance together accounted for a substantial part of the differences in susceptibility observed among host plants. Some unexplained inconsistencies may be attributable to possible genetic differences among English and Swiss carrot fly populations and to plant traits not controlled in the field trials. Host finding is assumed to be strongly affected by pronounced differences in apparency (size, growth stage), which clearly occurred among the plants in the field (Hardman & Ellis, 1982). Our findings suggest that antixenosis is probably a more important component of host-plant resistance to carrot fly than antibiosis. Analogous findings have also been reported for two other root flies (Baur et al., 1996) and for a gall midge (Åhman & Lövgren, 1995), only to mention two examples. However, at the level of carrot cultivars we demonstrated rather a prevalence of antibiotic resistance, which corroborates the assumption of Guerin & Ryan (1984) that root resistance to the larvae is the crucial prerequisite in breeding carrot varieties resistant to *Psila rosae*. In an attempt to achieve carrot cultivars with higher levels of resistance, breeding lines were developed from crosses between *Daucus capillifolius* and *Daucus carota sativus* (Ellis et al., 1993). The very low susceptibility of *Daucus capillifolius* is believed to be mainly due its low apparency (hair-like leaves) and small root size, both traits not useful for introduction into

cultivars. Yet, our laboratory data are inconclusive concerning the question of whether the low acceptability and suitability of this wild *Daucus* species are furthermore caused by particular allelochemicals. The mechanisms underlying host selection have been studied both in adult flies (e.g. Städler, 1977; Städler & Buser, 1984) and in larvae (e.g. Ryan & Guerin, 1982; Maki et al., 1989). Still, our understanding of the plant factors determining host finding and acceptance is far from comprehensive. Virtually nil is the knowledge about semiochemicals mediating antibiosis. The latter aspect – admittedly difficult to investigate – deserves more attention in future studies dealing with host-plant resistance to the carrot fly.

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Foliar form, colour and surface characteristics influence oviposition behaviour of the carrot fly

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Abstract

Various leaf models made of paper were presented to carrot flies, *Psila rosae* (F.) (Diptera: Psilidae) in choice assays to investigate the effect of non-chemical plant traits on oviposition behaviour. The surrogate leaves differed in colour, shape, surface coating, size and stem length. In the presence of host-plant extracts, physical factors strongly influenced oviposition. Green, yellow and orange three-dimensional models similar in shape to host-plant leaves (pinnately or ternately compound or dissected) and with a thin cover of paraffin wax were most acceptable to the females. Egg-laying was not affected by leaf size, but was negatively correlated with stem length. The results obtained by testing models with simple leaf silhouettes were confirmed in an experiment using more lifelike imitations of real host and non-host leaves. The findings are discussed by an extensive review of similar studies in three other phytophagous fly species (cabbage root fly, onion fly, Hessian fly).

Introduction

At close range, female carrot flies are presumably attracted to patches of host plants (e.g. carrot fields) by blends of host odours, e.g. propenylbenzenes and green leaf volatiles (Guerin et al., 1983). Once landed on a plant, they perform exploratory runs over the leaves before accepting it for oviposition (Bohlen, 1967). Finally, the eggs are deposited in crevices in the soil near the base of the plant.

Oviposition stimulants (e.g. propenylbenzenes, furanocoumarins, polyacetylenes) are crucial for host-plant recognition and acceptance in carrot flies (Städler & Buser, 1984). However, the general assumption that semiochemicals are of paramount importance in host finding and acceptance by herbivorous insects, whereas non-chemical plant stimuli play only a secondary role has been questioned (Miller & Strickler, 1984; Harris & Miller, 1988). Indeed, extensive studies with phytophagous fly species have revealed the potentially strong influence of physical plant properties on the host-selection behaviour (Harris & Miller, 1983; Harris & Miller, 1984; Harris & Rose, 1990; Roessingh & Städler, 1990). For the carrot fly, data on the effect of non-chemical plant features such as leaf shape, size, colour and surface characteristics on oviposition behaviour are scarce and sometimes ambiguous (Bohlen, 1967; Städler, 1972; Städler, 1977).

Surrogate leaves made of white filter paper have previously been used for testing the stimulatory activity of host-plant extracts and pure compounds (Städler, 1977; Städler & Buser, 1984). In a preliminary choice experiment, carrot flies deposited much fewer eggs below these filter paper surrogates than below green paper surrogates covered with a thin layer of paraffin or below commercially available green plastic parsley leaves (T. Degen, unpubl.). This outcome forced us to optimize a surrogate leaf for oviposition assays with respect to several physical factors, which at the same time allowed us to improve our understanding of the sensory information

involved in oviposition decisions made by the carrot fly. We evaluated the effects of shape, size, colour and surface properties on egg-laying behaviour using paper models that allow a convenient manipulation of cues.

Materials and methods

Insects. The flies for the oviposition tests were obtained from a permanent laboratory culture (Städler, 1971). They were 8–16 generations removed from field populations. The flies emerged continuously from pupae held in the cages in which the experiments were performed. Hence some proportion of the fly population had previously experienced surrogate leaves tested in other assays or natural host-plant foliage offered between the tests. However, we used exclusively naïve flies for the two experiments that involved landing counts (Figures 4 & 6) and for the experiment with the accurate leaf imitations (Figure 7). So far, there is no evidence that the oviposition preferences of carrot flies are notably modified by earlier experience (T. Degen, unpubl.; Figure 4).

Bioassays. The flies were kept in a climate controlled room (21 °C ± 1 °C, 80 ± 5% r.h.). We performed the oviposition experiments in screen cages (70 × 70 × 70 cm), which were surrounded by a four-sided box of white cardboard (85 × 85 × 85 cm) to provide even illumination. Four cool white (Osram L 20 W/20 S) and three yellow (Philips TLD 18W/16) fluorescent tubes were alternately placed on top of each cage and the following light-dark regime was administered: 12 h white and yellow light (8:00–20:00; 720–965 lux at the cage floor), two 2 h periods with just yellow 'twilight' (6:00–8:00 and 20:00–22:00; 290–370 lux) and 8 h dark. For the colour experiments we replaced the yellow lamps by white ones.

The oviposition devices consisted of plastic dishes (diameter 9 cm, height 4 cm) with a wetted foam rubber lining covered with a black cloth and a black polyethylene grid (mesh 1 mm) (Städler, 1971). The leaf models were fixed to the dish with two rubber bands (Degen & Städler, 1997). The dishes were topped by an inverted black plastic pot with a 5 cm × 5 cm wide opening that allowed the flies only restricted access to the oviposition substrate (see Figure 8). These covers were used because they increase the differences among the treatments by reducing 'stray' oviposition by flies that invade a dish when stimulated on a neighbouring instead of on the accompanying leaf (Degen & Städler, 1997).

Two, four or eight different treatments, as indicated in the figures, were presented simultaneously to the flies in choice assays. Invariably, eight oviposition dishes with the leaf models under investigation were arranged in a circle on the cage floor around a central potted apple seedling, which served as a resting and copulation site. The models were either randomly assigned to the positions, when eight different treatments were offered, or placed in a regular alternating sequence in the case of only two or four different treatments (with four and two repetitions for each treatment in the same block, respectively). The test leaves were exposed to the flies usually for one day, rarely for longer intervals (maximally four days) and re-introduced into the cage for the next experimental period, after the eggs laid onto the black cloth had been counted and removed. We permuted the positions in such a way that each treatment was located once at each position.

We analysed the egg counts for treatment effects with the Friedman test and the Friedman test modified for repetitions of each treatment within a block (one experimental period in a cage); multiple comparisons were made following a method based on rank sum differences among individual treatments (Conover, 1980). The number of replicates (n) is given by the number of leaf models with the same treatment (m) multiplied by the number of experimental periods (b) during which a single model was tested. The numbers of eggs are expressed in the figures as percent of the total laid in the block. Percentages were arcsine-transformed for the ANOVAs, that were carried out to test for main effects and interactions when more than one factor was varied in an experiment. Approximately two thirds of the experiments were conducted in two different cages, where the fly population typically consisted of 100–400 individuals (males and females). Since the treatments were exposed successively to the same fly population, with few exceptions during four consecutive one-day intervals, the replicates may not be considered independent in a strict sense. However, this problem is alleviated by the fact that the flies require on average three to four days between two oviposition events (Bohlen, 1967). Thus only a minority of the individuals would have contributed eggs to more than one block.

We are aware of the limitations of the multiple choice assay. The quantitative differences observed among particular treatments are clearly influenced by the range of all the treatments present in the test. Yet the ranking of the treatments probably remains unchanged irrespective of the experimental design (e.g. compare the results for identical treatments in Figures 3 and 5B). Preference hierarchies of the carrot fly for various plant species established both in dual and multiple choice assays (including a standard carrot cultivar) were in good accordance (Degen & Städler, unpubl.).

Landing counts. We counted the flies that landed on the leaf models chosen for comparison during the daily period of high oviposition activity (Städler, 1975). Two neighbouring models out of the eight present in the cage were observed simultaneously through a small opening in the cardboard box surrounding the cages with the ongoing oviposition experiment (Figures 4 & 6). Individuals that re-alighted on the same leaf immediately after flying off, without a stopover, were noted as arriving only once. We used the Wilcoxon signed rank test to compare the landing counts for both tested leaf models, the observational periods (15–30 min) serving as replicates.

Paper leaf models. Unless stated otherwise, the leaf models had the following properties. They were made of light green cardboard (300 g/m^2). The leaf area was 100 cm^2 including the stem, which was 8 cm long and 1 cm wide. The leaves were coated with a thin wax layer by dipping them into melted paraffin (Merck, melting point 42–44 °C). We supplied chemical oviposition stimuli by spraying the models with extracts of carrot leaves at a concentration of 3 or 4 gram leaf equivalents (gle) per 100 cm^2 area. The extracts were prepared either by dipping intact leaves in dichloromethane twice for 30 s (Städler & Buser, 1984) or by heating the leaves in hexane for 60 s to approximately 50 °C solvent temperature in a microwave oven (T. Degen, G. Poppy & E. Städler, unpubl.). Control leaves without chemical stimulants were only included in three instances (Figures 1, 6, & 7). The following leaf parameters were varied for the experiments:

– *Surface cover*. Models ('three-dimensional hogweed') with the following surface properties were tested: Paraffin (Merck, melting point 42–44 °C), Araldite (standard epoxide, Ciba Geigy), polyvinylpyrrolidone PVP (K 25; Fluka; purum), paper surface without coating. Paraffin and Araldite, but not PVP (dissolved in water), darken the colour of the paper, when applied to its surface. To avoid this darkening, we covered the leaves first with PVP before adding the paraffin or Araldite coating, respectively. Both Araldite and PVP provided a smooth, glass-like glossy surface. The PVP cover was fairly inelastic and slightly hygroscopic under high r.h. (e.g. close to the moist oviposition substrate). The paper and the paraffin surface were matt. The uncoated paper models slightly differed in hue and saturation, but not in brightness from the coated models as indicated by HSB-values (for explanation see next paragraph and Table 1).

– *Colour*. We compared models ('three-dimensional hogweed') with different colours (Figure 2A) and with different shades of green (Figure 2B). The models were produced from coloured cardboard (300 g/m^2). The colours tested are characterized in Table 1 by the reflectance at 560 nm (yellow) and 460 nm (blue). The ratio of these two values proved to correlate well with catches of coloured traps in a field study (Brunel & Langouet, 1970). The reflectance measurements were performed on a CAMAG TLC Scanner II (CAMAG, Muttenz, Switzerland) against MgO powder as a white standard. In addition, we recorded the colour attributes of the leaf models under the same light conditions as chosen for the experiments, with a video camera that was connected to a computer. Readout values of two colour models (HSB and CMYK) were obtained from the Adobe Photoshop™ software (Table 1).

– *Leaf silhouettes*: Five geometrical forms (circle, squares, triangles) were tested against three shapes that resembled host-plant leaves ('notched', 'hogweed', 'celery') (Figure 3).

Table 1. Colour values of the surrogate leaves based on two different colour models (HSB, CMYK) and percent reflectance at two different wavelengths, 460 nm (blue) and 560 nm (yellow)

Colour	HSB[1]			CMYK[2]				% reflectance at	
(data in figure)	H	S	B	C	M	Y	K	560 nm	460 nm
orange (2A)	18	84	96	0	71	88	0	13.7	2.5
yellow (2A)	59	97	97	11	0	95	0	56.5	3.7
light green (2A & B)	104	72	58	78	12	97	3	12.4	4.3
olive (2B)	113	54	41	80	27	91	24	14.3	9.3
dark green (2B)	144	35	22	73	32	63	65	2.5	3.1
turquoise (2B)	144	57	95	54	0	49	0	29.2	24.2
black (2A)	170	16	15	68	47	51	75	5.0	6.8
cyan (2A)	203	75	91	73	14	0	0	16.1	31.1
dark blue (2A)	232	70	48	95	84	0	0	3.1	11.8
white (2A)	312	2	99	1	3	0	0	75.2	69.6
red (2A)	358	70	52	31	91	80	21	3.7	5.6

[1] H = hue: 0° = red; 60° = yellow; 120° = green; 180° = cyan; 240° = blue; 300° = magenta; S = saturation: 0% = grey; 100% = fully saturated; B = brightness: 0% = black; 100% = white.
[2] C = cyan; M = magenta; Y = yellow; K = black; 0% = white; 100% = pure colour.

– *Flat and three-dimensional leaf models with cuts and/or folds*: We compared a flat leaf model ('square with tip down') to three-dimensional leaf models of the same silhouette, but with either folds (horizontal, vertical, directed towards the stem) or cuts (horizontal, vertical, upwards or downwards at an angle of 45° to the stem) (Figure 5A). The leaflets of the dissected models were bent alternately in two directions diverging at an angle of about 45° from each other. The models with folds had the same projection area, but a slightly bigger total area than the flat model. Hence the concentration of the host-plant extract per leaf area was somewhat lower on the folded models. In a second experiment four flat leaves ('square with tip down', 'notched', 'hogweed', 'celery') were tested against their three-dimensional counterparts with cuts and/or folds (Figure 5B).

– *Accurate leaf imitations*. We used scanned images of real leaves as templates for the models, which were cut from light green cardboard. The stems were reinforced with wire and covered with green adhesive tape, which was slightly lighter than the cardboard after coating with paraffin. Folds were designed to imitate leaf veins and added a third dimension to the models. We chose two host-plant species (Apiaceae: hogweed *Heracleum sphondylium*, parsley *Petroselinum crispum*) and three non-host species (Liliaceae: leek *Allium porrum*; Brassicaceae: cauliflower *Brassica oleracea* convar. *botrytis*, *Rorippa islandica*) as examples. Further details regarding the leaf dimensions (e.g. area, height) are given in Degen & Städler (1996). A low contrast environment was provided by covering the cage floor, the walls up to a height of 25 cm and part of the oviposition devices with green cardboard. In addition, sixteen grass dummies made of green paper were regularly interspersed between the test leaves to achieve an experimental set-up that resembled a natural plant assemblage. A high contrast environment was obtained by replacing the green background with a white one and by omitting the grass dummies. We used the same models for all 16 replicates (eight with green followed by eight with white background) and renewed the chemical treatment after four experimental periods with the green background. The amount of microwave extract applied was proportionate to the leaf area (4 gle/100 cm^2). Three models (*Allium*, *Brassica*, *Petroselinum*), one per plant family, were sprayed only with solvent (see Figure 7). The flies' discrimination between the extract-treated and the control leaves did not change significantly with the time elapsing after application of the extract.

– *Size*. The leaf models ('square with tip down') had the same proportions, but differed in area (25 cm^2, 50 cm^2, 100 cm^2, 200 cm^2). The amount of dichloromethane extract applied was adjusted to the leaf size (4 gle/100 cm^2), but the differences in height were not compensated for.

– *Stem length*. Four models ('three-dimensional hogweed') differing in stem length (1 cm, 4 cm, 8 cm, 12 cm) were tested. Here we compensated for the differences in height by varying the level of the oviposition dishes (Figure 8).

Results and discussion

Leaf surface cover. The surface coating of the surrogate leaves strongly influenced the absolute number of eggs laid as well as the discrimination between the chemical treatments (ANOVA; coating: $F = 19.5$; df = 3; $P < 0.0001$; chemical treatment: $F = 64.8$; df = 1; $P < 0.0001$; coating × chemical treatment: $F = 28.5$; df = 3; $P < 0.0001$). The paraffin-covered models elicited the strongest ovipositional response (Figure 1). The paraffin did not seem to be a chemical oviposition stimulus per se, since there were no significant differences between the control leaves with different coatings. Nevertheless, it enhanced the stimulative activity of the host-plant extract in comparison to the other surface covers. The nature of this interaction is unknown. The stimulus originating from the paraffin can be either chemical or tactile. The oviposition stimulants present in the extracts may be exposed to the sensory organs of the flies (e.g. antenna, tarsal sensilla) in different concentrations depending on the chemical properties of the coating substances. For example, paraffin could be an optimal slow release medium for these stimulating compounds compared to the other surface covers. However, the most important known oviposition stimulants (e.g. falcarindiol) are only slightly volatile and there was no detectable change in the preference pattern for the surface coatings over the experimental period (four days), which might be expected when volatile substances evaporate at different rates. Thus, the synergistic effect may alternatively be due to certain structural characteristics of the waxy paraffin layer, as has also been suggested for the cabbage root fly (Roessingh & Städler, 1990). Oviposition stimulants for the latter species (e.g. glucosinolates) are polar and hardly dissolve in paraffin. Nevertheless, the presence of a waxy coat is essential for an adequate ovipositional response of cabbage root flies to surrogate leaves. An alternative explanation could be a change of colour reflectance properties owing to the surface coating. Since the colour differences were only minor, we consider this possibility to be very unlikely.

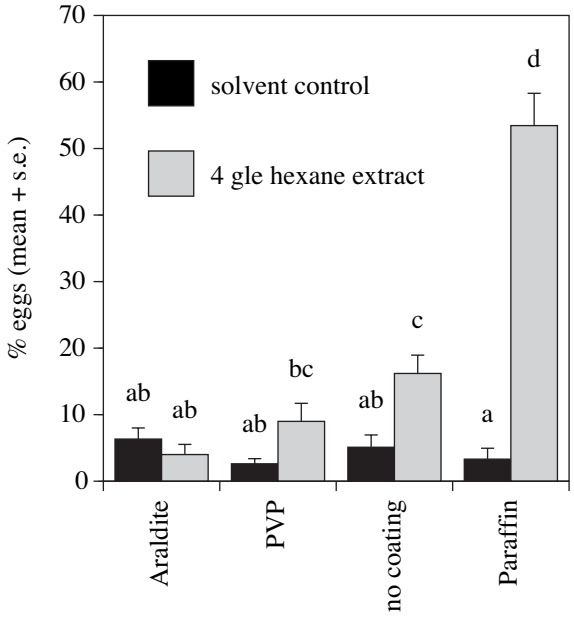

Figure 1. Influence of different surface coatings on surrogate leaves on oviposition in the presence and absence of host-plant extracts (treatment effect: $P < 0.0001$; Friedman test). Means accompanied by the same letter are not significantly different at the 5%-level. The eight treatments were simultaneously exposed to the flies (multiple choice assay). Total number of eggs = 2668; $n = 8$ (m = number of leaf models with the same treatment = 2, b = number of experimental periods per cage = 4).

Leaf colour. Oviposition was strongly affected by the colour of the surrogate leaves. The highest numbers of eggs were found with yellow, orange and green models (Figure 2A). Light green surrogate leaves received more eggs than leaves in darker shades of green, but also more than the turquoise surrogate, which had a higher brightness value (Figure 2B; Table 1). The percentage of eggs (as compared to the standard green used in both experiments) was significantly correlated only with the yellow CMYK-component (Spearman rank correlation: $n = 11$; $\rho = 0.73$; $P = 0.02$) and more strongly so with the

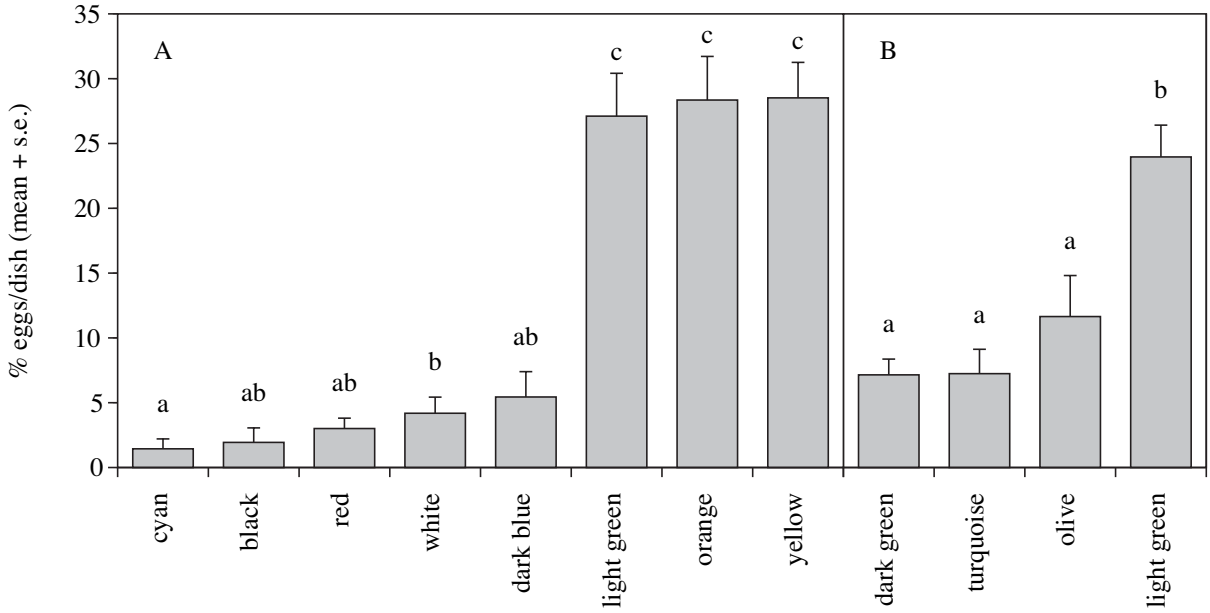

Figure 2. (A) Influence of colour of surrogate leaves on oviposition (treatment effect: $P < 0.0001$; Friedman test). Total number of eggs = 2641; $n = 8$ ($m = 2, b = 4$). (B) Influence of different shades of green on oviposition (treatment effect: $P = 0.025$; Friedman test). Total number of eggs = 1299; $n = 8$ ($m = 2, b = 4$). Columns topped by different letters differ significantly at $P < 0.05$.

ratio or the difference between the reflectance at 560 nm and 460 nm (ratio: $\rho = 0.88$; $P = 0.005$; difference: $\rho = 0.86$; $P = 0.007$). This is evidence for hue as an important cue for oviposition, most probably by influencing pre-alighting behaviour. However, the effects of hue, saturation and brightness were not examined separately in our experiments. A multiple regression with yellow and black CMYK-values as independent variables and with the percentage of eggs as dependent variable suggested that oviposition might also be positively correlated with brightness ($n = 11$; adjusted $r^2 = 0.65$; $F = 10.1$; $P = 0.006$; yellow: regression coefficient = 0.79; $P = 0.004$; black: regression coefficient = –0.68; $P = 0.04$).

Similar colour preferences have previously been recorded from a field trial (Brunel & Langouet, 1970) and from a laboratory study (Bohlen, 1967) by using coloured water traps. It is noteworthy that opposite results are reported from an investigation dealing with the colour of the oviposition substrate: carrot flies preferred oviposition sites with minimal reflectance at 500–650 nm (Städler, 1972).

Leaf shape. Carrot flies laid more eggs around models that had silhouettes similar in shape to host leaves ('hogweed', 'celery') than around models with simple geometric forms (Figure 3). There is no evidence so far that this apparently innate preference is modified by early experience. To test if a preference for leaf shape can be induced, we allowed the flies access to either the model 'square with tip down' or the model 'celery' for the first 1 to 5 days after hatching. The two fly subpopulations did not differ in the discrimination between these leaf shapes in the subsequent two-choice bioassay (Wilcoxon signed rank test with discrimination indices: $P = 0.95$; Figure 4A). However, it is possible that the flies were pre-exposed for a period too short for the induction of a preference, since only few eggs were deposited before the onset of the two-choice experiment. When we counted the flies landing on both leaf models, we distinguished the sexes according to their behaviour. Females typically performed prolonged exploratory runs over the leaf surface, moving their abdomen up and down. Males usually stayed only for a few seconds on the leaf, remained rather stationary and rapidly vibrated their body with their legs, a distinctive sexual behaviour (Städler, 1977). Landing rates were much higher for males than for females, though more than 50% of the males were removed from the cages. Pre-alightment preferences were at

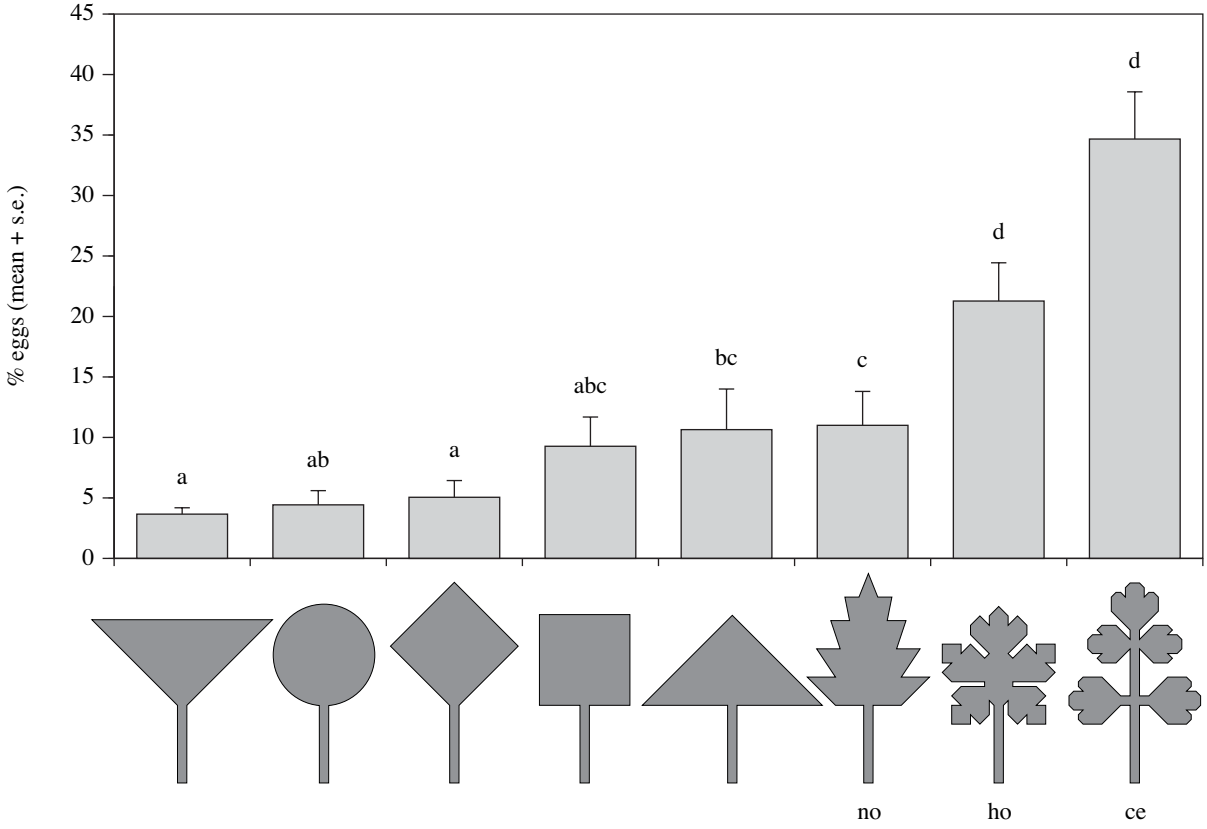

Figure 3. Influence of silhouette of flat leaf models on oviposition (treatment effect: P < 0.0001; Friedman test). Significant differences ($P < 0.05$) are indicated by different letters. no = 'notched'; ho = 'hogweed'; ce = 'celery'; total number of eggs = 2256; $n = 8$ ($m = 2$, $b = 4$).

least in part responsible for the higher numbers of eggs observed with the 'umbelliferous-like' leaf models. Females landed more frequently on the 'celery' leaf type than on the 'square with tip down' type (Figure 4B). The males did not differ from the females in the pre-alightment preferences (Wilcoxon signed rank test: $P = 0.12$). Since the 'celery' compound leaf has a slightly larger 'overall' area, more flies might be expected to alight on this model, when landings occur at random, but the pronounced difference observed between the models suggests that the carrot flies discriminate visually among the different leaf shapes before landing. This contrasts with the results presented by Bohlen (1967), who counted similar numbers of landing flies on both pinnate and non-pinnate leaf silhouettes painted onto a sticky pane of glass.

Three-dimensional properties of the leaf models also have an effect on the oviposition behaviour of carrot flies (Figure 5A). The flies generally laid more eggs around the three models with folds than around the flat model, though none of the differences was significant. The orientation of the folds was not of great importance, since the model with vertical folds did not receive more eggs than the model with horizontal folds, in contrast to the results that were reported for the cabbage root fly (Roessingh & Städler, 1990). On the other hand, oviposition was influenced by the orientation of the cuts in the dissected leaves: Higher numbers of eggs were recorded from the model with horizontal cuts than from the model with vertical cuts. The two models with cuts at an angle of 45° were intermediate. The addition of folds also enhanced the acceptability or attractiveness of the already highly preferred flat leaf models with a silhouette resembling the host leaves. Since we did not carry out landing counts, we cannot preclude that cuts and folds lead to differences in landing frequencies by changing the apparency of the models (variable light angles with three-dimensional,

but not with flat models). Further, the post-alighting behaviour may have been affected, too. When stimulated for oviposition, female carrot flies perform a geotactic run along the vertical axis of the leaf. Thus they can become 'trapped' on the tip of a leaflet that bends downwards. Often they do not find the way to the stem under such circumstances and tend to fly off (Städler, 1977). This could be one reason why the leaf model with vertical incisions and blades pointing downwards received fewer eggs. Yet, the leaves with leaflets inclined at an angle of 45° upwards and downwards yielded almost the same numbers of eggs. This indicates that 'guides' leading the flies to the stem are not very important. The results rather suggest that leaflets perpendicular to the main axis of a compound leaf act as oviposition stimuli. The model 'three-dimensional hogweed' elicited the highest oviposition responses (Figure 5B) and was chosen as standard leaf for further experiments in the present and in forthcoming studies (e.g. see Figure 8).

Both the leaf shape and the presence or absence of host-plant extract strongly influenced oviposition behaviour when tested in combination (Figure 6A); the impact of the chemical stimuli on the number of eggs laid was greater (ANOVA; chemical stimuli: $F = 119.1$; df = 1; $P < 0.0001$; leaf shape: $F = 17.7$; df = 1; $P = 0.0002$; chemical stimuli × leaf shape: $F = 5.1$; df = 1; $P = 0.03$). Leaf models with host-plant chemicals did not cause more flies to alight than did control leaves (Figure 6B). Hence the flies distinguished between these treatments only upon contact. More landings were recorded on the host-plant-like model 'three-dimensional hogweed' than on the model 'square with tip down', but the difference was less pronounced than with the leaf pair compared in Figure 4B.

The findings with the simplified leaf shapes were confirmed by an experiment using accurate leaf imitations, which came closer to a natural situation (Figure 7). Again the strong preference for compound leaves became manifest. However, this preference was not restricted to leaf shapes of host plants, since the flies did not differentiate between the leaves derived from umbellifers (hogweed and parsley) and another pinnate leaf originating from the cruciferous non-host *Rorippa islandica*. The relative number of eggs deposited below the five models changed only slightly, but significantly with the background (ANOVA including only the five leaf mimics treated

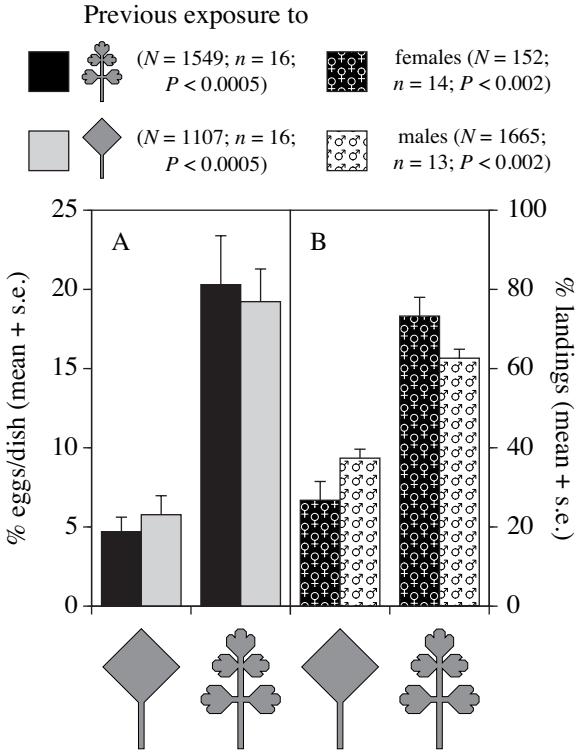

Figure 4. (A) Influence of pre-exposure to a leaf silhouette on subsequent oviposition in a two-choice experiment. The two subsets of flies were exposed to different leaf models ('square with tip down' or 'celery') for one to five days before the start of the two-choice experiment. (B) Relative numbers of landing females and males on the two leaf models. N = Total number of eggs or landings, respectively; n = number of replicates; P = significance with Friedman test (A) and Wilcoxon signed rank test (B).

with extract; leaf model: $F = 161.2$; df = 4; $P < 0.0001$; leaf model × background: $F = 4.8$; df = 5; $P = 0.0008$). Thus, apart from minor differences (e.g. with the *Allium*-mimic), the overall pattern of egg deposition was similar with both settings. This is indirect evidence that females can sense leaf shape after landing, since the low contrast environment should be expected to impair the ability of the flies to visually distinguish the foliar forms. Background colour and the presence of non-host plants (e.g. grasses) were shown to influence the landing frequency of cabbage root flies on host plants (Kostál & Finch, 1994). Nevertheless, it cannot be ruled out that visual discrimination among leaf shapes is still possible for the flies even in a low contrast environment, because there is some remaining contrast due to shadows and variable brightness of the leaf parts exposed at different angles to the light source.

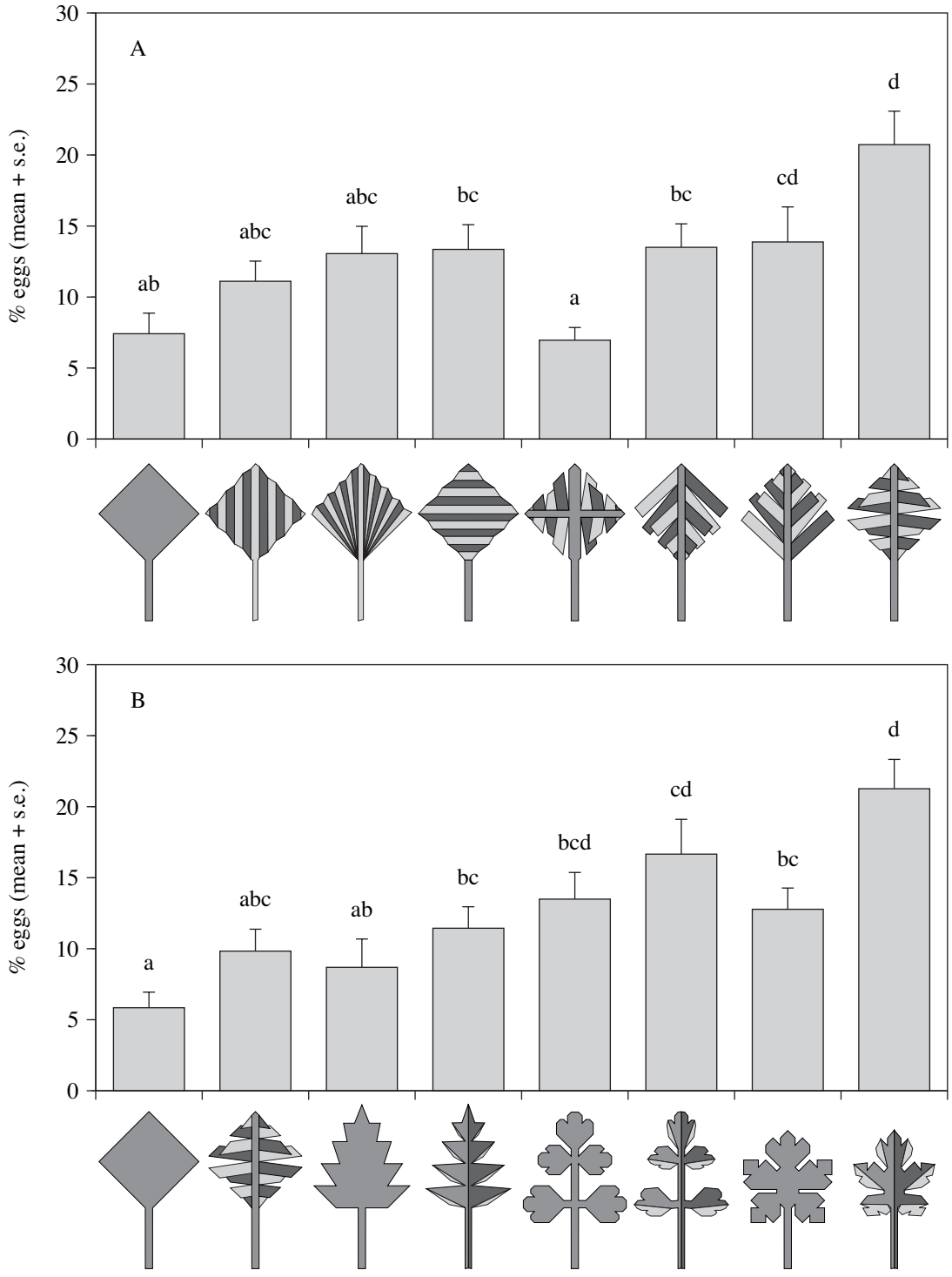

Figure 5. (A) Oviposition around surrogate leaves variable in the presence and orientation of incisions or folds, but with the same projection area (treatment effect: $P = 0.009$; Friedman test). Total number of eggs = 6854; $n = 8$ ($m = 2$, $b = 4$). (B) Oviposition on four different flat surrogate leaves and on the corresponding three-dimensional leaf models with cuts and/or folds (treatment effect: $P = 0.002$; Friedman test). Total number of eggs = 5314; $n = 8$ ($m = 2, b = 4$). Significant differences ($P < 0.05$) are indicated by different letters.

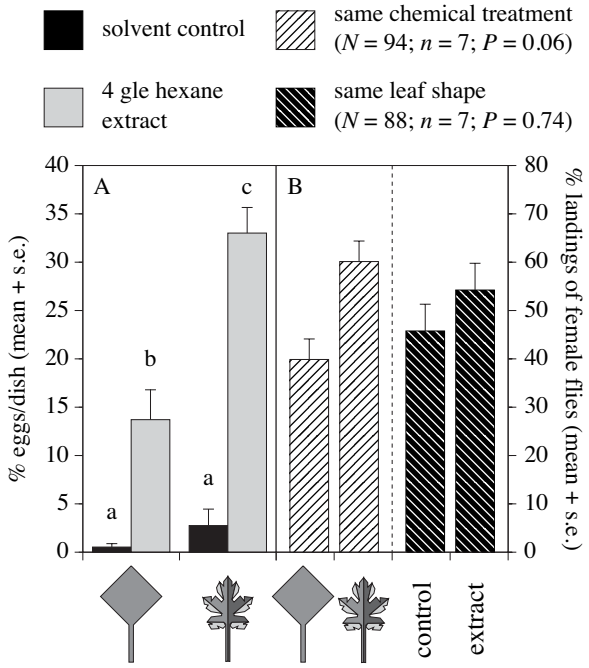

Figure 6. (A) Influence of leaf shape ('square with tip down' versus 'three-dimensional hogweed') and the presence or absence of host-plant extract on oviposition (treatment effect: $P = 0.0005$; Friedman test). Means accompanied by the same letter are not significantly different at the 5%-level. Total number of eggs = 1121; $n = 8$ ($m = 2$; $b = 4$). (B) Relative numbers of landing females on leaf models that differed either in shape (chemical treatment being the same for both models compared: extract or control) or in chemical treatment (leaf shape being the same for both models: 'square with tip down' or 'three-dimensional hogweed'). N = total number of landings; n = number of replicates; P = significance with Wilcoxon signed rank test.

Only single leaves were tested. This situation may be representative for plants producing leaf rosettes (e.g. many biennial umbelliferous plants in the first year). The arrangement of single leaves on a whole plant could, however, potentially provide further information about its identity. On the other hand, many small non-pinnate leaves arranged along the stem axis like leaflets in a compound leaf could be perceived by an insect as a single pinnate leaf.

Leaf size. Leaf size had no detectable influence on oviposition in spite of the eight-fold difference in leaf area between the smallest and the largest leaf model (Friedman test: $P > 0.8$; total number of eggs = 2680; $n = 16$; $m = 2$; $b = 8$). We presume that the greater number of landing flies on the large leaves was balanced by a higher percentage of flies completing the leaf run with subsequent oviposition on the small leaves, i.e. pre- and post-alighting behaviours had opposite effects. An explanation for this unexpected result might be found in our experimental design, which is probably biased towards flies that perform complete leaf and stem runs, whereas individuals stimulated for oviposition flying off the plant and landing near its base are less well represented within our bioassay, since they often miss the oviposition substrate. Under natural conditions, such downward flights could be a normal 'shortcut', occurring especially on bigger plants. There is also some evidence that disturbance by sexually displaying males, which may lead to a disruption of leaf runs, was more marked on large than on small leaves (T. Degen, unpubl.).

Stem length. Oviposition of carrot flies is negatively correlated with the stem length of the surrogate leaves (Figure 8; $r = -0.99$; $n = 4$; $P = 0.005$). This finding is in line with the results presented in the previous paragraph and hence can be interpreted in an analogous way. If we assume that the number of flies landing on a leaf model is independent of its stem length, fewer flies complete a 'regular' leaf run on the models with longer stems. Flies that are only weakly stimulated for oviposition have more opportunity to interrupt a run and fly off when they are on a long rather than on a short stem. A comparable effect was observed in a choice experiment, where the stems were left devoid of stimulants in half of the surrogates. Leaf models ('three-dimensional hogweed') fully treated with host-plant extract (including the stem) received more eggs than models with untreated stems: 16.2 ± 1.9% (s.e.) vs. 8.8 ± 1.1% (Friedman test: $P < 0.025$; total number of eggs = 1452; $n = 24$; $m = 4$; $b = 6$).

General discussion with reference to data from similar studies on other herbivorous flies

Do visual and tactile stimuli help the insects rather to find and distinguish plants from inanimate objects or do they supply host-plant specific information? In Table 2 we summarize the results obtained in this study and compare them with the results of related studies on three other oligophagous fly species with non-overlapping host ranges. Keeping in mind that the comparability of these data is limited because of

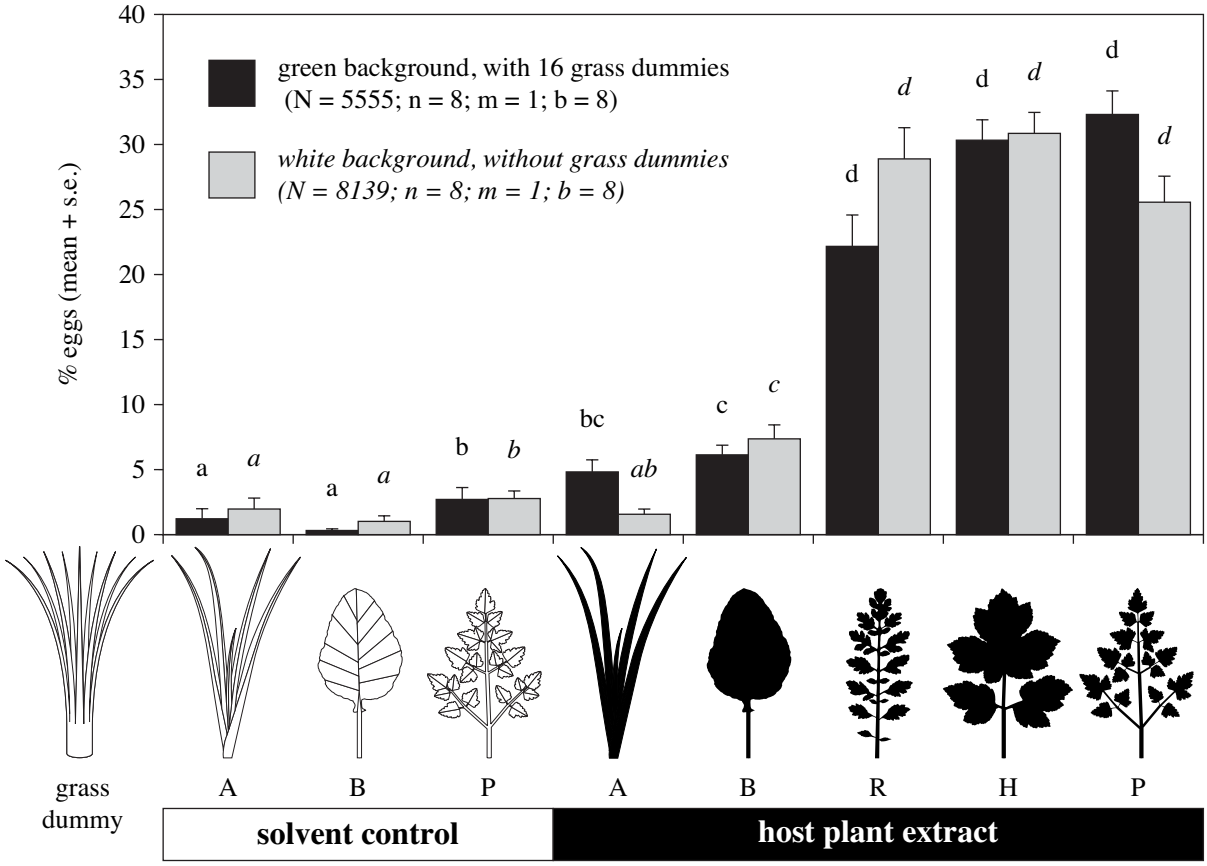

Figure 7. Oviposition around different mimics of natural leaves under a low and a high contrast environment (treatment effect with both backgrounds: $P < 0.0001$; Friedman test). The data on oviposition with the green background have already been presented by Degen & Städler (1996). Leaf areas ranged between 56 cm^2 (*Rorippa*) and 136 cm^2 (*Heracleum*). The multiple comparisons refer only to the leaf models within an experiment, either with a green (regular letters) or with a white (letters in italics) background. A = *Allium porrum* (leek); B = *Brassica oleracea* convar. *botrytis* (cauliflower); R = *Rorippa islandica*; H = *Heracleum sphondylium* (hogweed); P = *Petroselinum crispum* (parsley); N = total number of eggs.

the different experimental approaches, we argue that a very general uniformity of response to a particular non-chemical stimulus (e.g. spectral reflectance) among the four species implies that this cue is of little value to the flies for discrimination among host and non-host plants. Clearly it is important to consider the stage of host-plant selection during which a certain cue might exert an effect (finding plants, host-plant recognition, assessing the suitability of host plants).

Leaf surface cover. Since paraffin coating is not required for strong ovipositional responses in at least one of the examined species (onion fly), a waxy cuticle, as mimicked by a paraffin layer, does not seem to be generally essential for host-plant acceptance in herbivorous flies. Furthermore, for both carrot and cabbage root fly, it has not been unequivocally proven that the paraffin coat is indeed a non-chemical stimulus perceived by mechanoreceptors. The surface properties of the epicuticular wax crystals could vary in a discernible way among plants. The stronger response of the cabbage root fly to paraffin (Roessingh & Städler, 1990) as compared to the carrot fly might reflect differences among the respective host plants (e.g. waxy bloom in some cruciferous plants, yet there are also some umbelliferous plants with very waxy surfaces, e.g. fennel). Other possible tactile cues include hairiness and venation of leaves. Umbellifers differ widely in trichome density, hence it has been judged as improbable that leaf hairs play a significant role in host-plant recognition

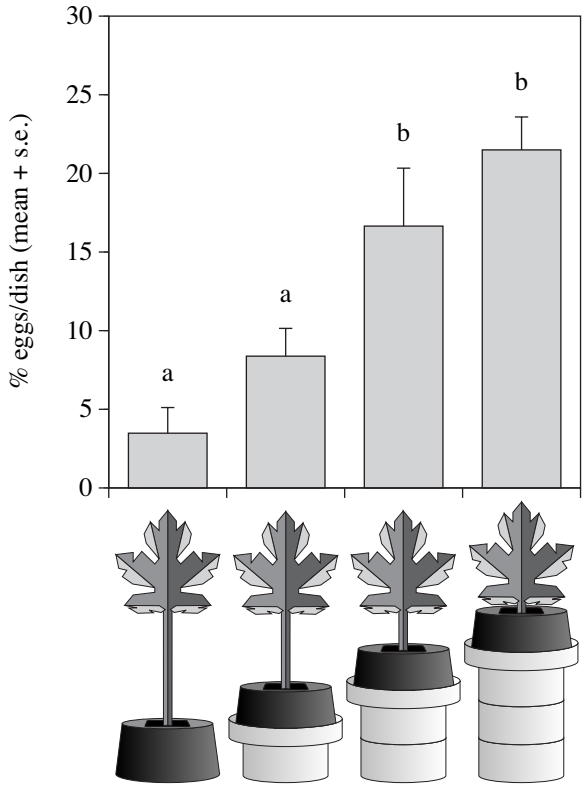

Figure 8. Influence of stem length of surrogate leaves on oviposition (treatment effect: $P < 0.005$; Friedman test). Columns topped by different letters differ significantly at $P < 0.05$. Total number of eggs = 983; $n = 8$ ($m = 2$; $b = 4$).

(Bohlen, 1967). Hessian flies are able to sense the orientation of small grooves in paraffin that were supposed to mimic leaf veins (Harris & Rose, 1990). Thus in this and potentially also in other species, leaf venation might be a cue that allows the flies a rough discrimination between mono- and dicotyledons.

Leaf colour. Carrot flies exhibit a positive response to leaves with a high reflectance in the yellow part of the visual spectrum as do the other three dipterous species. This widespread phenomenon seems to be characteristic of the majority of foliage-seeking insects (Prokopy & Owens, 1983). Thus there can be little doubt that colour vision or wavelength-dependent behaviour is important for location of living plant parts per se, rather than for finding specific plants. Spectral reflectance is probably only used as a cue for distinction among plants under particular circumstances. For example, cabbage root flies were shown to discriminate between green and red cabbage cultivars (Prokopy et al., 1983a). In most cases, however, it is unlikely that foliar spectral quality constitutes a host-plant specific character for insects, because of the high degree of similarity among plant species (Prokopy & Owens, 1983). Only comparative studies could reveal slight differences in colour perception and preference among insect species, that might reflect subtle differences in reflectance of the respective host plants (e.g. variation in reflectance due to waxy bloom or pubescence). Also variation in spectral quality may be more pronounced between different developmental stages within a plant taxon than between taxa. Judd et al. (1988) suggest that female onion flies may potentially select those plants most suitable for offspring survival (young plants vs. mature) on the basis of spectral reflectance.

Leaf shape. Plant dimensions and patterns are far more variable interspecifically than the diffuse spectral quality of foliage (Prokopy & Owens, 1983) and hence can be expected to elicit responses that more strongly diverge among specialized insect species. Indeed carrot flies, cabbage root flies and onion flies differ markedly in their oviposition preferences when given the same choice of host and non-host leaf mimics (Degen & Städler, 1996). This finding implies that the observed discrimination among leaf shapes may indeed have an adaptive significance for host-plant selection. Carrot flies appear to respond to morphological properties typical of their umbelliferous host plants: they were most stimulated for oviposition by pinnate leaves. As such compound leaves are common in many different plant families, it is difficult to assess to what extent leaf shape contributes to the specificity of host choice as compared to other cues. The preference of the grass-feeding Hessian fly for vertical elongate shapes can by interpreted along the same line (Harris et al., 1993).

Leaf size. We were not able to detect a correlation between leaf size and oviposition in carrot flies. As noted above, this could be a cage artefact due to our experimental set-up. There is some evidence from field studies, that the numbers of eggs laid increases with plant size (e.g. Petherbridge & Wright, 1943). In the other dipterous species leaf size almost invariably influenced either pre- or post-alighting behaviour or both.

Leaf stem. Although in none of our test leaves the stem was totally absent, our results indicate that the presence of a stem is less important for ovipositing carrot flies than it is for cabbage root flies. Kostál (1993) suggested that the stem enables the flies to deposit their eggs close to the food source of the larvae. Indeed the cabbage root fly tends to lay the eggs in close proximity to the host plant (Freuler & Goy, 1977), whereas the spatial distribution of the carrot fly eggs is much less restricted (Overbeck, 1978). This may reflect the stronger need of carrot flies to search for humid oviposition sites, because their smaller eggs and larvae are considered to be more susceptible to desiccation. A moist oviposition substrate is an important prerequisite for oviposition bioassays with carrot flies (Bohlen, 1967). In contrast, cabbage root flies readily accept dry sand as oviposition substrate (Zohren, 1968).

Most of the results summarized in Table 2 originate from laboratory experiments. This reflects the difficulties in observing small insects in the field and manipulating different traits of real plants independently of each other. Yet, field studies with more readily observable conspicuous insects suggest that phenomena manifested in the laboratory are potentially also relevant under natural conditions. For example, oligophagous butterflies were shown to develop search images based on visual perception of leaf shapes that result in an increased discovery of host plants (Rausher, 1978). Similar results are reported from two monophagous butterfly species (Mackay & Jones, 1989).

The carrot fly is another example of an insect whose host choice is considerably affected by non-chemical plant features. However, visual, tactile and chemical stimuli need to be tested in combination in order to estimate their relative importance for host selection and reveal possible interactions among the sensory modalities. In many of the laboratory experiments cited in Table 2, the insects were exposed to a wide range of tactile and visual stimuli that they may rarely or never encounter in nature. This shortcoming makes it difficult to assess the ecological relevance of the observed phenomena. When the variation in characteristics of leaf models extensively surpasses what is found in the field, the role of the concerned plant trait in the host choice of the insect is liable to be overestimated. Thus, in our view the naturally occurring variation in plant cues (e.g. shape, colour) should be thoroughly taken into account, when multiple input experiments are designed and conclusions are drawn from the results.

Acknowledgements

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Table 2. Comparison of the ovipositional responses of four phytophagous fly species to a range of physical plant properties. Whenever the information was available, we noted if pre- (b = before) and/or post-alighting (a = after) behaviour was affected (+) or not affected (–) by the given factor (pre- and post-alighting behaviour may act together or have opposite effects with respect to number of eggs). SF = semifield experiment; F = field experiments. All other data originate from laboratory studies

	Carrot fly, *Psila rosae* (F.) (Psilidae)	Cabbage root fly, *Delia radicum* (L.) (Anthomyiidae)	Onion fly, *Delia antiqua* (Meigen) (Anthomyiidae)	Hessian fly, *Mayetiola destructor* (Say) (Cecidomyiidae)
host-plant family; attacked plant organ; oviposition site	– Apiaceae (umbellifers) – roots – soil	– Brassicaceae (crucifers) – roots, rarely stems and sprouts – soil	– Liliaceae (genus *Allium*) – roots, bulbs, (microorganisms?) – soil	– Poaceae (grasses) – leaves – leaves
leaf surface cover	– Paraffin preferred to other surface coatings: Araldite, polyvinylpyrrolidone, paper without cover (this study)	– Paraffin strongly preferred to other surface coatings: Araldite, polyvinylpolypyrrolidone, paper without cover (Roessingh & Städler, 1990)	– Paraffin is not itself important (A. Peters & J. Miller, unpubl.)	– Paraffin used in one experiment, but not compared to other surface covers (Harris & Rose, 1990)
leaf colour	– Yellow, orange, green preferred to other colours (this study) – Yellow preferred to white (Städler, 1977)	– Bright green, yellow preferred to red, blue; little discrimination among shades of green (Roessingh & Städler, 1990) – Differential alighting based on colour of host-plant leaves and leaf mimics (Prokopy et al., 1983a). SF; b+	– Yellow, green preferred to other colours (Harris & Miller, 1983). b+; a+	– Yellow, green, orange preferred to red, blue (Harris & Rose, 1990; Harris et al., 1993). b+; a–
leaf shape	– Compound leaves preferred to surrogates and leaf mimics with other leaf shapes (this study; Degen & Städler, 1996). b+; (a+) – Dissected filter paper leaves preferred to undissected (Städler, 1977) – No difference in landings on pinnate vs. non-pinnate leaf silhouettes (Bohlen, 1967). b–	– No discrimination among leaf mimics based on foliar form (Degen & Städler, 1996) – No discrimination among surrogates with different simple geometric shapes (Roessingh & Städler, 1990) – Number of landing flies not influenced by foliar form of multi-leaved plant mimics (Prokopy et al., 1983b). SF; b–	– Host leaf mimics not generally preferred to non-host leaf mimics (Degen & Städler, 1996) – Narrow vertical cylinders preferred to other three-dimensional objects (Harris & Miller, 1984). (b–); a+ – More females alight on cylindrical than on spherical traps against a background of bare soil, the opposite is true against a background of real onions (Judd & Borden, 1991). F;	– More landings on vertically oriented long and narrow rectangular targets than on horizontally oriented rectangular targets and squares (Harris et al., 1993). b+

Table 2. Continued

three-dimensional properties, folds, leaf vein mimics	– Models with folds receive more eggs than flat models (this study)	– Models with vertical folds receive more eggs than models with horizontal folds (Roessingh & Städler, 1990). a+		– 'Microgrooves' parallel to the long axis of wax-covered filter paper strips increase egg-laying (Harris & Rose, 1990)
leaf or plant size	– No or only weak influence detected with both surrogate and natural leaves (this study; T. Degen, unpubl.) – oviposition increases with the size of the carrot foliage (Petherbridge & Wright, 1943). F – root attack correlated with plant size (Ellis et al., 1978). F	– Bigger leaf model receive more eggs (Roessingh & Städler, 1990) – No influence of size of leaf model (Kostál, 1993) – Landings on artificial mimics increase linearly with leaf area (Prokopy et al., 1983b). SF; b+ – Bigger plants receive more landings and eggs (Kostál & Finch, 1994). b+	– Oviposition is optimal on cylinders with diameters of 4–6 mm and increases with cylinder length (asymptotically to about 10 cm) (Harris & Miller, 1984; Harris et al., 1987). b+; a+ – Oviposition increases with size of onion plants (Harris et al., 1987)	– Landing frequency increases with target area (Harris et al., 1993). b+
leaf stem	– Oviposition decreases with stem length (this study)	– The presence of a stem enhances oviposition (Roessingh & Städler, 1990; Kostál, 1993)	– Preferred surrogates were stem-like (see above)	

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Influence of natural leaf shapes on oviposition in three phytophagous flies: a comparative study

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Introduction

Host finding and acceptance in herbivorous insects involves multimodal perception of chemical and physical properties characteristic of the host plants. Simplified artificial leaves have been used to demonstrate the influence of leaf shape on oviposition of phytophagous flies (e.g. Harris & Miller, 1984; Roessingh & Städler, 1990). However, to our knowledge only one attempt has been made to expose the flies to precise copies of host and non-host leaves. Prokopy *et al*. (1983) recorded landings of cabbage root flies on imitations of real plants and concluded that leaf shape is insignificant compared to leaf area and colour. Yet, these authors did not assess oviposition, thereby excluding possible post-alighting effects. We investigated whether the previously detected preferences for particular simple geometrical shapes (e.g. for narrow vertical cylinders in the case of the onion fly) are analogously manifested in a more complex context that comes closer to a natural situation. Therefore, we compared the egg-laying of three oligophagous fly species, the onion fly, *Delia antiqua* (Meigen) (Anthomyiidae), the cabbage root fly, *Delia radicum* (L.) (Anthomyiidae) and the carrot fly, *Psila rosae* (F.) (Psilidae), around five different models with leaf shapes copied from nature. These included examples from the host-plant families of the three insect species (Liliaceae, Brassicaceae, Apiaceae).

Materials and methods

Paper leaf models. Either one or two plant species with different foliar forms were chosen as representatives of the host-plant families: *Allium porrum* [Liliaceae], *Brassica oleracea* (convar. *botrytis*) and *Rorippa islandica* [Brassicaceae (Cruciferae)], *Heracleum sphondylium* and *Petroselinum crispum* [Apiaceae (Umbelliferae)]. We used scanned images (flat bed scanner) of real leaves as templates for the models, which were cut from green cardboard. The stems were strengthened with wire, which was covered with green adhesive tape. Folds mimicked leaf veins and added a third-dimension to the models. We provided a cuticle-like 'waxy' coat by dipping the leaf models into melted paraffin (Merck, melting point 42–44 °C). To supply chemical oviposition stimuli, host-plant extracts were sprayed onto the leaves at a concentration of 4 g leaf equivalents per 100 cm^2. For the onion fly and the cabbage root fly, we used methanol leaf surface extracts (Städler & Roessingh, 1991) of leek *Allium porrum* cv. 'Zefa Plus' and of kale *Brassica oleracea acephala* cv. 'Fribor', respectively. For the carrot fly, an extract was prepared by heating carrot leaves *Daucus carota sativus* cv. 'Tip-Top' submerged in hexane for one minute (to about 50 °C) in a microwave oven (T. Degen & G. Poppy, unpubl.). In the case of the onion fly, the leaves were further sprayed once with *n*-dipropyl disulphide at a concentration of 10 mg per 100 cm^2 (Harris *et al*., 1987) and 10 g of chopped onion was placed below the sand of the oviposition devices (Harris & Miller, 1984). The same models were used for all eight replicates and the chemical treatments were renewed after four experimental

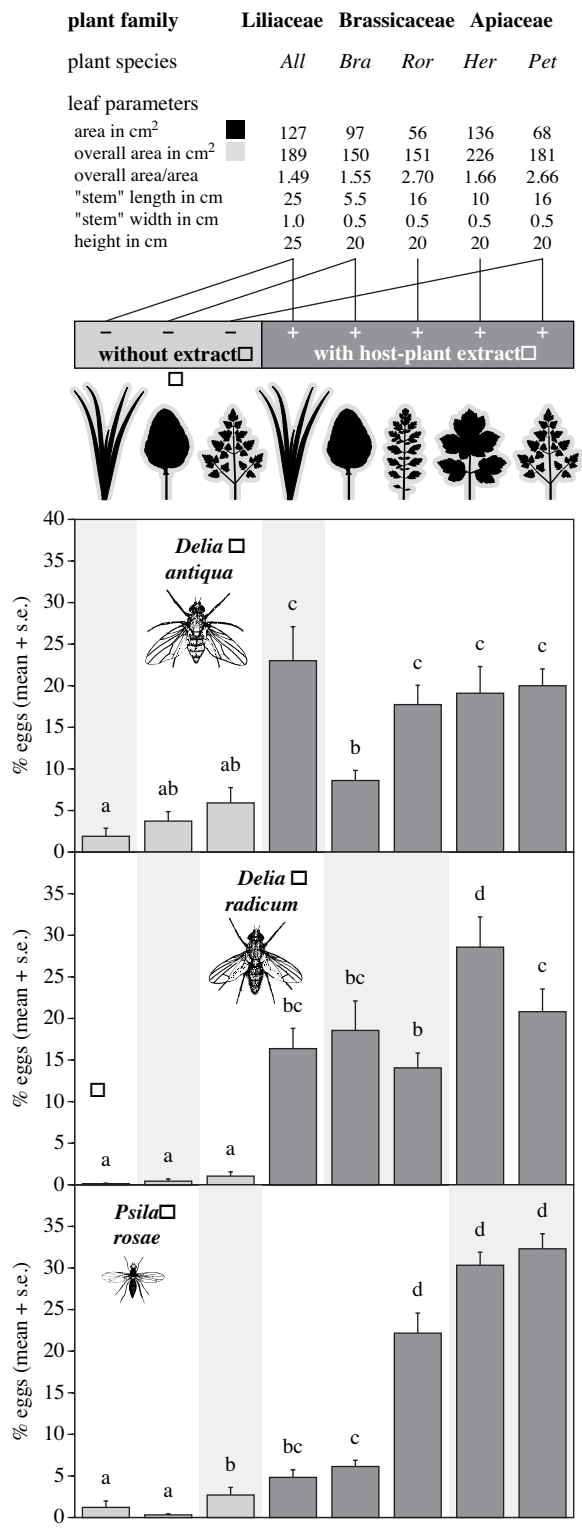

Figure 1. Influence of leaf shape and host-plant chemicals on oviposition in three fly species. Differences among the eight treatments are significant at $P<0.0001$ in all three cases (Friedman test). Significant differences at the 5%-level between individual treatments are indicated by different letters. Shaded areas refer to the respective host plants. *All* = *Allium porrum*; *Bra* = *Brassica oleracea*; *Ror* = *Rorippa islandica*; *Her* = *Heracleum sphondylium*; *Pet* = *Petroselinum crispum*. 'Stem' length = total length of the main stem or of the longest *Allium* leaf blade; area = actual area of the flat leaf model; overall area = area produced by arbitrarily adding a 1 cm wide strip to the leaf edge. The overall area/area ratio is a tentative measure for the degree to which the leaf is pinnate. Number of replicates = 8; total number of eggs: 2056 (*Delia antiqua*), 6105 (*Delia radicum*), 5555 (*Psila rosae*).

periods. Three leaves (*Allium*, *Brassica*, *Petroselinum*), one per plant family, were only sprayed with solvent (Figure 1).

Insects. The flies for the bioassays were obtained from continuous laboratory cultures (≥ nine generations removed from field populations). The freshly emerged flies (about 500 per cage) had access to the model leaves, but no experience with real host plants before the start of the experiment.

Bioassays. The choice experiments were performed in cubic cages (0.34 m^3) in a climate controlled room (21 °C ± 1 °C, 70–80% r.h., L16:D8). The leaf models were fixed with rubber bands to the oviposition devices. These consisted of plastic dishes (diameter 9 cm, height 4 cm) covered with a moist black cloth and a black polyethylene grid in the case of the carrot fly. For the onion fly the dishes were filled with moist sand, and for the cabbage root fly with dry sand. They were topped with a green cardboard cover, allowing the flies access to the oviposition substrate through an opening about 5 cm in diameter. The eight oviposition devices were arranged in a circle around an apple seedling (resting place). The 'adaxial' side of the leaf models faced the centre of the cage. To avoid any position effects, the treatments were randomly assigned to the eight positions and changed in such a way that each of them was located once at each position (= 8 replicates). We covered the cage floor and the walls up to a height of 25 cm with green cardboard and regularly interspersed 16 grass dummies made of green paper between the test leaves, to achieve an experimental set-up that resembled a natural plant assemblage. Depending on the egg-laying rates, the leaf models were exposed to the flies for one to two days (carrot

Table 1. ANOVA with leaf shape, chemicals (host plant extract or solvent control) and fly species as independent variables and with transformed percentage of eggs as dependent variable: arcsin √(% eggs/100). Two sets of test leaves are considered separately, since the chemical treatments were not balanced: there were no solvent controls for the leaf types '*Heracleum*' and '*Rorippa*'

	only the three leaf types with treated and untreated models			only the five leaf models treated with extract		
	df	*F*-Value	*P*-Value	df	*F*-Value	*P*-Value
all three fly species						
leaf shape	2	34.3	<0.0001	4	22.3	<0.0001
leaf shape × species	4	11.6	<0.0001	10	8.4	<0.0001
chemicals	1	444.9	<0.0001			
chemicals × leaf shape	2	8.5	0.0003			
chemicals × species	2	7.8	0.0006			
chemicals × leaf shape × species	4	10.3	<0.0001			
Delia antiqua						
leaf shape	2	3.9	0.03	4	4.9	0.003
chemicals	1	67.4	<0.0001			
chemicals × leaf shape	2	5.5	0.01			
Delia radicum						
leaf shape	2	1.5	0.23	4	3.3	0.02
chemicals	1	224.3	<0.0001			
chemicals × leaf shape	2	0.0	0.99			
Psila rosae						
leaf shape	2	74.9	<0.0001	4	72.6	<0.0001
chemicals	1	215.7	<0.0001			
chemicals × leaf shape	2	32.3	<0.0001			

fly), two days (cabbage root fly) and three days (onion fly) on average, before the eggs were counted. Number of eggs was expressed as proportion of total oviposition and percentages were arcsine-transformed for the ANOVA (Table 1) and the correlations.

Results and discussion

None of the fly species showed an exclusive preference for host-plant leaf shapes (Figure 1). An attempt to link the relative number of eggs with the leaf parameters presented in Figure 1 showed that none of the correlations was significant at the 5%-level, but this might be partly due to the small sample size ($n = 5$; only the five models treated with extract were considered). Nevertheless, the strongest correlations (given in parenthesis below) indicated that leaf models with morphological features typical of the respective host plants were favoured by onion flies and carrot flies. The four leaves with long and narrow structures (long leaf blades or stems) elicited more oviposition in the onion fly than the model with the shortest stem, i.e. the cabbage leaf ('stem' length: $r = 0.83$; $P = 0.08$). Carrot flies laid most of the eggs below leaves that were dissected to some degree (overall area/area ratio: $r = 0.66$; $P = 0.23$). Oviposition of the cabbage root fly appeared to be more strongly affected by 'overall' leaf size than by any structural quality of the leaves (overall area: $r = 0.82$; $P = 0.09$).

The three fly species clearly differed in their preference for particular leaf shapes. The presence or absence of host-plant chemicals had a stronger impact on acceptance for oviposition in all three fly

species than did the foliar form (Figure 1, Table 1). In the carrot fly and onion fly, however, the least preferred leaf type treated with extract did not receive significantly more eggs than the most favoured untreated leaf model.

The results presented here accord well with earlier findings. The preference for pinnate leaves in the carrot fly has already been shown with the aid of simple leaf models (Städler, 1977; T. Degen, unpubl.). For the cabbage root fly, leaf pattern plays only a subordinate role, but leaf size has a clear effect (Prokopy *et al*., 1983; Roessingh & Städler, 1990). Narrow vertical cylinders stimulated more egg-laying in the onion fly than a range of other geometrical 'foliar' shapes, and cylinder diameters of 4 and 6 mm proved to be most acceptable (Harris & Miller, 1984). In our study, the onion flies did not lay significantly more eggs around the host leaf than around three of the non-host leaves, perhaps because the *Allium porrum*-imitations were suboptimal in several respects. For example, the 'stem' was too wide at the base, the leaf blades were inclined and, though folded, were flat rather than cylindrical as in the onion *Allium cepa*, the main host plant. Another reason might be that the *Allium*-models looked very similar to the grass dummies thus making them the least apparent of all the tested models.

The discrimination between leaf shapes was less pronounced for *Delia radicum*. This may be explained by the fact that its crucifer hosts have various leaf forms. The other species are associated with host-plant families that are characterized by rather typical, uniform leaf shapes, i.e. *Psila rosae* (Apiaceae – pinnately or ternately compound leaves) and *Delia antiqua* (Liliaceae – long narrow leaves).

By offering a choice of fairly sophisticated imitations of leaves we confirmed that flies use leaf shape as a cue for host plant selection in addition to chemical (e.g. host odour, Guerin & Städler, 1982) or other physical plant properties (e.g. spectral reflectance). The relative importance of the leaf shape for host selection depends on the insect species and might be related to the degree of similarity in leaf morphology among the host-plant species.

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Extracting oviposition stimulants for the carrot fly from host-plant leaves

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Abstract

Extracts of carrot foliage obtained with various extraction methods were compared regarding their effectiveness in stimulating oviposition in the carrot fly. In choice assays, surrogate leaves treated with a hexane surface extract produced with a new microwave-assisted procedure almost reached the acceptability of real host leaves. The high stimulatory activity of this extract was attributable to the raised solvent temperature, since cold hexane extracts were much less stimulatory than warm extracts. The microwave extract elicited about twice as much oviposition as the previously used dichloromethane surface extract and the diethyl ether fraction of an extract that was obtained by a short immersion of the leaves into almost boiling water. The ovipositional responses to crude methanol and hot water extracts were only weak because of the presence of yet unidentified polar deterrent compounds. Total extracts of ground foliage (vacuum distillation and extraction with liquid carbon dioxide) had no net stimulatory effect on oviposition.

Introduction

The carrot fly, *Psila rosae* (F.), is an oligophagous pest insect whose larvae feed on the roots of a wide range of cultivated (e.g. carrot, celeriac, parsley) and wild umbelliferous species (Ellis et al., 1992). The female flies perform exploratory runs over the leaves of potential host plants and – when stimulated – follow down the stem axis to deposit the eggs in the soil near the plant's base (Bohlen, 1967). The first recorded oviposition stimulant for the carrot fly, *trans*-methylisoeugenol, was isolated from steam-distilled carrot leaves (Berüter & Städler, 1971). Later Städler and Buser (1984) identified five additional stimulatory compounds in dichloromethane washings of undamaged carrot foliage. Yet, surrogate leaves treated with high concentrations of these surface extracts elicited much less egg-laying than real carrot leaves (Städler, 1977). Apart from deficiencies in the physical properties of the artificial leaves made of filter paper, this result may also reflect qualitatively or quantitatively inadequate chemical stimuli originating from the extract of the carrot leaves. In line with this, previous investigations failed to demonstrate a correlation between the amounts of the known oviposition stimulants (phenylpropenes, furanocoumarins, polyacetylenes) and antixenosis resistance of different carrot cultivars (Visser & de Ponti, 1983; Guerin & Städler, 1984; Städler et al., 1990). This prompted us to re-evaluate several standard extraction procedures (for a survey see Städler & Roessingh, 1991) together with a new microwave-assisted method (Paré et al., 1991). We directly compared the stimulatory activity of these extracts in oviposition choice assays using surrogate leaves optimised regarding foliar form, color and surface characteristics (Degen & Städler, 1997a). This investigation is a further step towards the identification of all essential chemical stimuli that determine the host-plant preferences of ovipositing carrot flies.

Methods and materials

Extracts. We used foliage from carrot plants grown in a greenhouse at Rothamsted Experimental Station

(UK) for the following three extracts whose activities were compared in a choice assay (Figure 1A):

– *Vacuum distillation*. Carrot foliage was ground up in a mortar in liquid N_2. The resultant ground mixture was extracted in a conical jar with 500 ml of diethyl ether for 24 hours. The dark green crude extract was poured through a filter paper (Whatman No 1) and dried by adding a quantity of $MgSO_4$. The filtered ether solution was rotary evaporated down to about 20 ml. This concentrated extract was distilled under a vacuum of < 0.05 mm Hg for 24 hours in an apparatus similar to that described by Pickett and Griffiths (1980). By differential heating (20 °C/–196 °C) the ether was allowed to reflux onto the regions of the glass where any of the remaining volatiles had condensed. The distillate was then drawn out of the apparatus with an elongated pasteur pipette and placed at a concentration of 20 gram leaf equivalents per ml into an ampoule sealed under N_2.

– *Carbon dioxide*. Carrot leaves were crushed in liquid N_2. The resultant ground mixture was extracted with liquefied CO_2 for 90 minutes at a pressure of 60 bar and a temperature of 11–19 °C. Diethyl ether was then added to the CO_2 vessel to obtain a solvent extract. This solution was dried with anhydrous $MgSO_4$, filtered, concentrated under N_2 and stored in a sealed ampoule.

– *Microwave assisted extraction with hexane*. Entire carrot foliage was placed into extraction vessel, covered with hexane and introduced into a microwave oven that was run for 60 s at a power of 800 W. Hexane does not itself absorb microwave radiation, but it is heated indirectly by the water-containing plant material (Paré et al., 1991). A distillation apparatus was placed on top of the extraction vessel to ensure that no solvent vapour could enter the oven body. The distillation column traverses the ceiling of the oven where an antenna and a choke were used to prevent the microwaves from escaping.

To prepare the other extracts, we used leaves from carrot plants grown outside in seed beds at the Swiss Federal Research Station Wädenswil and foliage from wild hogweed plants collected locally. Cut, but otherwise undamaged leaves were either held by the stems and dipped into beakers filled with solvent (water, methanol, dichloromethane) or totally submerged (hexane). The examined extraction methods differed in duration, solvent polarity and temperature (Table 1):

– *Hot water*. The leaves were dipped for about 2 s into a beaker filled with almost boiling distilled water (Zobel & Brown, 1988). A part of this crude extract was separated into a water and a diethyl ether fraction (by extracting it three times with diethyl ether in separation funnels).

– *Methanol*. The leaves were successively immersed for 10 s respectively 30 s each into two beakers containing methanol. Between the dippings they were kept in the air for 5 s. An aliquot of the 2 × 30 s crude extract was evaporated to a smaller volume, taken up with water and extracted five times with diethyl ether to obtain a water and a diethyl ether fraction.

– *Dichloromethane*. The leaves were washed twice for 30 s with CH_2Cl_2 (Städler & Buser, 1984). During the time between dippings (5 s), evaporation of the solvent considerably cooled down the leaves and consequently the solvent in the second beaker.

– *Hexane in microwave oven*. Microwave-assisted extractions were carried out in a similar manner as described above. Carrot foliage was covered with hexane in a 1000-ml beaker topped with a Petri dish to prevent the escape of hexane vapours. Half a minute after first contact with the solvent the beaker was introduced into an unmodified household microwave oven that was set up in a fume hood and run for 30 s or 60 s at a power of 700 W or 900 W. Thereafter the solvent was immediately poured off. The leaves were in contact with the solvent for total of approximately 90 s and 120 s, respectively. The temperature reached 47–53 °C (with extractions lasting for 60 s) just after the beaker had been taken out of the microwave oven.

– *Hot and cold hexane*. For comparison with the microwave technique, two additional extracts were prepared by covering the leaves in a beaker with cold (22 °C) and heated (50–56 °C) hexane respectively for 120 s in the fume hood.

Table 1. List of leaf extracts tested in oviposition experiments and some extraction parameters

Abbreviations: vac = vacuum distillation; CO_2 = carbon dioxide extraction; dip = leaves dipped into solvent; imm = leaves entirely immersed in solvent; imm mw = leaves entirely immersed in solvent and heated in a microwave oven; D = *Daucus carota sativus*; DT = *Daucus carota sativus* cv. "Tip-Top"; DD = *Daucus carota sativus* cv. "Danvers"; H = *Heracleum sphondylium*

method	plant species	weight (g)	solvent	volume (ml)	extraction time	temperature in °C	fractions	results of experiment in Figure / Table
vac	D	170	ether	500	24 h	–196/20		1A
CO_2	D	170	ether	10	90 min	< 20		1A
imm mw[1]	D	100	hexane	500	60 s	(>> ambient)		1A
dip	DT[4]	100	water	500	~ 2 s	~ 96	water/ether	1B, 5B / 2
dip	DT[4]	100	methanol	2 × 300	2 × 10 s	(ambient)		1B / 2
dip	DT[4]	100	methanol	2 × 300	2 × 30 s	(ambient)	water/ether	1B, 5A / 2
dip	DT[4]	200	CH_2Cl_2	2 × 600	2 × 30 s	(< ambient)		1B / 2
imm mw[2]	DT[4]	100	hexane	600	30 (~ 90) s	(> ambient)		5A / 2
imm mw[2]	DT[4]	100	hexane	600	60 (~ 120) s	(>> ambient)		1B / 2
imm mw[3]	DD	100	hexane	600	60 (~ 120) s	max. 52		4
imm mw[3]	H	100	hexane	600	60 (~ 120) s	max. 47		4
imm mw[3]	DT[5]	100	hexane	600	60 (~ 120) s	max. 53		2, 3
imm	DT[5]	50	hexane	300	120 s	50–56		3
imm	DT[5]	50	hexane	300	120 s	~ 22		3

[1] microwave oven: Panasonic NN6452B run at 800 W
[2] microwave oven: Bauknecht MCCD 1820 Duo run at 900 W
[3] microwave oven: Panasonic NN-6807 run at 700 W
[4] sown 19.5.93, extracts prepared 63–80 days later
[5] sown 2.6.94, extracts prepared 115 days later

The dichloromethane and hexane solutions were dried with anhydrous Na_2SO_4 and filtered (Schleicher & Schuell No. 595 1/2). The crude extracts and the fractions were concentrated down to 20 ml or 40 ml (1.25–10 gle/ml) in a rotary evaporator at reduced pressure.

Volumes of 1.6 ml of the test solutions were applied with a glass chromatographic sprayer to surrogate leaves ("hogweed" shape; Figure 4) made of green paper and coated with a thin layer of paraffin (Merck, melting point 42–44 °C) (Degen & Städler, 1997a). If not stated otherwise, 4 gram leaf equivalents (gle) of the extracts were sprayed onto each paper leaf model, which had an area of 100 cm^2. Hence the concentration was only slightly above the average weight/area ratio of about 3.2 g/100 cm^2 for leaves of *Daucus carota sativus* cv. "Tip-Top". For the oviposition bioassay whose results are presented in Figure 1A we used plastic parsley leaves provided with a coat of paraffin wax.

Insects and oviposition choice assays. The insects for the experiments were obtained from a laboratory culture reared on carrots (Städler, 1971) for ≤ 13 generations after collection of pupae in the field at Wädenswil. The adult flies were fed a standard artificial diet containing sugar and yeast hydrolysate and were offered separately water and a 10% sugar solution. They were kept in a climate controlled room at 21 ± 1 °C and 80 ± 5% relative humidity. The oviposition experiments were performed in cubic screen cages (0.34 m^3), in which the flies continuously emerged from pupae, with the same illumination and photoperiod as chosen for previous assays (Degen & Städler, 1997a).

The oviposition devices consisted of plastic dishes (diameter 9 cm, height 4 cm) with a wetted foam rubber lining covered with a black cloth and a black polyethylene grid (Städler, 1971). Two rubber bands fixed the surrogate leaves at a metal wire, which perpendicularly protruded from the stem base, to the dishes. The real leaves were inserted through an opening in the centre of the grid into a small plastic vial filled with water. An inverted black plastic pot with a 5 × 5 cm wide opening was placed over the dishes to reduce "stray" oviposition by females

stimulated on another than the accompanying leaf (Degen & Städler, 1997b).

Eight oviposition dishes were spaced on the cage floor, midpoints about 20 cm distant from each other, around an apple seedling in the centre of the cage (resting and copulation site). Depending on the number of treatments (two, four or eight), the leaf models were assigned to the positions in a regular alternate sequence or in a random manner. An experimental period lasted usually one day, rarely up to three days. Positions were changed for each experimental unit in such a way that each treatment was located once at each position. Between the bioassays with surrogate leaves, the flies were regularly exposed to carrot leaves, to avoid non-discriminating behaviour due to a build-up of eggs. The population in the cages usually consisted of 100–400 individuals (males and females).

Statistics. The results of the egg counts were expressed as percentage per dish of total oviposition during an experimental period. We used the Friedman test and the Friedman test modified for repetitions of each treatment within a block (= one experimental period in a cage) to compare the results of the egg counts. Multiple comparisons were made following the method ("least significant rank sum difference") given by Conover (1980). The number of replicates (n) is given by the number of leaf models with identical treatment (m) multiplied by the number of experimental periods (b) during which they were tested (N = total number of eggs).

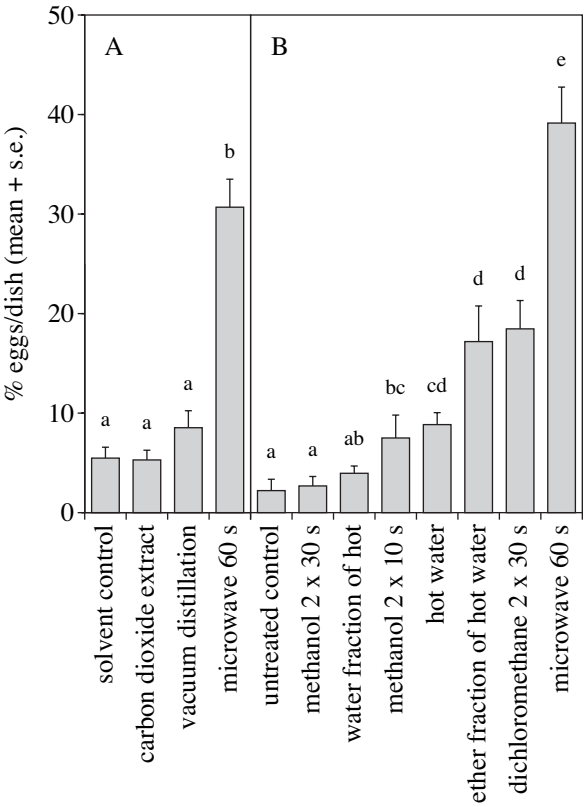

Figure 1. Oviposition stimulating activity of different extracts of carrot leaves as measured in two multiple choice experiments (A and B). Friedman test for treatment effect: A: $P < 0.0005$; $m = 2$; $b = 6$; $n = 12$; $N = 3747$; B: $P < 0.0001$; $m = 2$, $b = 4$; $n = 8$; $N = 3273$. The leaf models were sprayed each with 5 gram (A) or 4 gram (B) leaf equivalents of the respective extracts. Columns topped by different letters differ significantly at $P < 0.05$.

Results

Microwave extracts with hexane as solvent elicited the strongest ovipositional response in both multiple choice experiments (Figure 1A and B). The vacuum distillate and the CO_2-extract did not stimulate more egg-laying than the solvent control. The leaf models treated with the ether fraction of the water extract or with the previously used dichloromethane washes received only half as many eggs as the models treated with microwave extracts. The stimulatory activity of the extracts tended to decline with increasing polarity of the solvent.

When we tested the extracts separately against the solvent control in dual or triple choice experiments, we obtained essentially the same results as with the multiple comparison (Table 2). The extract/control ratios for oviposition recorded with both experimental approaches – dual/triple choice and multiple choice – were highly correlated ($r = 0.98$; $P = 0.003$; $n = 5$). The only major discrepancy was observed with the hot water extract: no net stimulatory effect could be detected in the dual choice experiment as opposed to the findings of the multiple choice experiment. Stronger ovipositional responses were attained when the extraction process in the microwave oven lasted 60 s instead of only 30 s. In this case the effect is probably due both to the duration of the extraction and the higher temperatures achieved as a consequence of the prolonged extraction time. Extraction times could be prolonged further with a modified microwave oven allowing a reflux of the boiling hexane.

Table 2. Oviposition by carrot flies below surrogate leaves treated with extract and solvent. results of dual or triple choice assays

extract	% eggs/dish (mean ± s.e.)	*P* (Friedman test)	surrogates(*m*)	experimental periods (*b*)	total replicates (*n*)	total eggs (*N*)
solvent control	11.9 ± 1.3	NS	4	4	16	1156
water	13.1 ± 1.2		4	4	16	
solvent control	9.0 ± 1.0 a	< 0.05	4	8	32	2308
methanol 2 × 10 s	19.4 ± 2.8 b		2	8	16	
methanol 2 × 30 s	12.6 ± 2.1 a		2	8	16	
solvent control	3.7 ± 1.7	< 0.005	4	2	8	730
dichloromethane	21.3 ± 3.4		4	2	8	
solvent control	1.9 ± 0.7 a	< 0.0005	4	4	16	1138
microwave 30 (~ 90) s	16.5 ± 3.0 b		2	4	8	
microwave 60 (~ 120) s	29.7 ± 3.6 c		2	4	8	

Different letters indicate significant differences among the treatments at $P < 0.05$.

Carrot flies always laid significantly more eggs around leaf models treated with microwave extract than around control leaves, even when the concentration of the extract was as low as 0.125 gle (Figure 2). For comparison, real carrot leaves of equal size weigh about 3 g. The fact that the 0.125 gle treatment elicited about four times more egg-laying than the solvent control suggests that measurable behavioural effects might be evoked with even lower concentrations. On the other side, the dose-response curve reaches asymptotically a plateau around 4 gle or – alternatively – has an optimum in this region. The latter possibility cannot be ruled out, because concentrations above 8 gle were not included in the assays.

In the microwave oven, the water-containing plant tissues absorb the radiation and transfer the heat to the surrounding medium. A cold hexane extract proved to be much less stimulating than the microwave extract (Figure 3). However, when already heated hexane was added to the carrot leaves – the extraction parameters (temperature and duration) otherwise being comparable – the resulting extract was as active as the microwave extract (Figure 3). This implies that the solubility of the oviposition stimulants present in the leaf surface is strongly enhanced by increasing the temperature of the solvent, irrespective of the heat source.

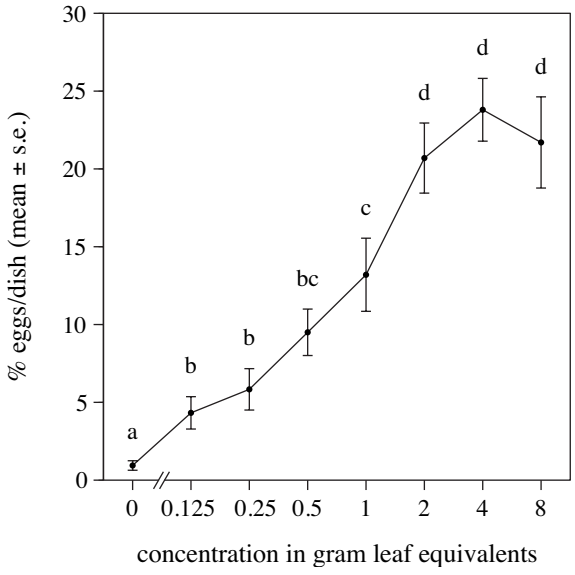

Figure 2. Oviposition of carrot flies around surrogate leaves as influenced by the concentration of microwave extract. Friedman test for treatment effect: $P < 0.0001$; $m = 1$; $b = 8$; $n = 8$; $N = 4980$. Means accompanied by the same letter are not significantly different at the 5%-level.

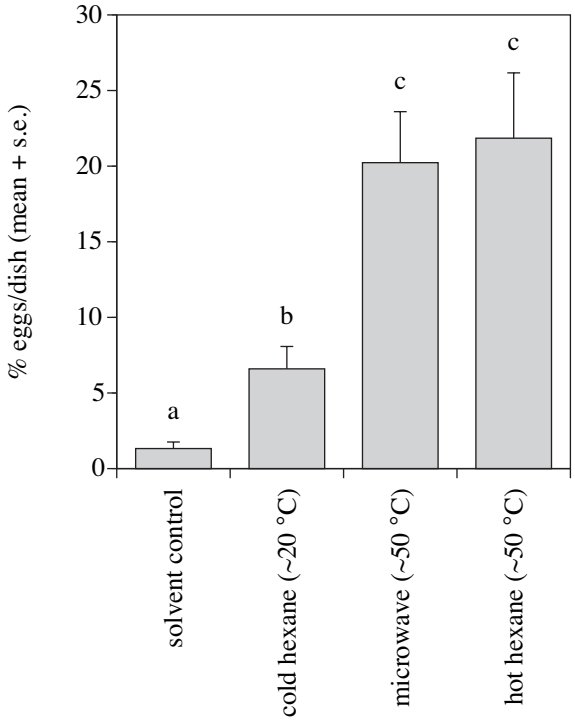

Figure 3. Effect of temperature during extraction on the stimulatory activity of three hexane extracts. Friedman test for treatment effect: $P < 0.0005$; $m = 4$; $b = 2$; $n = 8$; $N = 2465$. Significant differences ($P < 0.05$) among the treatments are indicated by different letters.

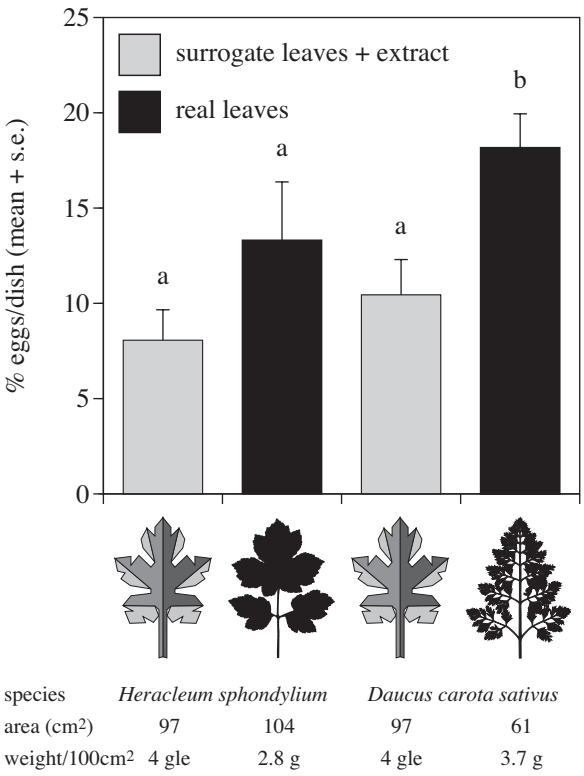

Figure 4. Oviposition around surrogate leaves sprayed with microwave extracts of carrot leaves, *Daucus carota sativus* cv. "Tip-Top", and hogweed leaves, *Heracleum sphondylium*, as compared to oviposition around real leaves of the corresponding plant species (Friedman test for treatment effect: $P < 0.025$; $n = 8$; $N = 2879$). Significant differences ($P < 0.05$) among the treatments are indicated by different letters.

Surrogate leaves sprayed with microwave extracts of carrot and hogweed plants at approximately "natural" concentrations (with respect to gle/leaf area) were only slightly less acceptable to the carrot flies than the corresponding real leaves (Figure 4). The difference between artificial and genuine leaves was only significant in the case of the carrot plant and it was least pronounced on the first day of the experiment. This could be due to the fact that we supplied fresh leaf material for each experimental period, whereas we used the same leaf models throughout the whole experiment lasting four days. Hence evaporation of more volatile compounds or degradation may have led to a loss of stimulatory activity in the surrogate leaves.

The methanolic extract with the shorter extraction time was slightly stimulating oviposition, whereas the one with the longer extraction time did not differ from the solvent control. Assuming that the latter contained at least the same quantity of stimulants as the former, we may conclude that methanol extracted along with stimulants deterrent compounds possibly from further inside the leaf, and the more so, the longer the extraction lasted. In accordance, we were able to separate the "inactive" methanol extract into a stimulatory ether fraction and an inhibitory water fraction (Figure 5A). The stimulatory activity was not extractable from the methanol phase with hexane, which indicates that the active compounds must be of intermediate polarity. An analogous situation was found with the hot water extract whose ether fraction showed stronger stimulatory activity than the crude extract (Figure 1B). The corresponding water fraction had an inhibitory effect as evidenced by the fact that the combined water and ether fraction elicited much less egg-laying than the ether fraction alone (Figure 5B).

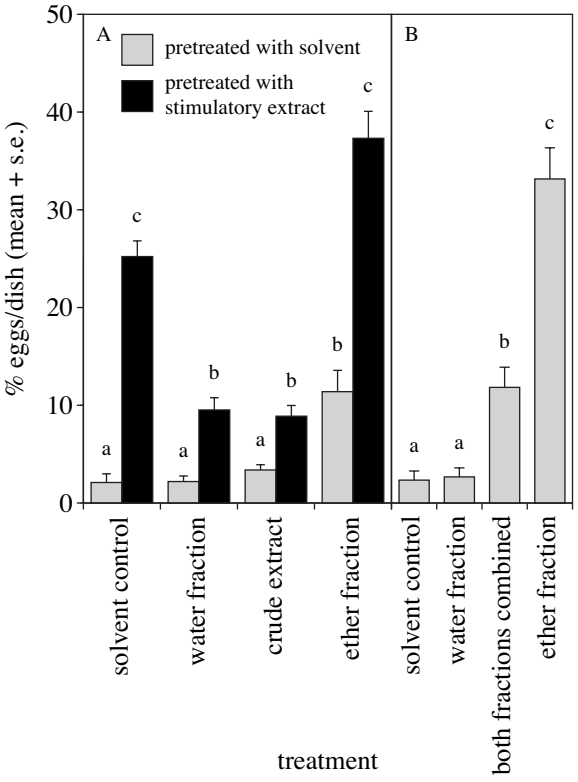

Figure 5. Oviposition deterring activity of polar fractions of A) methanol (2 × 30 s; Friedman test for treatment effect: $P < 0.0001$; $m = 2$; $b = 4$; $n = 8$; $N = 4321$) and of B) hot water extract ($P < 0.0005$; $m = 2$; $b = 4$; $n = 8$; $N = 1735$). In experiment A half of the surrogate leaves were pretreated with a stimulatory microwave extract of carrot leaves. Different letters indicate significant differences among the treatments at $P < 0.05$.

Discussion

Almost all the extracts prepared from undamaged leaves stimulated oviposition to a varying intensity as can be deduced from the review of all the extraction methods tested so far to isolate oviposition stimulants from carrot leaves (Table 3). In contrast, with the exception of the steam distillate (Berüter & Städler, 1971), none of the total extracts with ground leaf material had an overall stimulatory effect, irrespective of the polarity of the solvent. The most plausible explanation for this phenomenon may be a masking effect of inhibitory substances located inside the leaf that are co-extracted with the stimulatory compounds, when the leaves are ground up. Such inhibitory compounds were assumed to be present in a steam distillate of celery foliage (Städler, 1972). Here we can confirm the occurrence of deterrents or – less likely – repellents in another host-plant species.

The microwave-assisted extraction procedure appears to yield optimal results in terms of stimulatory power since the treated leaf models approached the stimulation achieved by real leaves. However, a high overall stimulatory activity is not the only criterion for a suitable extraction method. In studies of host-plant selection, extracts are expected also to reflect differences among the plants in the contents of semiochemicals (e.g. stimulants, deterrents), while the absolute yield of active compounds is of secondary importance in this case. In general, surface extracts may prove to be most suitable for this purpose, since they should be more representative than total extracts of the compounds that are actually perceptible to the insects (Städler & Roessingh, 1991). The stimulatory activity of microwave extracts prepared from various host plants explained a fair amount of the variation observed in the acceptability of these plant species to egg-laying carrot flies (Degen & Städler, in prep.). The results obtained in this study for hogweed and carrot (Figure 4) seem to confirm this finding, though the differences were only significant between the real leaves and not between the microwave extracts.

An ideal extraction method should selectively target active compounds present in the uppermost wax layers whilst avoiding leakage of substances from the leaf interior as much as possible. Clearly some solvents do not meet such a requirement. For example, the exposure of leaves to methanol will inevitably lead to the extraction of polar compounds from inside the cells unless the time of solvent contact is only very short. The brief dipping of leaves into almost boiling water removes substantial amounts of the cuticular wax including compounds that are associated with deeper layers of the cuticle, but tests failed to reveal any significant leakage from inside the leaf due to cell disruption, as the epidermal cells seem to remain alive (Zobel & Brown, 1988). Likewise, Städler and Buser (1984) claimed that dichloromethane washings do not disrupt the epidermal cells and therefore the resulting crude extracts contain little material from the leaf interior. By contrast, the sudden rise in temperature inside the plant material that accompanies the microwave-assisted extraction causes severe damage to the cells (Paré et al., 1991). However, it is questionable, whether

substances of cytoplasmic or vacuolar origin, which are set free, penetrate the cuticle and are taken up by the apolar extraction medium in substantial amounts. The hot hexane extracts without microwave irradiation were similar in appearance (almost uncoloured) and stimulated oviposition as effectively as the microwave extracts, though they most probably were much less detrimental to the leaf cells. Even if these extracts could also comprise some fat-soluble compounds from inside the leaf (e.g. from oil ducts, intercellular space), the bulk of extracted substances may still originate from the leaf surface. In any case, there is no experimental evidence or theoretical reason supporting the assumption frequently made that chloroform or any other apolar solvent does not enter the cuticle during short dippings or rinsings (Riederer & Schneider, 1989). This may cause no serious problems as long as the distribution of secondary metabolites is fairly consistent throughout the apoplast and the cuticle. According to Derridj et al. (1996) the chemical information available to insects on the leaf surface may be very similar to the apoplastic composition.

The yield of soluble cuticular lipids (alkanes, alkanols, acids, esters) varies not only quantitatively, but also qualitatively with extraction methods, e.g. dipping versus rinsing (Stammiti et al., 1996), solvent type, duration of immersion and temperature (Riederer & Schneider, 1989). It seems plausible that this holds also true for secondary compounds embedded in the matrix built up by cuticular lipids. Since we did not analyse our extracts for the known oviposition stimulants, we do not know to which degree variation in stimulatory power among the different extracts is explained by the quantities extracted of these compounds alone. These semiochemicals previously identified in dichloromethane extracts are undoubtedly as well responsible to some extent for the activity of extracts using other solvents (e.g. hexane). There is evidence that host choice of the carrot fly is also affected by more polar inhibitory and additional stimulatory compounds not yet identified. This implies that there is probably no single representative extraction method for all the key compounds determining oviposition preferences in the carrot fly. For a complete picture it may be necessary to make comparative extractions on a range of host plants, since different host species may contain distinct sets of stimulants and deterrents with potentially differing solubility properties (Städler et al., 1990; Degen & Städler, in prep.).

Since oviposition stimulants for different insects differ in polarity and hence solubility, and since even insects that share the same host plants do not necessarily react to the same compounds (Städler, 1994), no recommendation can be made a priori which extraction method should be favoured (Städler & Roessingh, 1991). The present study highlights the need to look at various methods. Furthermore, we want to stress the point here that surface extracts, i.e. extracts prepared from intact plant material, should always be considered in the first place for studying the chemical aspects of insect-plant interactions, when the sampling behaviour of the insect (e.g. before oviposition) does not involve damaging the leaf. Surface extracts are very easy to produce and fewer steps are required for purification and subsequent analysis of the extracts because of the lower number of compounds present. Hot hexane extracts may prove to be suitable in many cases where the essential semiochemicals are of low or intermediate polarity. The microwave technique is also applicable with other, partially absorbing solvents such as methanol or dichloromethane (Paré et al., 1991) and offers a very rapid extraction method for bulk production without the need to heat up large amounts of solvents in water baths.

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Table 3. Stimulatory and deterrent effects of total and surface extracts of carrot leaves on oviposition of the carrot fly

Abbreviations:

effect on oviposition:
– = no activity
± = weak activity
+ = moderate activity
++ = strong activity

stimulants:
pp = phenylpropenes
fc = furanocoumarins
pa = polyacetylenes

References:
a = this study
b = Bohlen (1967)
c = Berüter & Städler (1971)
d = Städler (1972)
e = Städler & Buser (1984)
f = Städler (1986)
g = Städler et al. (1990)
h = Degen & Städler (in prep.)

leaf parts	method	solvent	temperature[3]	fraction	overall stimulatory activity	stimulants	deterrent activity	reference
total	vacuum distillation	ether	–196/20	crude	–			a
(leaves	carbon dioxide	ether	11–19	crude	–			a
ground)	steam distillation		120	petrol ether	+	pp[5]		c
	ground with solvent	ether	35 (bp)	crude	–			d
	ground with solvent	benzene	80 (bp)	crude	–			d
	ground with solvent	pentane	36 (bp)	crude	–			d
	ground with solvent	ethanol	ambient	water	–			d
				ether	–			d
				hexane	–			d
	("leaf juice")	(water)	ambient	crude	–			b
surface	immersion	water with detergent[1]	ambient	crude	±			e, f
(leaves cut,		or complexing agent[2]						
otherwise	dippings	water	~ 96	crude	±			a
undamaged)				water	–		+	a
				ether	+	fc[6]		a
	dippings	methanol	~ 20	crude	±			a, f
				water	–		+	a
				ether	+			a
				hexane	–			a
	dippings	dichloromethane	< ambient	crude	+			a
				ether[4]	+	pp, fc, pa		e, g
	immersion	petrol ether	ambient	crude	+			d
	immersion	hexane	~ 20	crude	+			a
	immersion	hexane	~ 50	crude	++			a
	microwave	hexane	~ 50	crude	++		(–)	a
	(immersion)			ether[4]	++	pp, fc, pa		h
				methanol[4]	+			h
	dippings	melted paraffin	70	crude	+			d

[1] 1% tween 80 or 1% triton X-100
[2] 1‰ β-cyclodextrin
[3] temperature during rotary evaporation may be higher, e.g. for aqueous solutions up to 70 °C
[4] fractionation on silica gel column (Städler et al., 1990)
[5] *trans*-methylisoeugenol
[6] good method for extraction of furanocoumarins (Zobel & Brown, 1988), yet carrot plants usually contain only low concentrations of furanocoumarins (Städler & Buser, 1984; Ceska et al., 1986; Städler et al., 1990) that may not account for the comparatively high stimulatory activity of the ether fraction.

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Oviposition of the carrot fly (*Psila rosae*) in response to foliage and leaf surface extracts of various host-plant species

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Summary

Foliage of twelve host and two non-host species and surrogate leaves treated with the respective leaf extracts were presented to laboratory populations of the carrot fly (*Psila rosae*) in oviposition choice assays. The stimulatory activity of dichloromethane surface extracts and the diethyl ether fraction of hot water extracts did not reflect accurately the differences in acceptability observed among intact leaves. A better correlation was found using hexane extracts prepared in a microwave oven. Two out of five fractions of this crude hexane extract obtained by a silica gel column purification were shown to stimulate oviposition. The diethyl ether fraction, which comprised the previously identified oviposition stimulants (propenylbenzenes, furanocoumarins, polyacetylenes), could only account for a minor part of the variation in the acceptability of host leaves. The preference hierarchy for the intact leaves corresponded better to the ranking of the species according to the activity of the methanolic fraction, which must contain still unknown stimulatory compounds.

The water fractions of the hot water extracts were shown to reduce egg-laying underneath surrogate leaves treated with a stimulatory extract. This oviposition-deterring effect was particularly strong with the non-preferred species *Pimpinella major*, which is also highly resistant in the field. Hence, yet unidentified inhibitory compounds may also contribute to differential acceptability of host plants. It is concluded that antixenotic (non-preference) resistance of host plants to carrot fly attack depends on complex mixtures of semiochemicals.

Introduction

Host-choice in herbivorous insects is often viewed as a process that is based on an integration of manifold positively and negatively interpreted signals originating from the plant (e.g. Huang & Renwick, 1993). The importance of positive host-specific stimuli such as attractants and oviposition stimulants has particularly been stressed in the case of "specialist" insects (Schöni et al., 1987; Städler, 1992). The carrot fly, *Psila rosae* (F.) (Diptera: Psilidae), is an oligophagous insect almost completely confined to members of the family Apiaceae, the umbellifers (Hardman et al., 1990). Both chemical and non-chemical plant traits have been shown to influence host acceptance by ovipositing females (Städler, 1977; Degen & Städler, 1996; Degen & Städler, 1997a). Since only plant secondary compounds seem to be specific enough to explain the restricted host range, major emphasis has been laid on studies in semiochemicals. Investigations into chemical aspects of the host-selection process have focused so far mostly on secondary plant metabolites originating from carrot *Daucus carota* (e.g. Guerin et al., 1983; Guerin & Städler, 1984). Allelochemics in the surface of carrot foliage were shown to synergistically stimulate oviposition (Städler & Buser, 1984). These oviposition stimulants belong to three compound classes (phenylpropenes, furanocoumarins, polyacetylenes) that are in combination characteristic of the Apiaceae (Städler, 1986). However, neither single compounds (methylisoeugenol, asarone) nor the complete set of identified stimulants could account for the differences in antixenotic resistance ("non-preference") among carrot cultivars (Visser & de Ponti, 1983; Guerin & Städler, 1984; Städler et al., 1990). Thus,

some important factors mediating oviposition were missing. We decided to expand the chemical studies to a wider range of host-plant species differing more distinctly in acceptability from each other than do carrot cultivars (Degen & Städler, in prep.). Along with re-evaluating the ecological significance of the known oviposition stimulants, this investigation intended to gather evidence for the occurrence of further behaviourally active compounds not yet identified. To this end, we tested to which degree the stimulatory activity of foliar extracts and of fractions thereof reflects the acceptability of the corresponding intact host-plant foliage. As polar fractions of methanol and hot water extracts prepared from carrot foliage were shown to exert an inhibitory effect on egg-laying (Degen, Poppy & Städler, in prep.), this study was also designed to examine whether oviposition deterrents might contribute to the observed host-plant preferences of the carrot fly.

Material and Methods

Insects. The flies were reared on carrots (Städler, 1971) and maintained in culture for ≤ 16 generations after pupae were collected from an infested field in Wädenswil, Switzerland. Most assays were run with two separate cage populations consisting typically of 100–400 individuals (males and females), which continuously hatched from pupae located inside the experimental cages. Between two successive choice assays with extract-treated surrogate leaves (lasting up to four days), we presented the flies with real host-plant leaves to prevent the potential building up of oviposition drive due to not entirely adequate physical or chemical stimuli arising from the leaf models. For this purpose, the flies were usually allowed access to carrot foliage (e.g. with all dual choice assays) or to all leaf types the extracts of which were subsequently tested (with some multiple choice assays).

Plants. The foliage used for the oviposition assays and for preparing the extracts was obtained either from plants grown in seed beds or from wild plants (Table 1). In rare cases (e.g. *Pimpinella major*) the leaves originated from plants grown in pots outdoors. Most of the seeds for the sowings were supplied by the Genetic Resources Unit of Horticulture Research International Wellesbourne. Some additional seed material (*Anethum graveolens*, *Anthriscus cerefolium*, *Pastinaca sativa sativa*) was acquired from commercial Swiss seed producers (Samen Mauser). The plants used for the multiple choice assays were heavily damaged by hail about two months after sowing, but most of them had recovered and looked healthy by the time the two oviposition assays with the foliage were carried out, i.e. about 90 and 125 days after sowing, respectively. Only the annual plants (*Anethum graveolens*, *Anthriscus cerefolium*) had to be resown and hence were about two months younger than the other test plants included in the same multiple choice assay. The foliage tested in dual choice assays was harvested 52–189 days after sowing, with test plants either having the same age as or being older than standard plants.

The leaves to be compared in a choice assay were cut to the same length: 24 cm (dual choice assays) or 20 cm (multiple choice assays). The surface of each leaf was determined with an area meter LI-3100 (Li-Cor, inc., Lincoln, Nebraska) or calculated from its weight (in the dual choice experiments) by using "weight-area coefficients" obtained from linear regressions (without intercept) as a simple approximation. Normally each oviposition dish was equipped with only one leaf, in rare cases (multiple choice assays: *Anthriscus cerefolium*, *Anethum graveolens*, *Foeniculum vulgare*, *Cichorium intybus*) with two or three to compensate at least partly for pronounced differences in leaf mass respectively area.

Foliar extracts. All the extracts were prepared from leaves that were cut at the petioles, but were otherwise undamaged. The samples were collected from several (≥ 4) individual plants. The extraction methods differed in duration, in solvent polarity and in the temperatures reached during the extraction process.

– *Dichloromethane extracts*. This extraction procedure was previously used for isolating oviposition stimulants from the surface waxes of carrot leaves (Städler & Buser, 1984). The extracts were prepared from five days before to maximally 38 days after the dual choice oviposition experiments with the respective leaves were performed. The total amount of plant material extracted was variable (40–200 g; mostly 100 g), but the solvent volume was adjusted to foliage weight (600 ml/100 g). The leaves were held with the stems and immersed successively for 30 s into two beakers containing

Table 1. Umbelliferous and non-umbelliferous (nu) plants tested in dual or multiple choice assays and origin of the leaves used for the oviposition experiments and for preparing extracts (CH_2Cl_2, hexane, hot H_20). w = wild plants; s = grown in seed beds; p = grown in pots

Plant species	choice assay: solvent:	dual CH_2Cl_2	multiple hexane	multiple hot H_20
Aegopodium podagraria L., ground elder		w	w[2]	w
Anethum graveolens L., dill		s	s[1]	
Anthriscus cerefolium (L.) Hoffm., garden chervil		p	s[1]	s
Apium graveolens var. *rapaceum* (A. W. Hill) cv. "Balder", celeriac			s[2]	
Carum carvi L., caraway		s	s[1]	
Conium maculatum L., hemlock		s	s[2]	s
Daucus carota L. ssp. *sativus* (Hoffm.) Arcangeli cv. "Danvers", standard carrot cultivar		s/p	s[1,2]	s
Daucus carota L. ssp. *sativus* (Hoffm.) Arcangeli cv. "Sytan", carrot			s[2]	
Foeniculum vulgare Miller, fennel			s[1]	
Heracleum sphondylium L., hogweed		w	w[2]	
Pastinaca sativa ssp. *sativa* L. cv. "Halblange", parsnip		s	s[1]	s
Pastinaca sativa ssp. *sylvestris* (Miller) Rouy & Camus, wild parsnip		s		
Petroselinum crispum (Miller) A. W. Hill, parsley		s	s[2]	s
Pimpinella major (L.) Huds., greater burnet saxifrage		s	p[1]	p
Athyrium filix-femina (L.) Roth, lady fern (nu)			w[2]	
Cichorium intybus L., chicory (Catalogna) (nu)			s[1]	

[1] set 1
[2] set 2

dichloromethane (Fluka for HPLC). Between the dips the leaves stayed in the air for about 5 s. Evaporation of the adherent solvent cooled down the foliage and consequently the liquid in the second beaker. The combined solutions were dried by adding Na_2SO_4, filtered (folded paper filter Schleicher & Schuell No. 595 1/2), concentrated (rotavapor: 30 °C, 580 mbar) to 5 gram leaf equivalent (gle) per ml and stored in the deep freezer at –20 °C until they were tested in dual choice assays.

– *Microwave-assisted hexane extracts*. The method has been developed by Paré et al. (1991) and was shown to produce highly stimulatory extracts from carrot leaves (Degen, Poppy & Städler, in prep.). We prepared the extracts 2–4 days after completion of the multiple choice assays conducted with actual leaves of the corresponding plant species. Foliage (100 g) was covered with 600 ml hexane (Fluka for HPLC) in a 1000-ml beaker that was subsequently topped with a Petri dish and placed into a microwave oven (Panasonic NN-6807) 30 s after first contact with the solvent. The leaves were heated for 60 s at a power of 700 W, which caused the temperature of the hexane – itself transparent to microwaves – to rise to 47–53 °C. Afterwards the solvent was immediately poured off into another beaker so that the foliage was immersed in the solvent for a total of about 120 s. The resulting solution was dried with Na_2SO_4, filtered, rotary evaporated (30 °C, 240 mbar) to a concentration of 2.5 gle/ml and stored at –20 °C.

The crude hexane extracts were subjected to a fractionation on a silica gel column (75 × 11 mm, silica gel 70–230 mesh, Merck "Kieselgel 60"). This purification method was adopted from an earlier study, where it was used to analyze dichloromethane surface extracts for six identified oviposition stimulants (Städler et al., 1990). Aliquots of the crude extracts (40 gle) were concentrated to a volume of about 1 ml under a N_2-stream, brought onto the column with a pasteur pipette and eluted successively with 20-ml portions of 100% hexane (fraction 1), 5% diethyl ether (Fluka puriss.; stab. with 0.0005% 2,6-di-tert-butyl-p-cresol) in hexane (fraction 2), twice 100% diethyl ether (fraction 3.1 and 3.2) and 100% methanol (Merck pro analysi; fraction 4).

– *Hot water extracts*. This method was described as highly efficient in removing furanocoumarins

from the leaf surface of rutaceous and umbelliferous plants without any significant leakage of substances from the interior due to cell disruption (Zobel & Brown, 1988; Zobel & Brown, 1990). The extracts were prepared 20–44 days after completion of the multiple choice assays with the respective leaf types. The leaves (50 g per plant species) were held by the stems and dipped for a short time of about 2 s into a beaker filled with 250 ml of distilled water near its boiling point (94–97 °C). After 7–11 minutes, when all portions of foliage had been immersed, the solution was filtered (Schleicher & Schuell No. 595 $^1/2$) and stored in the deep freezer before further processing. The thawed crude extracts were transferred into separation funnels and extracted three times with diethyl ether (total volume 250 ml). The resulting ether fraction (dried with Na_2SO_4) and water fraction were concentrated (rotavapor: 600 mbar, 30 °C and 100 mbar, 60 °C, respectively) to 2.5 gle/ml and stored at –20 °C.

– *Methanol extract*. Leaves (67 g) of *Pimpinella major* were extracted by dipping them successively for 10 s into two beakers each filled with 200 ml methanol (Merck gradient grade). The crude extract was filtered, concentrated (rotavapor: 180 mbar, 40 °C) to 2.5 gle/ml and stored in the deep freezer.

The crude extracts and fractions thereof were applied in volumes of 1.6 ml with a chromatographic sprayer to paraffin-coated paper surrogate leaves (Degen & Städler, 1997a). Unless stated differently, we provided the leaf models, which had a surface area of 100 cm^2 (including the stems), with 4 gle of the test solutions, thereby taking into consideration that real leaves of equal size on average weigh about 4 g (there is some variation in weight per area depending on leaf morphology).

Oviposition choice assays. The oviposition experiments were conducted in screen cages (70 × 70 × 70 cm) with perspex at the front and rear side, which were located in a controlled environment room (21 ± 1 °C; 70–80% r.h.). Light conditions and diurnal photoperiod were the same as chosen for previous oviposition experiments (Degen & Städler, 1997a). The acceptability of real leaves and of test solutions was determined in dual or multiple choice assays. In both cases the susceptible carrot cultivar "Danvers" served as standard plant to which the test plants were compared. Dichloromethane extracts and the corresponding real leaves were tested in dual choice assays. In all the remaining experiments, four or eight different treatments were simultaneously displayed to the flies.

Real leaves and surrogates were attached to the egg-laying devices (Städler 1971) as described by Degen & Städler (1997b). The oviposition dishes were topped by an inverted black plastic pot with a 5 × 5 cm wide opening through which the flies could reach the oviposition site (Degen & Städler, 1997b). Eight dishes were arranged in a circle around a non-host plant (apple seedling) in the centre of the cage, which served as a resting and copulation site. In the dual choice situation the positions of the four repetitions for the standard and test leaves were alternated, in the multiple choice situation the eight different treatments were randomly assigned to the positions. Single experimental periods lasted mostly one day, rarely two days. After the eggs were counted and removed, the dishes were re-introduced into the cages for the next interval. Positions were permuted in such a way, that each treatment was located once at each position, except for the assays comparing the various fractions of the hexane extract (Figure 4; eight treatments, but only four replicates and hence four positions per treatment). Regardless, position effects were if anything weak and thus negligible. In the dual choice assays, the same real leaves were tested during two successive periods on different positions, whereas they were replaced by fresh foliage after each period in the multiple choice experiments. Usually, there were two surrogate leaves treated with the same test solution and exposed four times in the multiple choice assays and four models with the same treatment tested twice in the dual choice assays, resulting in eight replicates per treatment in both cases.

Statistics. The numbers of eggs laid per dish are expressed in the figures as percent of total oviposition per period. They were analyzed for treatment effects by the Friedman test (eight treatments tested together) or by the Friedman test modified for repetitions in a block, i.e. one experimental period in a cage (two or four treatments tested together). Multiple comparisons among treatments were made following a method based on rank sum differences (Conover,

1980). The problem of "pseudo-replication", i.e. the repeated exposure of the treatments to the same cage population on (maximally four) consecutive days, is presumably of minor relevance, since the interval between two successive oviposition bouts of individual carrot flies is on average 3–4 days (Körting, 1940; Bohlen, 1967; Collier & Finch, 1996). To approximate a normal distribution, the percentages of the numbers of eggs deposited were arcsine-transformed and the correlation matrix (product-moment correlation coefficients) given in Table 2 was calculated from the means of these transformed values.

Results

Dichloromethane extracts. Leaves and the corresponding dichloromethane extracts sprayed onto surrogate leaves were tested separately in dual choice oviposition assays against leaves and extracts of the standard carrot cultivar, respectively. Oviposition underneath the intact foliage (mean percent eggs test leaves/mean percent eggs standard leaves) was not significantly correlated to both leaf size (mean area of test leaves/mean area of standard leaves) and leaf morphology as reflected in the number of "first order"-leaflets perpendicular to the leaf axis (mean for test leaves/mean for standard leaves): $r = -0.46$, $P = 0.14$ and $r = 0.31$, $P = 0.34$, respectively ($n = 12$ in both cases). The results obtained with intact foliage and the dichloromethane extracts were not in good accordance ($r = 0.30$; $n = 12$; $P = 0.34$): the strongest deviations from the expected values based on oviposition below real leaves were observed with the extracts of *Pimpinella major*, *Pastinaca sativa* (both ssp. *sativa* and *sylvestris*) and one extract of *Anthriscus cerefolium* (Figure 1). Even when the latter extract was dismissed as a dubious outlier, the correlation did not become significant ($r = 0.56$; $n = 11$; $P = 0.07$). A sample of the extracts involved – seven out of the twelve test-plant and four out of the six standard plant extracts – were tested against solvent controls and all of them, including the least acceptable *Anethum graveolens* extract, elicited significantly more egg-laying than the control (Friedman test: $n = 8$; $P < 0.005$ in each case).

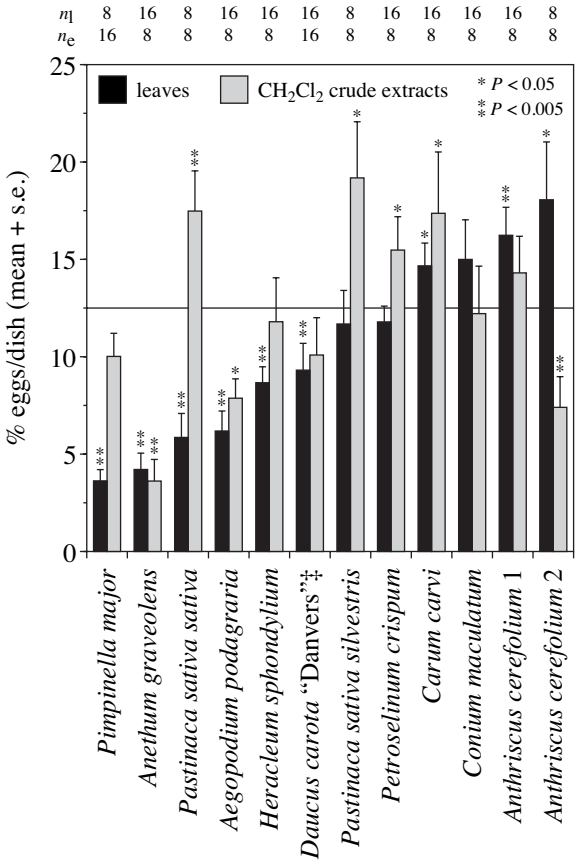

Figure 1. Oviposition of the carrot fly in response to intact leaves and leaf models treated with the corresponding CH_2Cl_2 surface extracts. Test leaves and extracts were always compared in dual choice assays to standard carrot leaves and extracts, respectively (*Daucus carota sativus* cv. "Danvers"). The space between the columns and 25% refers to the complementary percentage of eggs deposited with standard treatments (leaves or extracts). Since four repetitions of test and standard leaves were present during each experimental period (block), mean percentages add up to 25% instead of to 100%. Significant differences between test and standard treatments are indicated by stars on top of the columns (Friedman test for repetitions within a block). n_l = replicates for leaves; n_e = replicates for extracts; ‡ = standard carrot leaves of older plants (~120 days after sowing) tested against standard carrot leaves of younger plants (~60 days after sowing).

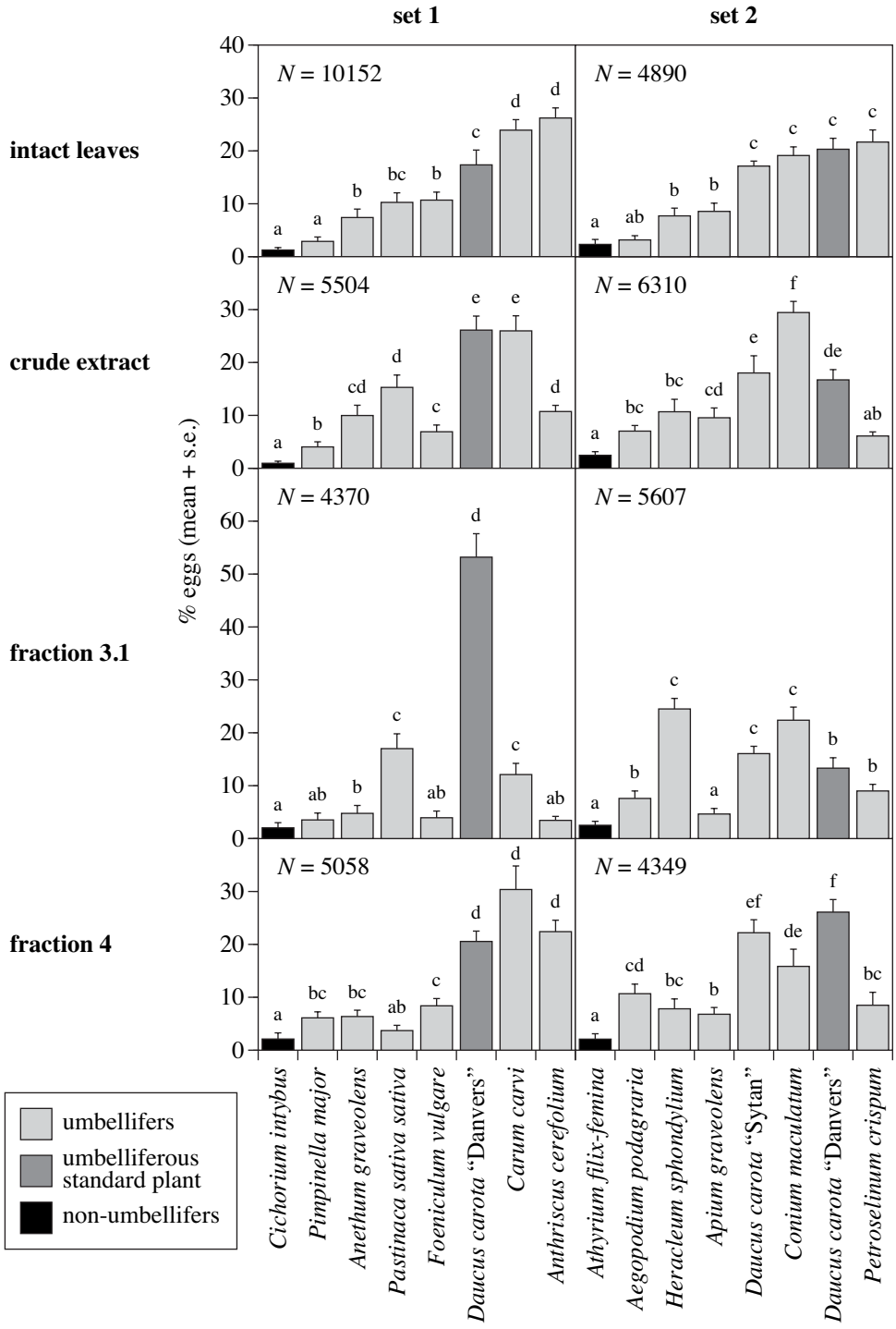

Figure 2. Oviposition of the carrot fly in response to host and non-host foliage and to surrogate leaves treated with the corresponding hexane crude extracts and with the fractions 3.1 and 4 thereof. Two sets of plants each including the standard carrot cultivar "Danvers", a non-umbelliferous and six umbelliferous species were tested in multiple choice assays. The treatment had a significant effect (Friedman test: $P < 0.0001$) in all eight experiments. Means accompanied by the same letter are not significantly different at the 5%-level. Eight replicates per treatment in each experiment. N = total number of eggs. The data obtained with the leaves have already been published (Degen & Städler, in prep.) and are shown for comparison.

Microwave-assisted hexane extracts. The relatively unsatisfactory findings with the dichloromethane extracts prompted us to perform analogous experiments with hexane microwave extracts, which showed a higher stimulatory activity when directly compared to dichloromethane extracts (Degen, Poppy & Städler, in prep.). Leaves and the corresponding crude extracts of two sets of seven test plants including each a non-umbelliferous plant were tested in multiple choice assays together with standard leaves and extracts, respectively (Figure 2). In set 1, ovipositional responses to foliage and to extract-treated leaf models were only moderately, but significantly correlated, in set 2 the correlation was close to significant (Table 2). A major outlier was present in each set: *Anthriscus cerefolium* (set 1) and *Petroselinum crispum* (set 2). As with the dual choice assays, mean oviposition per leaf type was not significantly affected by the mean leaf area ($n = 8$; set 1: $r = -0.42$, $P = 0.32$; set 2: $r = -0.67$, $P = 0.07$) and the mean number of leaflets ($n = 8$; set 1: $r = 0.64$, $P = 0.09$; set 2: $r = -0.10$, $P = 0.82$).

Samples of crude extracts of both sets were tested against a solvent control in an additional multiple choice assay (Figure 3). The two standard carrot extracts were similar in activity and no longer significantly superior in stimulating oviposition over extracts of *Aegopodium podagraria* and *Petroselinum crispum*. Hence the relative differences in activity changed depending on the selection of plants present in the assay. However, the rank order of the species belonging to the same set remained identical. The two non-host extracts as well as the extract of *Pimpinella major* did not elicit significantly more egg-laying than the solvent control.

Four extracts were chosen for comparing the stimulatory activity of the fractions obtained with the silica gel column separation. On the whole, the fractionation procedure did not lead to a notable loss in stimulatory activity as all fractions applied in combination did not significantly differ from the crude extract (Figure 4). Invariably, fraction 1 (hexane) and fraction 2 (5% diethyl ether in hexane) were inactive, whereas fraction 3.1 (diethyl ether) and fraction 4 (methanol) were stimulating oviposition as compared to the solvent control. With the extracts of *Daucus carota* and *Conium maculatum*, fraction 3.1 showed higher activity than fraction 4, with *Carum carvi* the inverse situation was observed. Only with the extract of *Conium maculatum*, leaf models sprayed with

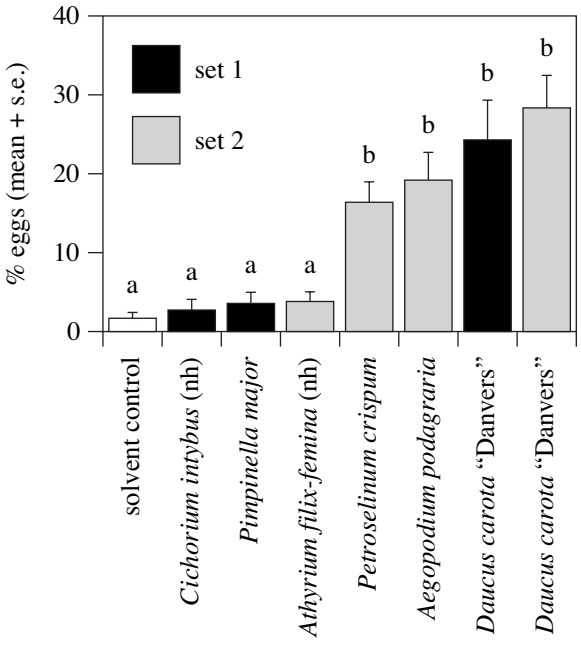

Figure 3. Stimulatory activity of crude hexane extracts sprayed onto surrogate leaves relative to the solvent control. For comparison, examples of both sets of plant species (Figure 2) were included in the multiple choice assay. The treatment effect was significant at $P < 0.0001$ (Friedman test). Columns topped by different letters differ significantly at $P < 0.05$. Eight replicates per treatment; total number of eggs laid = 2049; nh = non-hosts, i.e. non-umbelliferous species.

fraction 3.2 received significantly more eggs than solvent controls. The low activity of fraction 3.2 indicated that the stimulatory effect of fraction 4 was not due to compounds in common with fraction 3.1 that were carried over into fraction 4. With both sets of plant species, the preference hierarchy obtained for fraction 4 accorded fairly well with the ranking for the corresponding real leaves, even slightly better than did the activity of the crude extracts, whereas the activity of fraction 3.1 was not significantly correlated with the acceptabilities of real foliage (Figure 2; Table 2). It is notable that all fractions 3.1 of the test species included in set 1 stimulated less oviposition relative to the standard than did the test fractions 3.1 comprised in set 2. Fraction 3.1 and fraction 4 together accounted for most of the variation in the activity of the crude extracts (multiple regression: set 1: $r^2 = 0.83$, $P = 0.01$; set 2: $r^2 = 0.73$, $P = 0.04$; $n = 8$ in both cases).

Table 2. Correlation matrix for oviposition data (mean arcsine-transformed proportions of eggs per plant species) obtained with leaves, crude extracts, fractions 3.1 and fractions 4 (in parentheses Fisher's *r* to z *P*-value)

set		leaves	crude extract	fraction 3.1
1	crude extract	0.80 (0.02)		
	fraction 3.1	0.40 (0.34)	0.77 (0.02)	
	fraction 4	0.90 (0.001)	0.76 (0.03)	0.41 (0.33)
2	crude extract	0.68 (0.06)		
	fraction 3.1	0.54 (0.17)	0.75 (0.03)	
	fraction 4	0.71 (0.05)	0.76 (0.03)	0.56 (0.15)

The potential occurrence of deterrents in the hexane extracts was examined by adding the crude extracts of the two non-umbellifers and of one of the least acceptable umbellifers, *Pimpinella major*, to a hexane extract of carrot foliage. The stimulatory activity of the latter extract was slightly, but significantly reduced only by the chicory extract, whereas it was strongly increased by the *Pimpinella major* extract (Figure 5). The lady fern extract was neutral in this respect.

Hot water extracts. Three species each of both plant sets were chosen together with the standard plant for the hot water extracts. When prepared from carrot leaves, these extracts were shown earlier to contain stimulatory activity in the diethyl ether fraction as well as deterrent activity in the water fraction (Degen, Poppy & Städler, in prep.). The diethyl ether fractions of all species except for *Pimpinella major* elicited more egg-laying than the solvent control, when sprayed onto surrogate leaves (Figure 6). However, the oviposition data (mean percent eggs with test plant/mean percent eggs with standard plant) obtained with the diethyl ether fractions and with real leaves were not significantly correlated ($r = 0.43$; $n = 7$; $P = 0.34$). Much smaller numbers of eggs than would have been predicted from the acceptabilities of the intact foliage were laid underneath leaf models treated with the fractions of standard *Daucus carota* and – as with the hexane extracts – of *Anthriscus cerefolium*. *Petroselinum crispum* leaves yielded a highly acceptable diethyl ether fraction, in contrast to the situation observed with the hexane extracts.

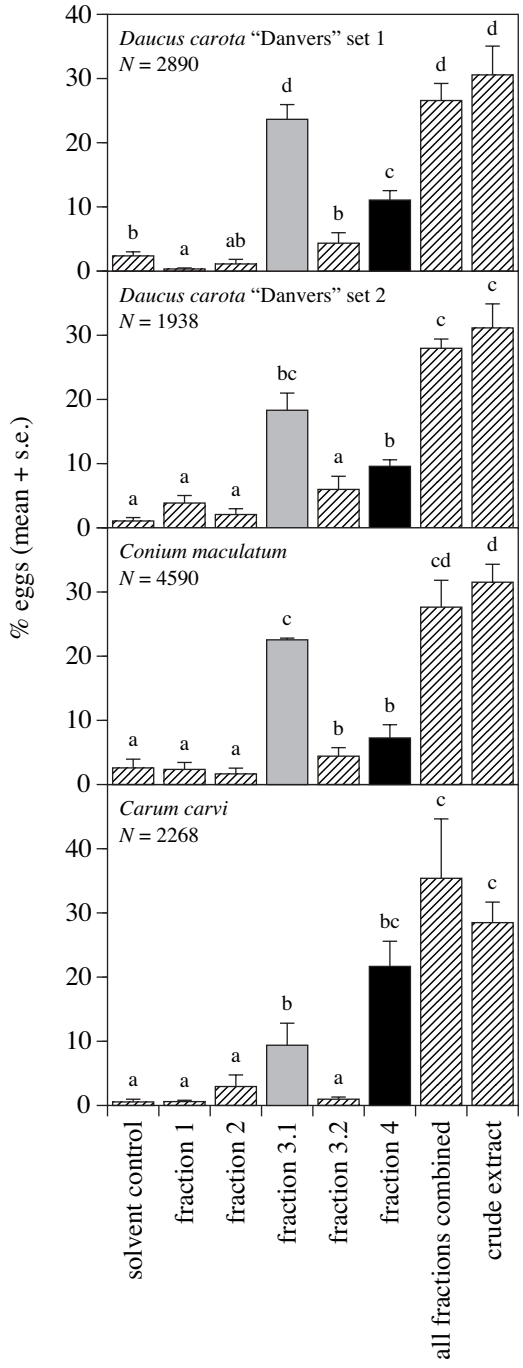

Figure 4. Stimulatory activity of the silica gel fractions of the hexane extracts as determined by multiple choice oviposition assays. Two species from each plant set (see Figure 2) are represented. The treatment effect was significant in all four cases (Friedman test: $P < 0.001$). Significant differences ($P < 0.05$) among particular treatments are indicated by different letters. Four replicates for each experiment; *N* = total number of eggs.

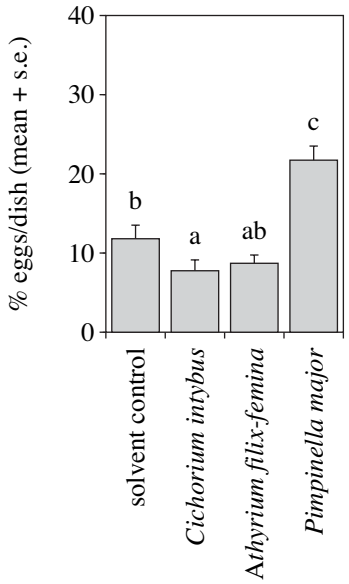

Figure 5. Test for an inhibitory effect of three crude hexane extracts, which had no or only low stimulating activity, in a multiple choice oviposition assay. The extracts were sprayed at a concentration of 1.75 gle onto surrogate leaves pre-treated with a stimulatory hexane microwave extract (1.75 gle) prepared from carrot foliage (*Daucus carota sativus* cv. "Tip-Top"). The treatment effect was significant at $P < 0.001$ (Friedman test for repetitions within a block). Means accompanied by the same letter are not significantly different at the 5%-level. Eight replicates per treatment; total number of eggs laid = 3104.

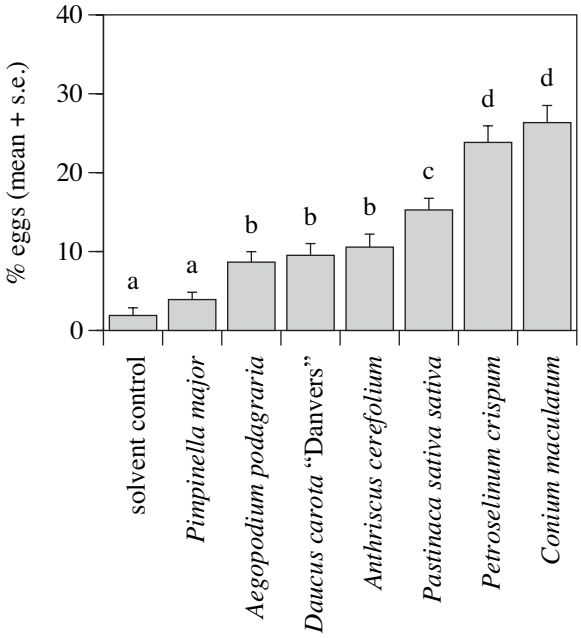

Figure 6. Oviposition stimulated by the Et_2O fractions of hot H_2O extracts of seven host-plant species in a multiple choice assay. The treatment effect was significant at $P < 0.0001$ (Friedman test). Significant differences ($P < 0.05$) among particular treatments are indicated by different letters. Eight replicates per treatment; total number of eggs laid = 6905.

The water fractions of all seven plant species significantly reduced the number of eggs deposited compared to the solvent control, when sprayed onto leaf models that had been pre-treated with a stimulatory hexane extract (Figure 7). The inhibitory effect was very strong with burnet saxifrage *Pimpinella major*, but only moderate with the other species, which did not differ significantly from each other in the power to diminish egg-laying. However, if we allow for the not significant differences found among the leaf models before the treatment with the water fraction, variation among these species becomes more pronounced: *Petroselinum crispum* is no longer different from the control, but more prominently so from *Conium maculatum*, *Pastinaca sativa* and *Anthriscus cerefolium*. The water fraction of burnet saxifrage *Pimpinella major* was still highly deterrent at a concentration of only 0.25 gram leaf equivalents and totally counteracted the stimulatory effect of the hexane extract prepared from carrot leaves, when applied at the same concentration (4 gram leaf equivalents; Figure 8). Since the effect of the hot water extract arguably may be an artefact due to chemical changes occurring at the high temperatures reached during extraction (94–97 °C), we prepared an additional surface extract of *Pimpinella major* foliage using a more gentle method, short leaf dippings (2 × 10 s) into methanol. Both water and methanol extract turned out to be highly effective in decreasing the number of eggs laid underneath real celeriac leaves (Figure 9). Nevertheless, foliage of the non-host plant *Ranunculus repens* (creeping buttercup) was still distinctly less acceptable than the treated leaves of *Apium graveolens*.

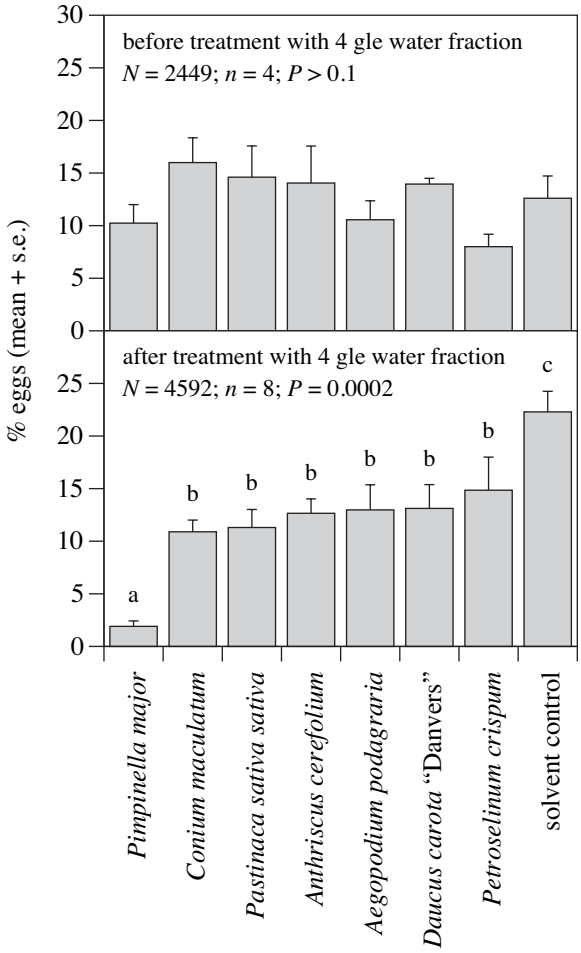

Figure 7. Inhibitory effect of H_2O fractions of the hot H_2O extracts prepared from seven host-plant species and tested in a multiple choice oviposition assay. The test solutions (4 gle per leaf model) were sprayed onto surrogate leaves previously treated with a stimulatory hexane microwave extract (2 gle per leaf model), which was produced from a mixture of foliage of the seven host-plant species in equal portions (total 100 g). The significance of the treatment effect was assessed with the Friedman test. Significant differences ($P < 0.05$) among particular treatments are indicated by different letters. N = total number of eggs laid; n = number of replicates.

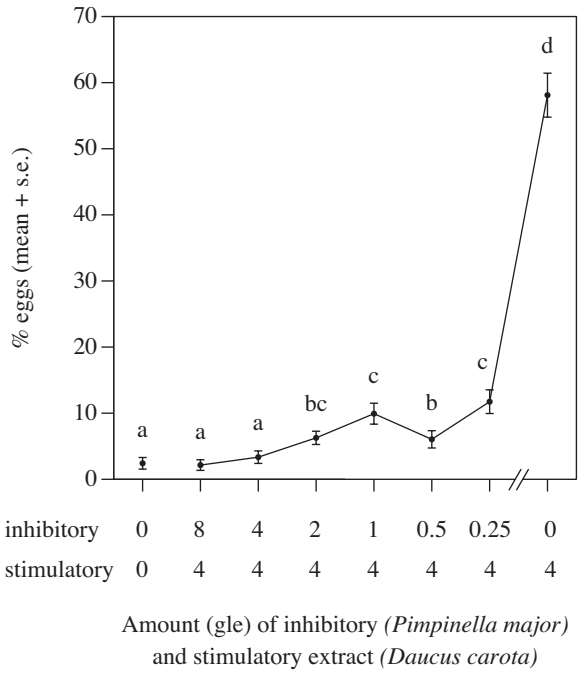

Figure 8. Inhibitory effect of the H_2O fraction of a hot H_2O extract prepared from *Pimpinella major* foliage, when applied at different concentrations (in gle) to surrogate leaves treated beforehand with a stimulatory hexane (microwave) extract from carrot leaves (*Daucus carota sativus* cv. "Tip-Top"). The treatment effect was significant at $P < 0.0001$ (Friedman test). Significant differences ($P < 0.05$) among particular treatments are indicated by different letters. Eight replicates per treatment; total number of eggs laid = 3400.

Discussion

The preference hierarchy established for the foliage of the various host plants is similar, but not identical to the ranking of the species according to the susceptibility in the field (Hardman et al., 1990). For example, these authors found only relatively low numbers of adult flies emerging from soil samples of *Carum carvi*. Yet, leaves of this plant were highly preferred in our oviposition assays. Clearly, under field conditions additional factors are effective, such as plant apparency (i.e. plant size) and larval performance (i.e. antibiosis). Yet, our data suggest that differential susceptibility to carrot fly attack can at least partly be attributed to antixenotic resistance. This has also been shown for carrot cultivars in the field (Maki & Ryan, 1989). Our laboratory study focused on host acceptance of the carrot flies upon direct contact to foliage, thus by-passing host finding which can be crucial in the field, though the separation of these two steps may be somewhat artificial as particular host volatiles may act both as attractants and oviposition stimulants (e.g. *trans*-asarone). Foliage proved to be fairly representative for whole plants in earlier comparative assays (Degen & Städler, in prep.). Nevertheless, there is some evidence that

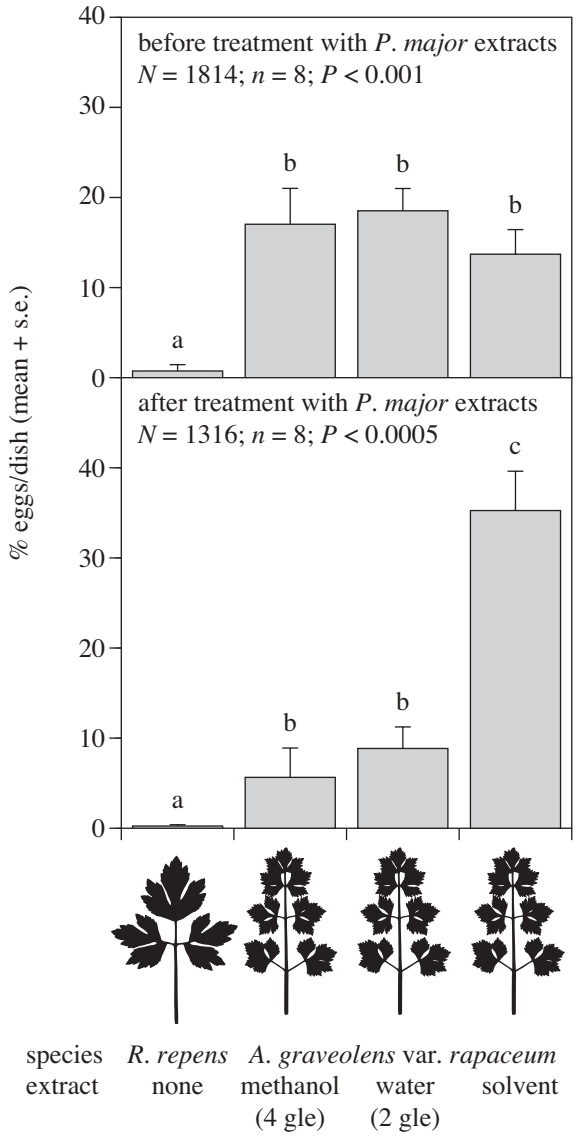

Figure 9. Inhibitory effect of a MeOH crude extract (4 gle) and of the H_2O fraction of a hot H_2O extract (2 gle) prepared both from foliage of *Pimpinella major*, when applied to celeriac leaves *Apium graveolens* var. *rapaceum* cv. "Monarch". Untreated non-host leaves *Ranunculus repens* (creeping buttercup) were included for comparison. The significance of the treatment effect was assessed with the Friedman test. Significant differences ($P < 0.05$) among particular treatments are indicated by different letters. N = total number of eggs laid; n = number of replicates.

volatiles emanating from the roots may as well contribute to antixenotic resistance in carrot cultivars (Maki & Ryan, 1989). Our investigations concentrated on leaf chemistry.

Even acknowledging a very general positive relationship among the stimulatory activities of the extracts and the acceptabilities of the corresponding leaves, the correlation was not very strong and not always significant. With all extraction methods, there were some discrepancies among the ovipositional responses to extracts and to real foliage. For example in *Anthriscus cerefolium*, all the extracts exhibited only a moderate stimulatory activity, whilst the foliage was highly effective in eliciting oviposition. Strong deviations were also found with the dichloromethane extracts of *Pastinaca sativa* (both wild and cultivated parsnip) and of *Pimpinella major*, possibly due to deficiencies of the corresponding standard carrot extracts. Some other major outliers involve the comparatively low activity of the hot water extracts (ether fraction) of *Daucus carota* and of the hexane extract of *Petroselinum crispum*.

The imperfect fit among the rankings obtained for extracts and for real leaves may have several causes. The most likely explanation is a qualitatively inadequate composition of the extracts, that does not reflect appropriately the whole range of chemical stimuli exposed to the flies at the leaf surface. Semiochemicals crucial in mediating antixenotic resistance may not be present in the extracts in correct proportions or may be completely lacking. Clearly, such a situation is not unexpected, when we assume that the diverse behaviourally active compounds differ largely in polarity and hence solubility. For example, the dichloromethane extraction method might be biased towards furanocoumarins, as most plants with extracts that stimulated more egg-laying than expected are known to contain moderate to high amounts of furanocoumarins, whereas carrots generally exhibit quite low levels (Ceska et al., 1987; Degen, Buser & Städler, in prep.).

Variation in non-chemical leaf traits (e.g. morphology, colour, hairiness) among the host species may be another potential reason for the limited accordance between ovipositional responses to foliage and to the respective extracts. We controlled for size by cutting the leaves to the same length. A more accurate alternative might have been to keep leaf area constant by adjusting weight of the tested foliage according to weight-area coefficients. Notwithstanding, leaf area tended to have if anything, then rather a negative effect on the numbers of eggs laid. This implies that the minor differences in leaf size presumably were negligible. Leaf shape may be

of higher relevance in this context. Carrot flies prefer compound leaves over non-compound leaves (Degen & Städler, 1996; Degen & Städler, 1997a). All the umbelliferous species tested are characterized by pinnate foliage, yet the leaves differed markedly in the degree of dissection as reflected for instance in the number of leaflets perpendicular to the leaf axis. Plant species with "higher order" compound leaves (e.g. *Daucus carota, Carum carvi, Conium maculatum*, *Anthriscus cerefolium, Petroselinum crispum*) were more acceptable than species with less "dissected" foliage, (e.g. *Aegopodium podagraria*, *Apium graveolens*, *Pastinaca sativa, Heracleum sphondylium*). Thus, variable leaf morphology was a parameter potentially affecting oviposition in our assays using real foliage. Moreover, for convenience, the concentrations of the leaf surface extracts were referred to leaf weight equivalents (gle) instead of to leaf surface equivalents. Mean leaf weight per 100 cm^2 was somewhat variable (mean ± s.e. = 4.5 ± 0.8) and ranged between 2.1 (*Athyrium filix-femina*) and 14.4 (*Foeniculum vulgare*). Therefore the concentration of stimulants in the surface of some species (e.g. *Foeniculum vulgare*) may have been relatively underestimated with our method.

Since leaf shape was eliminated as an influencing factor from the assays of the extracts, we conclude that differential acceptability of real foliage must be largely attributed to variation in leaf chemistry. There are several other studies on host choice of phytophagous insects that succeeded in correlating the stimulatory activity of surface extracts to anti-xenotic resistance of the respective host plants. Examples involve the root flies *Delia radicum* and *D. floralis* with four *Brassica* genotypes (Baur et al., 1996), the Hessian flies, *Mayetiola destructor*, with four cereal grasses (Foster & Harris, 1992). and the corn earworm, *Heliothis zea*, with wild and cultivated tomato plants (Juvik et al., 1988).

The results obtained with the three extraction methods cannot be compared directly due to different experimental approaches (dual versus multiple choice assays) and to not totally identical sets of test plants (e.g. inclusion or exclusion of non-hosts). Nevertheless, according to the correlation coefficients, the preference ranking for the hexane extracts seemed to match best the preference ranking for genuine leaves. Also, hexane extracts of carrot foliage proved to be more stimulatory than hot water and dichloromethane extracts in comparative experiments, and surrogate leaves treated with hexane extracts were almost as acceptable as real foliage in choice assays (Degen, Poppy & Städler, in prep.). Thus, the hexane extracts were chosen for further analysis. Since we assessed the relative activity of the silica gel column fractions only with four extracts, we do not know whether oviposition stimulants are confined to the fractions 3.1 and 4. It cannot be excluded that the fractions 1, 2 and 3.2 also contained stimulatory or synergistic compounds in some other cases. However, fractions 3.1 and 4 together explained the activity of the crude extract fairly well. So, it seems to be justified to suppose that the results of the examined plants are representative for most species. Fraction 3.1, which contained the earlier identified oviposition stimulants (Städler & Buser, 1984), could only account for a small part of the variation in the acceptabilities of the host leaves. Using a broad range of host plants, we thus come to the same conclusion as put forward in earlier studies dealing with carrot cultivars (Guerin & Städler, 1984; Städler et al., 1990): an explanation of the host-plant preferences of the carrot fly cannot be based on the so far known stimulants alone. Additional yet unidentified stimulatory compounds in fraction 4 apparently are more promising in this respect because of the better correlation found between the activity of fraction 4 and the acceptability of real leaves.

There is no evidence for the occurrence of inhibitory compounds in the hexane extracts of host plants, as indicated by the fact, that the non-preferred *Pimpinella* extract did not decrease the activity of the carrot extract, but rather considerably enhanced it, possibly owing to the presence of moderate amounts of furanocoumarins that normally are detected in carrot foliage only in trace amounts (Degen, Buser & Städler, in prep.). Even when the two non-host extracts were added to the stimulatory extract, egg-laying was reduced only slightly. By contrast, all the hot water extracts prepared from host-plant leaves deterred oviposition to some degree. By far the strongest inhibitory effect was observed with the extract of *Pimpinella major*, which was one of the least acceptable host species in our choice assays, did not support larval development in antibiosis experiments (Degen & Städler, in prep.) and is also highly resistant in the field (Hardman et al., 1990). This is circumstantial evidence for the potential significance of deterrents in the host-selection process. On the

other hand, *Pimpinella major* contains only low levels of stimulants. Therefore the presence of deterrent compounds is not necessary to explain the low acceptability of this plant. Further research is needed to ascertain whether deterrents are responsible for variation in host acceptability that cannot be explained by the contents in stimulants.

The identified oviposition stimulants are of intermediate polarity. Some are even volatile enough to act as attractants in the field (Guerin et al., 1983). It has been proposed that the boundary layer surrounding the leaves is saturated with these compounds, as they are known to be perceived by olfactory sensilla on the antennae of the insects (Städler & Roessingh, 1991). Using a slightly modified method described by Guerin & Visser (1980) we recorded electroantennogram (EAG) responses to the crude hexane extracts from a single female carrot fly. In contrast to the host extracts, non-host extracts evoked no significant EAG activity (T. Degen, unpublished). This suggests that the antennal receptors are selectively tuned to host-specific compounds, supporting earlier findings (Guerin & Städler, 1982). Within plant set 2, EAG amplitudes were significantly correlated with the stimulatory activity of fraction 3.1, which implies that the responses are mainly attributable to the known stimulants. By contrast, within set 1, the non-preferred hosts *Anethum graveolens* and *Pimpinella major* evoked higher EAG responses than the preferred hosts *Daucus carota* and *Carum carvi*. Recordings from sensilla on the tarsi as yet gave no indication for contact chemoreceptors sensitive to the identified oviposition stimulants. However, ablation experiments and electrophysiological investigations using methanolic extracts of carrot foliage provided some hints that tarsal sensilla might be involved in the perception of host-plant stimuli (Städler, 1977; Städler, 1982). Therefore it seems likely that the unknown polar stimulants and deterrents are perceived by tarsal contact chemoreceptors.

Our findings confirm the hypothesis that leaf chemistry is central to the understanding of host selection in the carrot fly. As in many other insects, the oviposition behaviour of the carrot fly is affected by complex mixtures of stimulatory and probably also inhibitory compounds, which are perceived through different channels (olfactory and contact chemoreceptors). Only a part of these semiochemicals has been characterized yet. Forthcoming research should focus on the identification of further behaviourally active compounds – stimulants and deterrents – as well as on the elucidation of the modes by which these compounds are perceived.

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Patterns of oviposition stimulants for the carrot fly in leaves of various host plants

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Key Words: phenylpropenes, furanocoumarins, polyacetylenes, coumarins, Apiaceae, *trans*-methylisoeugenol, *trans*-asarone, xanthotoxin, bergapten, falcarinol, falcarindiol, *Psila rosae*, Diptera, Psilidae

Abstract

Undamaged leaves of twelve host-plant species differing widely in acceptability to ovipositing carrot flies were extracted with a microwave-assisted method using hexane as solvent. The highly stimulatory diethyl ether fraction of these extracts obtained by a separation on a silica gel column was semiquantitatively analysed by GC-MS for six previously identified oviposition stimulants of the carrot fly (phenylpropenes, furanocoumarins, polyacetylenes). The various plant species exhibited widely differing profiles of these stimulatory compounds. In choice assays, moderate numbers of eggs were deposited underneath surrogate leaves sprayed with fractions that contained high amounts of just one type of compounds and low amounts of the other two types (e.g. polyacetylenes in *Carum carvi*, furanocoumarins in *Pastinaca sativa*). Only fractions with medium to high levels of at least two compound classes elicited strong ovipositional responses (e.g. phenylpropenes and polyacetylenes in *Daucus carota*, furanocoumarins and polyacetylenes in *Heracleum sphondylium* and *Conium maculatum*). None of the examined plants contained high quantities of all three compound classes. Thus, the contents of the stimulants accounted in a synergistic manner for the variation in activity of the diethyl ether fraction. However, they could not explain adequately the observed preference hierarchy of the carrot fly for the host-plant species. Therefore, the oviposition behaviour of the carrot fly must be affected by additional, yet unidentified semiochemicals.

Introduction

The carrot fly, *Psila rosae* (F.) (Diptera, Psilidae), is an oligophagous insect: more than a hundred plant species, exclusively belonging to the family Umbelliferae (Apiaceae), have been recorded as hosts (Hardman et al., 1990; Ellis et al., 1992). The female flies perform an exploratory run over the leaves before accepting a host-plant and depositing their eggs in the soil underneath. The larvae feed on the roots and may cause severe damage to various crops (carrots, celeriac, parsnip). Consistent differences among carrot cultivars in resistance against carrot fly attack have been established (Ellis & Hardman, 1981; Ellis et al., 1984), but relatively few studies have dealt with the underlying mechanisms. Evidence has been put forward for the involvement of both antibiosis (Guerin et al., 1981; Maki & Ryan, 1989) and antixenosis (Guerin & Ryan, 1984; Guerin & Städler, 1984).

Host selection in the carrot fly is influenced by physical (leaf shape and colour) and chemical plant cues (Städler, 1977; Degen & Städler, 1996). Defense chemicals in the surface wax of carrot leaves were shown to stimulate oviposition (Städler & Buser, 1984). The compounds thus identified as stimulants belong to three classes (phenylpropenes, furanocoumarins and substituted coumarins, polyacetylenes) that are in combination characteristic of the umbellifers (Städler, 1986). Yet, previous studies failed to correlate the varying level in antixenotic resistance among carrot cultivars with the amounts of the known oviposition stimulants in the leaf surface (Visser & de Ponti, 1983; Guerin & Städler, 1984; Städler et al., 1990). In addition, it was demonstrated that celeriac, another important host, exhibited a

pattern of stimulating compounds in its leaf surface very distinct from that of carrot (Städler et al., 1990). This prompted us to expand the investigation to various other host-plant species that differ more markedly in acceptability than carrot cultivars (Degen and Städler, in prep.). In the present study, leaf surface extracts of twelve host-plant species were semiquantitatively analysed by high-resolution gas chromatography/mass spectrometry (HRGC-MS) for the six previously identified oviposition stimulants. The acceptabilities of real leaves and the relative stimulatory activities of the examined fractions were compared to the respective amounts of stimulants detected in order to assess the importance of these compounds for host acceptance of the carrot fly.

Methods and materials

Plants. Twelve host-plant species were chosen for examination (Table 2), including the two previously studied carrot cultivars "Danvers" and "Sytan" (Guerin & Städler, 1984; Städler et al., 1990). Foliage for the extracts and the oviposition assays was collected from locally occurring wild plants (*Heracleum sphondylium*, *Aegopodium podagraria*, *Athyrium filix-femina*) and from plants grown in pots (*Pimpinella major*) or in seed beds (the eight remaining species) at the Swiss Federal Research Station Wädenswil. The young plants were heavily damaged during a hailstorm, but recovered and looked healthy by the time they were used for oviposition assays and extractions, i.e. about one and two months later, respectively). Only the annual plants *Anethum graveolens* and *Anthriscus cerefolium* had to be resown.

Foliar extracts. The extracts were prepared about 60 days (*Anethum graveolens* and *Anthriscus cerefolium*), 100 days (set 2) and 130 days (set 1) after sowing of the plants, and 2–4 days after completion of the respective multiple choice oviposition assays with the intact leaves. We adopted a microwave-assisted extraction procedure (Paré et al., 1991) using hexane as a solvent medium that does not absorb radiation. This method was shown to produce extracts from carrot leaves that were highly effective in stimulating egg-laying in the carrot fly (Degen, Poppy and Städler, in prep.), superior to the dichloromethane dippings previously used to isolate oviposition stimulants (Städler & Buser, 1984; Städler et al., 1990). Cut, but otherwise undamaged leaves (100 g each per plant species) were covered with 600 ml hexane (Fluka puriss. p.a.) in a 1000-ml beaker, that was topped by a Petri dish to prevent solvent vapours from entering subsequently the oven body. Half a minute after the first contact of the foliage with the solvent, the beaker was placed into a microwave oven (Panasonic NN-6807) run for 60 s at a power of 700 W. The heating of the water-containing plant material caused the temperature of the hexane to rise to 47–53 °C. Afterwards the solvent was immediately decanted into another beaker. The foliage was immersed in the solvent for a total of about 120 s. The resulting solution was dried with Na_2SO_4, filtered (Schleicher & Schuell No. 595 1/2 folded paper filter) and concentrated on a rotary evaporator at 30 °C and 240 mbar to a volume of 40 ml, i.e. to 2.5 gram leaf equivalents/ml (2.5 gle/ml).

Purification. Each crude extract was subjected to fractionation on silica gel according to Städler et al. (1990). A 16-ml aliquot corresponding to 40 gle was concentrated to ≈ 1 ml under a N_2-stream, transferred with a pasteur pipette onto the column (75 × 11 mm, silica gel 70–230 mesh, Merck "Kieselgel 60") that was eluted successively with 20-ml portions of 100% hexane (Merck zur Rückstandsanalyse; fraction 1), 5% diethyl ether (Fluka puriss.; stab. with 0.0005% 2,6-di-tert-butyl-p-cresol) in hexane (fraction 2), twice 100% diethyl ether (fraction 3.1 and 3.2) and 100% methanol (Merck pro analysi; fraction 4). The diethyl ether fraction contained the previously identified oviposition stimulants (Städler & Buser, 1984) in a study using dichloromethane surface extracts (Städler et al., 1990) and therefore the corresponding fraction 3.1 was chosen for the GC-MS analysis. All solutions were stored in a deep freezer at –20 °C before further use (oviposition assays, fractionation, GC-MS analysis).

Semiquantitative GC-MS analysis of fraction 3.1. The diethyl ether was removed from 20-gle aliquots of fraction 3.1 and replaced with ethyl acetate to reach a final concentration of 2 gle/ml. Aliquots of 1 μl of these solutions corresponding to 0.002 gle were on-column injected.

GC-MS analysis was performed with a VG Tribrid mass spectrometer (VG Fisons, Manchester, England). The ion source was operated in the electron ionization mode (EI, 70 eV, 180 °C). Full-scan mass spectra (m/z 35–435; 1.05 s/scan; mass resolution $M/\Delta M$ = 500) were recorded. For the

Table 1. Ions used for selected ion monitoring (SIM), other important ions and elution temperatures for the six oviposition stimulants

compound	*m/z* quantitation ion	*m/z* other important ions	elution temperature
trans-methyl-isoeugenol	178 (M^+)	163, 147, 135, 115, 107	171 °C
trans-asarone	208 (M^+)	193, 165, 150	182 °C
xanthotoxin	216 (M^+)	201, 188, 173, 145	212 °C
bergapten	216 (M^+)	201, 188, 173, 145	213.5 °C
falcarinol	159	244 (M^+) very weak, 229, 173	210 °C
falcarindiol	157	260 (M^+), 242, 171, 129, 128, 115	225 °C

quantification of particular target compounds present in low amounts, analysis was also carried out with selected-ion-monitoring (SIM) using the molecular ions (*trans*-methylisoeugenol, *trans*-asarone, bergapten, xanthotoxin) or a major fragment ion (falcarindiol, falcarinol; see Table 1). A lock mass of *m/z* 207.033 from the silicon bleed of the HRGC column was used in SIM.

The analyses were carried out with a 24 m OV1 fused silica (0.25 mm i.d.) HRGC column that was temperature programmed as follows: 70 °C, 2 min isothermal, 20 °C/min to 160 °C, then at 5 °C/min to 280 °C, followed by an isothermal hold at this temperature. Data acquisition was started when the column reached 160 °C. The compounds of interest eluted between ~2 min (methylisoeugenol) and 13 min (falcarindiol) from that point on.

The amount of analyte was determined from the peak height measurements relative to that from known amounts injected (SIM or total ion data). The concentration of the polyacetylene standards was not reliably known, as the signals obtained for falcarindiol and falcarinol were much lower than expected when compared to equal amounts of furanocoumarins. Thus we adjusted the originally calculated amounts for these two compounds with correction factors, assuming that identical quantities of polyacetylenes and furanocoumarins result in identical peak heights (total ion data). Major additional compounds present in fraction 3.1 were identified according to their mass spectra and retention times, when a standard was available, or tentatively from the interpretation of the mass spectra alone. The amounts of these compounds were estimated by comparing the peak height to the peak heights of standards or of the key compounds belonging to the same or a related compound class.

Insects and oviposition assays. The flies for the oviposition experiments were obtained from a laboratory culture reared according to Städler (1971) and were kept in a controlled-environment chamber at 21 ± 1 °C, 70–80% r.h. and 16:8 hr light-dark regime. Oviposition preference hierarchies were established separately for two sets of eight plants, each including the standard carrot cultivar "Danvers" and a non-host. The multiple choice assays were conducted in cubic screen cages (0.34 m^3) containing 100–400 individual flies (males and females). Eight oviposition devices (Städler, 1971) equipped with foliage or with surrogate leaves were arranged in a circle around an apple seedling (resting place) in the centre of the cage. The surrogate leaves made of green cardboard and coated with a thin layer of paraffin (Degen & Städler, 1997) were sprayed with 4 gram leaf equivalents of the respective fraction 3.1. The positions of the eight treatments were permuted for each of the eight experimental periods, which usually lasted one day ("randomized complete block design"). The egg counts were tested for a treatment effect (plant species) with the Friedman test. Multiple comparisons among treatments were made following the method based on rank sum differences given by Conover (1980).

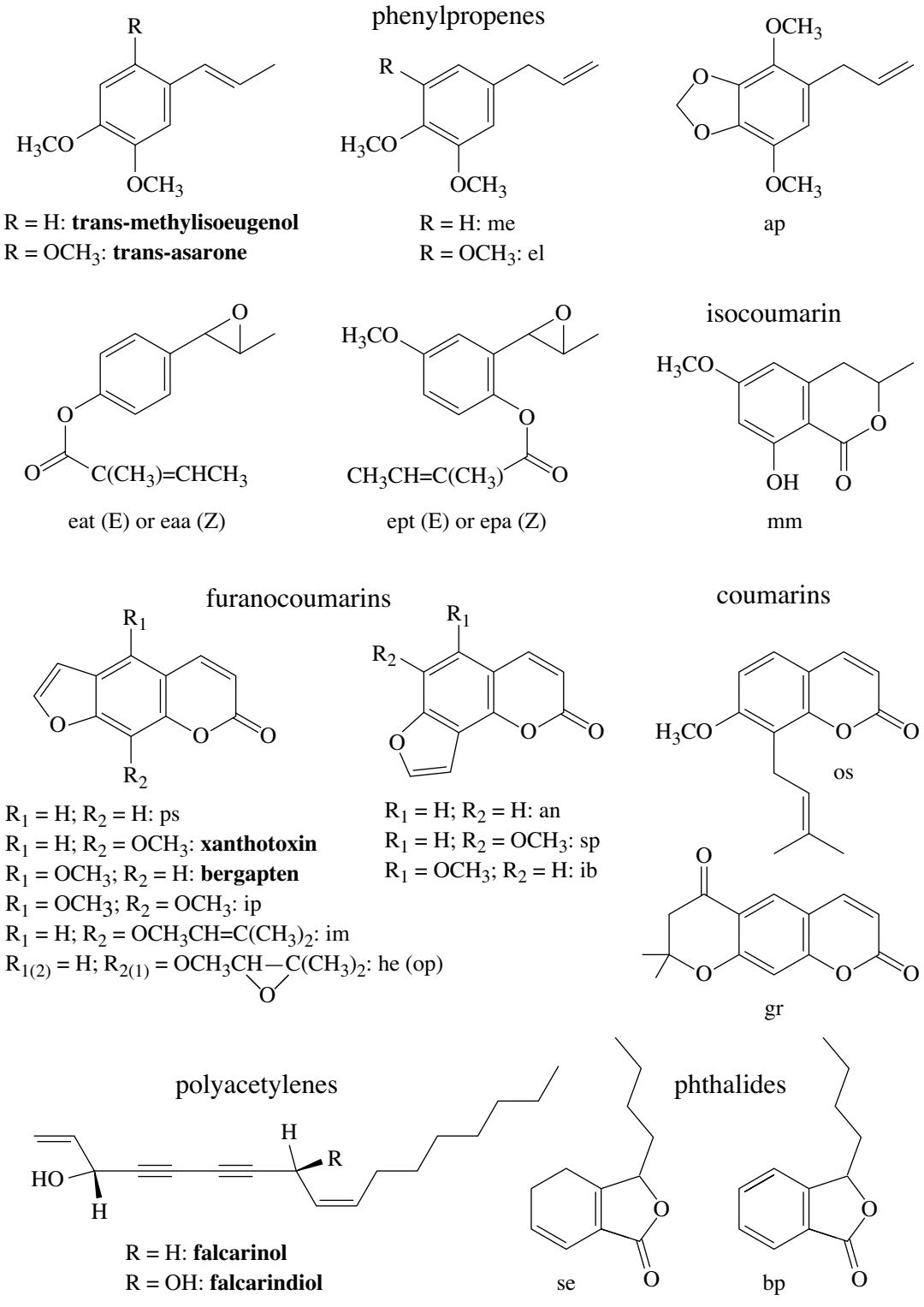

Figure 1. Structural formulae of the six oviposition stimulants (key compounds in bold letters) and of related compounds detected in the various host-plant species (for abbreviations see Table 4).

Table 2. Semiquantitative GC-MS analysis of fraction 3.1 for six previously identified oviposition stimulants. Amounts in ng per gram leaf equivalent

set	plant species	phenylpropenes		furanocoumarins		polyacetylenes	
		t-methyl-isoeugenol	*t*-asarone	xanthotoxin	bergapten	falcarinol	falcarindiol
1	*Anthriscus cerefolium* (L.) Hoffm., garden chervil	1	< 0.7	≤ 1	≤ 0.4	160 [b]	40
	Pimpinella major (L.) Huds., greater burnet saxifrage	1	≤ 0.5	20	60 [b]	< 20	< 30
	Foeniculum vulgare Miller, fennel	3	≤ 0.4	3	14	40	50
	Anethum graveolens L., dill	≤ 0.4	≤ 0.7	≤ 0.7	≤ 0.3	100	180 [b]
	Carum carvi L., caraway	1	≤ 0.6	4	3	90	5'300 [a]
	Pastinaca sativa ssp. *sativa* L. cv. "Halblange", parsnip	1	≤ 0.7	80'000 [a]	23'000 [a]	≤ 80 [d]	< 30
	Daucus carota L. ssp. *sativus* cv. "Danvers", carrot	300 [a]	30 [b]	≤ 1	≤ 1	110	120 [b]
2	*Apium graveolens* L. var. *rapaceum* cv. "Balder", celeriac	7	< 1	2'400 [a]	5'600 [a]	< 17	< 30
	Aegopodium podagraria L., ground elder	2	< 0.3	12	9	500 [b]	4'300 [a]
	Petroselinum crispum (Miller) A. W. Hill, parsley	5	1? [c]	400 [a]	5'600 [a]	< 4	14
	Daucus carota L. ssp. *sativus* cv. "Danvers", carrot	1'000 [a]	30	4	≤ 0.6	40	100
	Daucus carota L. ssp. *sativus* cv. "Sytan", carrot	3'700 [a]	500 [a]	1	6	80	200 [b]
	Conium maculatum L., hemlock	< 3 [a]	< 6 [a]	9'600 [a]	2'600 [a]	2'100 [a]	300 [a]
	Heracleum sphondylium L., hogweed	< 4 [a]	< 5 [a]	4'800 [a]	2'300 [a]	400 [a]	5'200 [a]
	estimated threshold for stimulation when applied as pure compound to filter paper leaf (Städler & Buser, 1984)	1 mg	1 mg	1 mg	100 µg	(≤ 30 µg)	100 ng

[a] Values are calculated from total ion counts (TIC), otherwise the values are obtained from SIM data
[b] Values are calculated from SIM data, but verified by complete mass spectra
[c] Expected value according to regression of *t*-methylisoeugenol on *t*-asarone because of coelution with apiole
[d] Maximal value, coelution with isobergapten, which has a small signal at *m/z* 159
< No signal three times greater than noise detected (detection threshold)
≤ Signal three times greater than noise detected (SIM)

Results

The fractionation procedure resulted in no obvious loss of stimulatory activity, since a combination of all fractions did not elicit significantly less oviposition than the crude extract in multiple choice assays carried out with four selected examples (standard carrot *Daucus carota sativus* cv. "Danvers" of set 1 and 2, *Conium maculatum*, *Carum carvi*). These experiments also showed that the stimulatory activity was confined to fraction 3.1 and fraction 4 (Degen and Städler, in prep.). The very low acceptability of surrogate leaves treated with the additional diethyl ether fraction 3.2 indicated that there was no substantial carry over of stimulants from fraction 3.1. Hence other compounds yet unknown must be responsible for the stimulatory activity of fraction 4.

The contents of oviposition stimulants and related secondary compounds (see Figure 1 for structural formulae) in fraction 3.1 varied widely among the host-plant species (Table 2 and 4, Figure 2 and 3) while the plants were quite similar to each other with respect to major wax components such as long-chained fatty alcohols and sterols. This is exemplified by three chromatograms depicted in Figure 2.

The concentrations of the six oviposition stimulants (on a log scale) were correlated within, but not among the compound groups, i.e. high amounts of *trans*-methylisoeugenol were detected together with relatively high amounts of *trans*-asarone ($r = 0.91$; Fisher's r to z P-value < 0.0001; $n = 13$; without *Petroselinum crispum*). The same was found for the two furanocoumarins ($r = 0.95$; $P < 0.0001$; $n = 14$) and the two polyacetylenes ($r = 0.63$; $P = 0.013$; $n = 14$). When the species with amounts below detection threshold (values with < in table 2) are excluded from the calculation of r, the correlation is still significant for the phenylpropenes ($r = 0.96$; $P < 0.0001$; $n = 8$), but no longer for the polyacetylenes ($r = 0.40$; $P = 0.26$; $n = 10$), since the ratio of falcarinol to falcarindiol was more variable. Nevertheless, the summation of the amounts of the stimulants belonging to the same compound class as shown in Figure 3 seems to be justified for a simplified comparison of the stimulant composition in the various host plants.

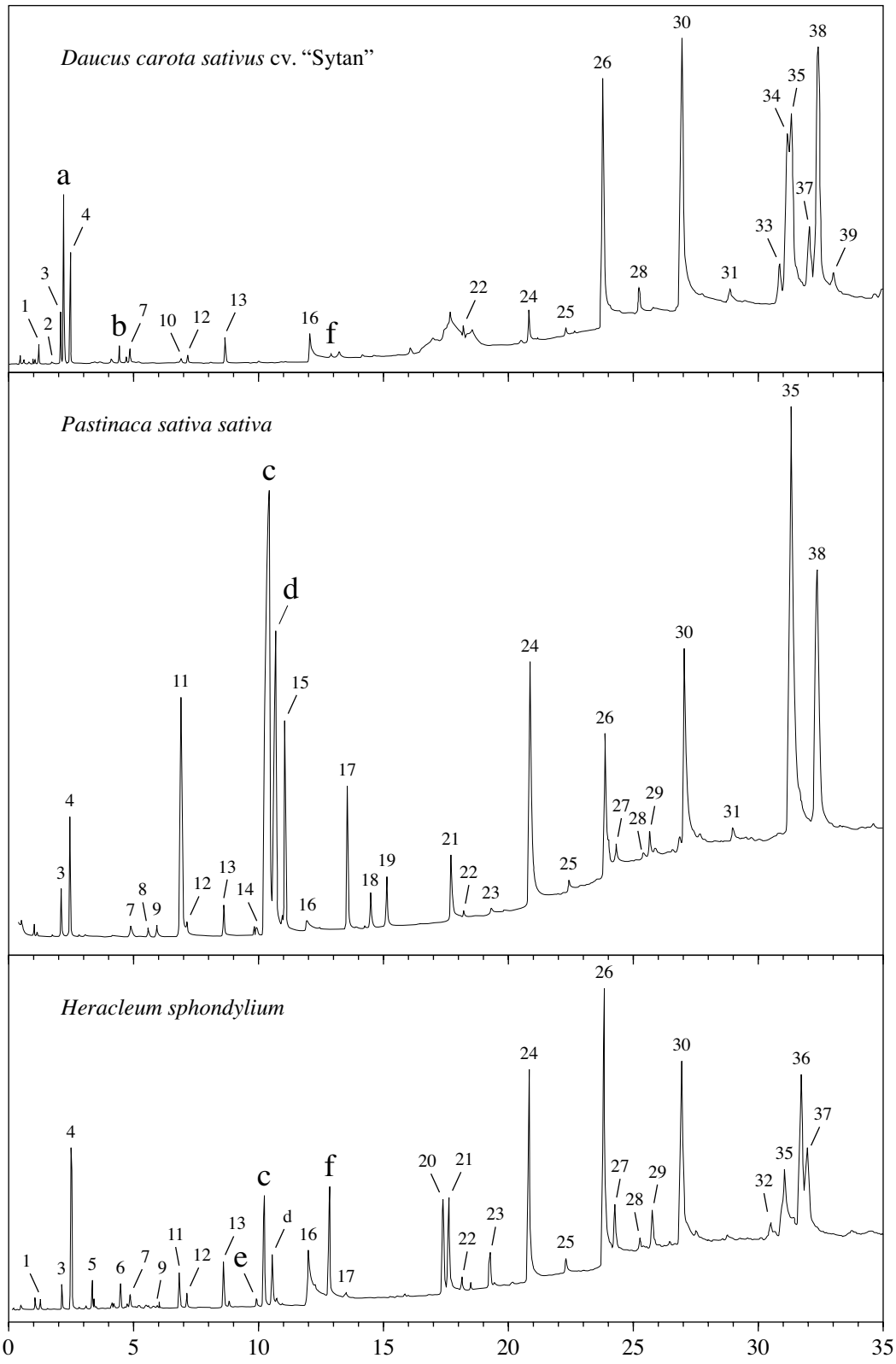
Daucus carota sativus cv. "Sytan"
Pastinaca sativa sativa
Heracleum sphondylium
0
5
10
15
20
25
30
35
retention time in minutes (starting at 160 °C column temperature)

Figure 2. Chromatograms from total ion scans of fraction 3.1 of three chosen host-plant species that differ markedly in their pattern of oviposition stimulants (key compounds).
Key compounds : a = *trans*-methylisoeugenol; b = *trans*-asarone; c = xanthotoxin; d = bergapten; e = falcarinol; f = falcarindiol. **Additional components:** 1 = methyleugenol; 2 = *cis*-methylisoeugenol; 6 = apiol; 9 = angelicin; 10 = 6-methoxymellein, 11 = psoralen; 14 = sphondin or isobergapten; 15 = 5-dodecyldihydro-2(3H)-furanone; 16 = linolenic acid; 17 = isopimpinellin; 19 = imperatorin; 20 = oxypeucedanin or heraclenin; 21= *n*-docosanol; 23 = *n*-tricosanol; 24 = n-tetracosanol; 25 = *n*-pentacosanol; 26 = *n*-hexacosanol; 28 = heptacosanol; 29 = β-tocopherol; 30 = *n*-octacosanol; 31 = *n*-nonacosanol. **Contaminants:** 3 = ionox; 4 = butylhydroxytoluol (BHT, antioxidant); 12 = diisobutylphthalate (DiBP); 13 = dibutylphthalate (DBP); 22 = dioctylphthalate (DOP). **Unidentified compounds:** 5 = oxygenated sesquiterpene; 7 = *m/z* 224 (M^+), 208, 193, 123; 8 = *m/z* 188 (M^+), 160, 146; 18 = *m/z* 280 (M^+?); 27 = *m/z* 402 (M^+), "desmethyl-tocopherol"; 32 = *m/z* 414 (M^+), sterol; 33 = *m/z* 426 (M^+), sterol; 34 = *m/z* 426 (M^+), sterol; 35 = *m/z* 426 (M^+), sterol (amyrin); 36 = *m/z* 414 (M^+), sterol; 37 = *m/z* 426 (M^+), sterol; 38 = *m/z* 426 (M^+), sterol (amyrin); 39 = *m/z* 426 (M^+), sterol.

We found essentially four major patterns in the distribution of the stimulants: 1) High levels of only furanocoumarins were observed with *Pastinaca sativa*, *Apium graveolens* and *Petroselinum crispum*. 2) High contents of only polyacetylenes were found with *Carum carvi* and *Aegopodium podagraria*. 3) The phenylpropenes *trans*-methylisoeugenol and *trans*-asarone were only present in substantial amounts in the three carrot extracts together with moderate amounts of polyacetylenes. 4) High quantities of both furanocoumarins and polyacetylenes were detected in *Conium maculatum* and *Heracleum sphondylium*. The remaining species contained at best moderate amounts of a single compound class (e.g. furanocoumarins in *Pimpinella major*, polyacetylenes in *Anethum graveolens* and *Anthriscus cerefolium*). There was no example with a combination of both phenylpropenes (i.e. *t*-methylisoeugenol, *t*-asarone) and furanocoumarins or of all three compound classes together occurring in high quantities.

In Figure 3 we compared the relative numbers of eggs deposited by the carrot flies underneath real leaves and surrogates treated with fraction 3.1 to the distribution of the six oviposition stimulants in the respective plant species. Leaf models treated with fractions containing solely high levels of a single compound class received only moderate numbers of eggs (e.g. polyacetylenes in *Carum carvi* and *Aegopodium podagraria*, furanocoumarins in *Pastinaca sativa*). Strong ovipositional responses were only elicited when two compound classes were present in relatively high amounts (e.g. phenylpropenes and polyacetylenes in *Daucus carota*, furanocoumarins and polyacetylenes in *Heracleum sphondylium* and *Conium maculatum*). Within plant set 1, the oviposition preferences of the carrot fly appeared to be determined by the phenylpropenes alone which were only present in notable amount in the highly favoured fraction of the standard carrot cultivar "Danvers", whilst the furanocoumarin and polyacetylene contents had no significant effect according to a multiple regression (Table 3), though they may be responsible for the fact that the fractions of *Pastinaca sativa* and *Carum carvi* stimulated more egg-laying than the remaining fractions. In contrast, all three compound classes seemed to contribute equally to the prediction of the numbers of eggs laid with the different plant species within set 2. However, the overall relationship is only close to significance ($P = 0.07$) in this case. This is probably a consequence of the small sample size (only seven plants), as suggested by the outcome of the analysis using data pooled for both sets (13 plants; mean of the two values for the standard plant). Yet, we are aware that such a combination of the two sets is statistically not correct and thus conclusions must be drawn very cautiously because the values within a set are not independent of each other. By chance, most plants chosen for set 1 contained less oviposition stimulants and consequently activities of fractions 3.1 relative to the standard were generally lower than in set 2. The effect of a particular treatment seemed to depend on the range of the other treatments present in the experiment, as evidenced by the higher proportion of eggs (relative to the standard) deposited with the non-host treatment in set 2. Thus the percentages for the plant species are not directly comparable between the sets, even though a standard was included in each of them. For example, *Carum carvi* significantly differed from the standard in set 1, whereas in set 2 the difference between the standard and *Aegopodium podagraria* with a pattern of stimulants very similar to the pattern of caraway was not significant. (However, experience shows that the ranking of the treatments is less affected by the experimental design, i.e. the range of other treatments included, than are the percentages.) The total content in the six stimulants explains a smaller part of the variation observed in the number of eggs laid than the three compound classes separately (Table 3).

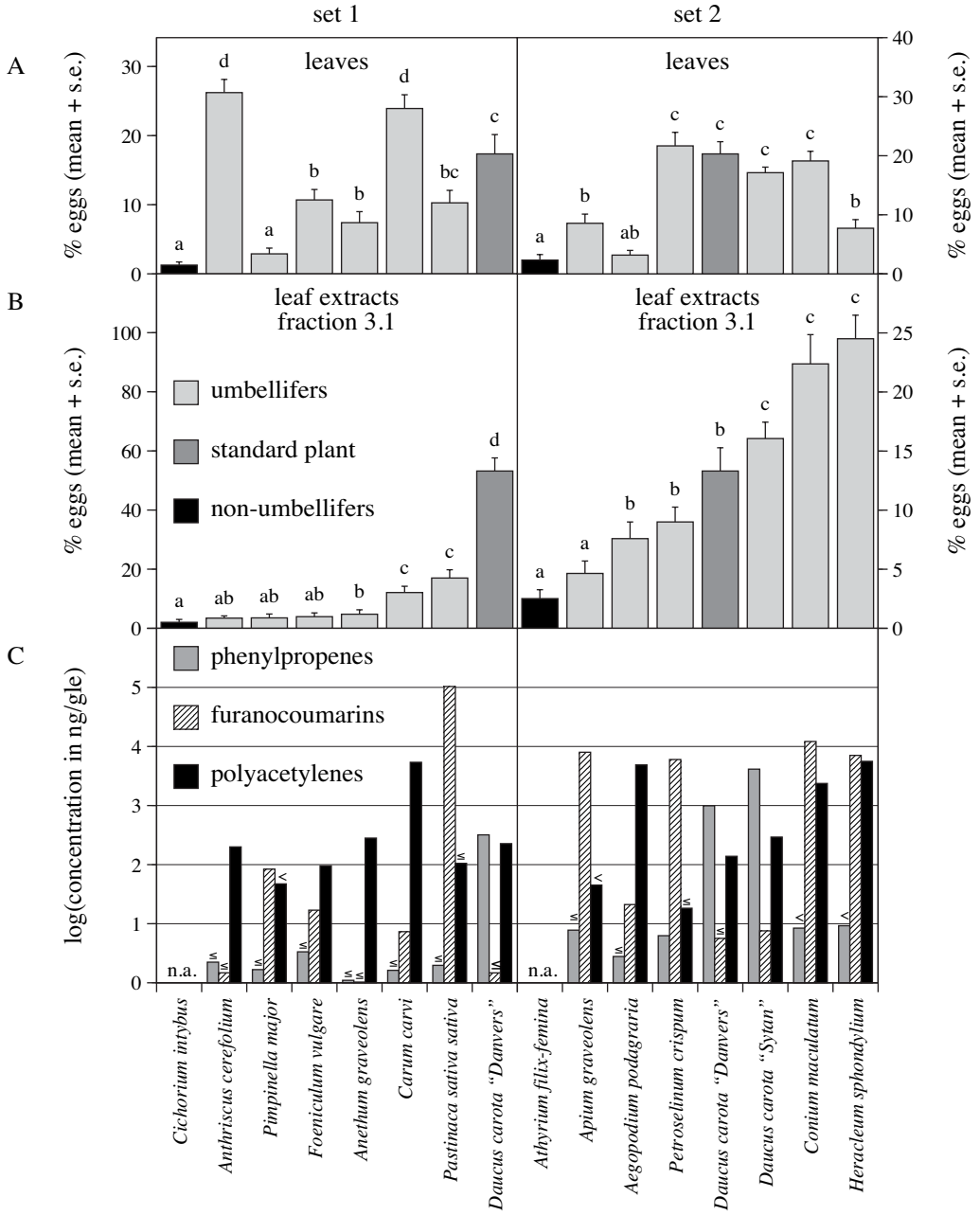

Figure 3. A and B: Oviposition of carrot flies underneath real leaves (A) and surrogate leaves treated with four gram leaf equivalents of fraction 3.1 of the respective leaf surface extracts (B). Two sets of eight plants each including the standard carrot cultivar "Danvers" were tested separately in multiple choice assays. These data are identical to those in Degen and Städler (in prep.) and are given here in a rearranged order for comparison. The numbers of eggs are expressed as percent of total oviposition during an experimental period. Y-axes are adjusted in such a way that columns of the standard plant are of equal height with both sets of host species. The treatment had a significant effect (Friedman test: $P < 0.0001$) in all four experiments. Means accompanied by the same letter are not significantly different at the 5%-level. Eight replicates per treatment in each experiment.

C: Amounts (logarithmic scale) of oviposition stimulants detected in the corresponding fractions 3.1 summarized for compound classes (phenylpropenes = *t*-methylisoeugenol and *t*-asarone; furanocoumarins = xanthotoxin and bergapten; polyacetylenes = falcarinol and falcarindiol). n.a. = not analysed. ≤ = maximal amount, signal detected with SIM; < = detection threshold, no signal three times greater than noise detected.

Table 3. Multiple regression from logarithmic concentrations (in ng/gle) of oviposition stimulants (summarized for compound classes, see Figure 3) on stimulatory activity of the fractions 3.1 (dependent variable: percent oviposition relative to standard carrot cultivar)

set	independent variables	coefficient	std. coeff.	t-value	P-value	R^2 (adjust.)	df (total)	*P*-value
1	phenylpropenes[a]	40.5	1.0	8.6	0.003	0.96 (0.92)	6	0.01
	furanocoumarins[b]	5.2	0.3	2.2	0.12			
	polyacetylenes[c]	12.5	0.2	2.0	0.14			
	intercept	–34.1	–34.1	–2.0	0.14			
	total stimulants[d]	6.8	0.2	0.5	0.64	0.05 (–)	6	0.64
	intercept	6.6	6.6	0.2	0.88			
2	phenylpropenes[a]	46.2	1.0	3.3	0.05	0.87 (0.75)	6	0.07
	furanocoumarins[b]	34.2	1.0	3.2	0.05			
	polyacetylenes[c]	49.8	0.9	4.0	0.03			
	intercept	–186.8	–186.8	–2.8	0.07			
	total stimulants[d]	57.3	0.4	0.9	0.41	0.14 (–)	6	0.41
	intercept	–111.0	–111.0	–0.5	0.66			
1+2	phenylpropenes[a]	37.4	0.6	4.4	0.002	0.82 (0.75)	12	0.001
	furanocoumarins[b]	19.5	0.5	3.7	0.005			
	polyacetylenes[c]	40.6	0.6	3.8	0.004			
	intercept	–115.1	–115.1	–3.5	0.007			
	total stimulants[d]	34.1	0.5	1.9	0.08	0.25 (0.19)	12	0.08
	intercept	–52.4	–52.4	–0.8	0.41			

[a] *trans*-methylisoeugenol and *trans*-asarone
[b] xanthotoxin and bergapten
[c] falcarinol and falcarindiol
[d] sum of all six compounds

Hence the effects of the different stimulants seem to be non-additive (note that the regression was performed with the logarithm of the concentrations).

The preference hierarchies obtained for real leaves and for the fractions 3.1 do not accord well (correlations using mean arcsine-transformed percentage of eggs; set 1: $r = 0.40$, $P = 0.34$; set 2: $r = 0.54$, $P = 0.17$). This is best illustrated by the very low stimulatory activity of fraction 3.1 in *Anthriscus cerefolium*, a highly preferred host plant (Figure 3).

Additional compounds present in fraction 3.1 were identified whenever possible (Table 4), but were not screened for systematically by selected-ion-monitoring (SIM). Traces of *cis*-methylisoeugenol accompanied the *trans*-isomer in the carrot cultivar "Sytan". On the other hand, the occurrence of the allyl-isomer methyleugenol (e.g. in *Anthriscus cerefolium)*, of related allylbenzenes (e.g. apiol in *Petroselinum crispum*, elemicine in *Conium maculatum*) or of structurally related compounds (epoxy-pseudoisoeugenol ester and epoxy-anol ester in *Pimpinella major*) did not seem to be linked to the contents of *trans*-methylisoeugenol or *trans*-asarone. By contrast, the incidence of further linear furanocoumarins (e.g. psoralen, isopimpinellin) was restricted to plants that comprised considerable amounts of xanthotoxin and bergapten. Angular furanocoumarins were only discovered in *Pastinaca sativa* and *Heracleum sphondylium*. Osthol, a

Table 4. Further related compounds identified in fraction 3.1

Phenylpropanoids: c-mi = *cis*-methylisoeugenol; *t*-ie= *trans*-isoeugenol; me = methyleugenol; el = elemicin; ap = apiole; eat/eaa = epoxy-anol tiglate or angelicate (isomers); ept/epa = epoxy-pseudoisoeugenol tiglate or angelicate (isomers)
Furanocoumarins: ps = psoralen; ip = isopimpinellin; im = imperatorin; op/he = oxypeucedanin or heraclenin (isomers); an = angelicin; sp/ib = sphondin or isobergapten (isomers)
Isocoumarins and coumarins: mm = 6-methoxymellein; os = osthol; gr = graveolone
Phthalides: se = sedanenolide; bp = butylphthalide
Approximate concentrations: – = < 10ng/gle; ± = 10–100 ng/gle; + = 100–1000 ng/gle; ++ = 1–10 µg/gle; +++ = 10–100 µg/gle
None of these additional compounds were detected in *Carum carvi* and *Daucus carota* "Danvers" (set 1) as well as in *Aegopodium podagraria* (set 2)

set	compounds	phenylpropanoids							furanocoumarins						(iso)coumarins			phthalides	
		c-mi	*t*-ie	me	el	ap	eat/eaa	ept/epa	ps	ip	im	op/he	an	sp/ib	mm	os	gr	se	bp
	identification according to	b	a	a	b	a	b	b	a	a	a	b	a	b	b	a	b	a	a
1	*Anthriscus cerefolium*	–	+	++	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Pimpinella major	–	–	–	–	–	+++[d]	++	–	–	–	–		–	++	–	–	–	–
	Foeniculum vulgare	–	–	±[c]	–	–	–	–	–	–	–	–	–	–	–	–	++	±	
	Anethum graveolens	–	–	–	–	–[e]	–	–	–	–	–	–	–	–	–	–	++	+	–
	Pastinaca sativa	–	–	–	–	–	–	–	+++	++	++	–	+	+	–	–	–	–	–
2	*Apium graveolens*	–	–	–	–	–	–	–	–	++	–	–	–	–	–	–	–	+++	++
	Petroselinum crispum	–	–	–	+	++	–	–	++	+	–	++	–	–	–	–	++	+	–
	Daucus carota "Danvers"	–		–	–									–	±[c]	–			
	Daucus carota "Sytan"	±	±	+	±[c]		–	–	–	–	–	–	–	–	+	–	–		–
	Conium maculatum	–	–	+	+	–	–	–	++	++	–	–	–	–		+	–	+?[f]	–
	Heracleum sphondylium	–	–	+	±	++	–	–	++	+	–	++	±	–		–	–	±?[f]	
	stimulatory activity	+	–[g]	–		–			–	+	+				–	+		–	–
	amount[h]	10mg	10mg	10mg		10mg			1mg	30µg	2mg				1mg	300µg			

[a] retention time and mass fragmentation (standard compound injected)
[b] mass fragmentation
[c] SIM
[d] + small amounts of Epoxy-anol 2-methylbutyrate (100–1000 ng/gle)
[e] traces
[f] possible carry-over from preceding injection of celery fraction
[g] inhibitory effect on oviposition
[h] minimal amount tested that was active (+) or maximal amount tested that was inactive (–) (E. Städler, unpublished)

substituted coumarin identified earlier as an oviposition stimulant in carrot extracts (Städler & Buser, 1984), could only be detected in *Conium maculatum*. Instead *Anethum graveolens*, *Foeniculum vulgare* and *Petroselinum crispum* contained substantial amounts of graveolone, another coumarin, which was first isolated and described from dill plants (Aplin & Page, 1967). The phytoalexin 6-methoxymellein, an isocoumarin, was found in carrots *Daucus carota* and in *Pimpinella major*. *Apium graveolens* was characterised by high levels of phthalides. Falcarinone, a further polyacetylene, was present in some of the samples in trace amounts only detectable by SIM, but not verifiable by full-scan mass spectra.

Discussion

Plant extracts have only rarely been analysed by GC-MS for falcarinol and falcarindiol, which are known to decompose easily (e.g. Städler & Buser, 1984; Mercier et al., 1993). Our study shows that a quantification of polyacetylenes by this method is feasible with certain reservations, e.g. possible non-linearity due to irreversible adsorption (Yates & England, 1982) and a higher detection threshold than for other compounds. Taking these difficulties into consideration, the amounts given in Table 2 probably are minimal values. In any case, the absolute concentrations should be compared among the compound

classes only with caution, since a standard with an exactly known concentration was not available for the polyacetylenes. On the other hand, the relative differences in the amounts detected among the plant species are fairly reliable especially because the variation in the contents ranged more than 2–5 orders of magnitude. Ultimately, the latter was more crucial for the conclusions to be drawn from this study. The data given in Table 2 originated from single GC runs in most cases, yet standard errors of means from injections of two independent samples proved to be quite small in an earlier study using the same method (Städler et al., 1990).

Although there is a wealth of studies dealing with secondary plant compounds in umbellifers, mostly using roots or seeds as a source for the extractions, relatively few comparative studies have included several umbelliferous genera belonging to different tribes (e.g. Berenbaum, 1981; Ceska et al., 1987). Here we present data on leaf surface contents of three characteristic compound classes from twelve species raised under the same or very similar environmental conditions. If we regard components in concentrations below 10 ng/gle as quasi absent, the frequency of the compound classes within the set of plants examined declines in the following order: polyacetylenes, furanocoumarins, propenylbenzenes. C-17 polyacetylenes have earlier been recorded from all the plant species examined in this study with the possible exception of *Anthriscus cerefolium* and *Foeniculum vulgare* (Bohlmann, 1971; Hegnauer, 1973). More recent investigations revealed the occurrence of falcarinol and falcarindiol in *Aegopodium podagaria* (Kemp, 1978) and in *Daucus carota* (e.g. Lund & Bruemmer, 1991; Mercier et al., 1993). Edible parsley root *Petroselinum crispum* ssp. *tuberosum* proved to be a rich source of both compounds, whereas ssp. *crispum* yielded only minor amounts (Nitz et al., 1990). While the falcarinone-type polyacetylenes apparently are more universally distributed among the umbellifers than the other stimulants, they also seem to be most specific of Apiaceae (Städler, 1986), though they also occur in the very closely related Araliaceae (Hansen & Boll, 1986; Boll & Hansen, 1987) and in the Asteraceae (Hegnauer, 1971). Falcarindiol surprisingly has also been reported as a phytoalexin in tomatoes (De Wit & Kodde, 1981). The distribution of furanocoumarins among the species found in this study is in fairly good accordance with earlier investigations inspecting leaf and seed contents respectively (Berenbaum, 1981; Ceska et al., 1987). According to Harborne (1971) phenylpropenes with a 2,4,5-substitution pattern such as in asarone are confined to *Daucus carota*. Our results confirm this notion.

It is well known that the production of allelochemicals in plant tissues is affected by developmental and environmental factors. For example, furanocoumarin content in umbelliferous species has been shown to vary with season (Zobel & Brown, 1990) and with light and nutrition (Zangerl & Berenbaum, 1987). Likewise, changes in furanocoumarin content may arise in response to stress by UV-irradiation or low temperatures (Beier & Oertli, 1983), to pathogen attack (Heath-Pagliuso et al., 1992) and to insect feeding (Zangerl, 1990). Therefore, it is also conceivable that the damage inflicted by the hail upon the young test plants in our study may have influenced the levels in the secondary substances under investigation. However, this is not of relevance here since the main objective was to demonstrate the effect of the semiochemicals on the ovipositional behaviour of the carrot fly, and not to quantify absolute contents of semiochemicals in the respective host-plants. Even acknowledging pronounced variability in response to environmental factors, the general pattern in semiochemical distribution shown by particular plant species is probably quite constant, as both constitutive and induced levels can be expected to be under genetic control (Zangerl & Berenbaum, 1990). For example, linear furanocoumarins were found in foliage of *Petroselinum crispum* together with graveolone in proportions very similar to the ones observed in our study (Beier et al., 1994). Likewise, the partially resistant carrot cultivar "Sytan" invariably exhibited higher concentrations of *trans*-methylisoeugenol and *trans*-asarone than the susceptible cultivar "Danvers" in this and two previous analyses (Guerin & Städler, 1984; Städler et al., 1990). Varietal differences in oviposition stimulants apparently can also be observed with *Apium graveolens*: the polyacetylene content of the cultivar "Balder" was below detection threshold in our study, whereas concentrations in the cultivar "Tropa" were found to be higher than those in carrots (Städler et al., 1990). This deficiency in polyacetylenes may be specific of the cultivar "Balder" and may explain its relatively low acceptability compared to other celeriac cultivars.

The variation in the stimulatory activity of fraction 3.1 seems to be reasonably accounted for by a synergistic interaction of the six known oviposition stimulants. Surveying blends of oviposition stimulants actually occurring in host plants, we can thus confirm earlier findings obtained by testing mixtures of pure compounds (Städler & Buser, 1984). Some unexplained differences in the numbers of eggs received (e.g. among *Apium graveolens* and *Petroselinum crispum*) may be due to additional components of known or unknown activity (Table 4), to unidentified constituents or to early eluting ($T_e < 160$ °C) compounds not analyzed (e.g. monoterpenes, further phenylpropenes). The non-preference of the carrot flies for the fractions of *Anethum graveolens*, *Foeniculum vulgare* and *Pimpinella major* provides circumstantial evidence for the lack of stimulatory activity of graveolone and the pseudoisoeugenols, respectively, which have not yet been tested as pure compounds. It should also be kept in mind that the relationship among the amounts of semiochemicals (on a logarithmic scale) and the behavioural response not necessarily needs to be linear. There might be optimal concentrations for particular stimulants or optimal ratios among different compounds.

The oviposition stimulants quantified in this study are perceived by olfactory sensilla on the carrot fly's antennae, but not by contact chemoreceptors of the tarsal sensilla (Städler & Roessingh, 1991). The synergistic effect of the compounds implies that there are probably at least two distinct receptors for the stimulants. Since none of the plants exhibited high levels of both phenylpropenes and furanocoumarins, we do not know whether these two compound groups also synergize in eliciting egg-laying and are bound to different receptors. The apparently higher ovipositional response to phenylpropenes than to furanocoumarins could be due to differences in receptor sensitivity. Alternatively, it could also be an artefact caused by an interaction among the paraffin covering the surrogate leaves and the oviposition stimulants, which potentially leads to varying release rates for different compounds.

While the known oviposition stimulants appear to be responsible for the varying activities of fraction 3.1, they do not properly reflect the differences in acceptability observed among real leaves. Hence we are still far away from a complete understanding of the factors governing host choice of the carrot fly. Further stimulatory compounds eluting into the methanol fraction of the hexane extract seem to play an important role in host acceptance. Moreover, it has been found that polar deterrents present in host plants potentially also affect oviposition preferences (Degen and Städler, in prep.). As long as these additional semiochemicals remain unidentified, it is difficult to assess the full ecological significance of the stimulants dealt with in this paper. Future research projects should concentrate on the identification of further behaviourally active compounds effective in this insect-plant relationship as well as on the sensory physiology underlying host recognition and acceptance. The existing evidence strongly suggests that the carrot fly is another example of an insect whose host choice is based on the integration of a whole complex of chemical and non-chemical plant stimuli (Städler, 1992).

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General Discussion

Semiochemicals and host selection by the carrot fly

Host finding, a topic not studied in this thesis, necessarily is a critical step in the behavioural sequence leading to oviposition and can contribute to variation in susceptibility to carrot fly attack in nature, provided that the hosts differ in "findability". Some plants may escape the "attention" (in the sense of Bernays, 1996) of the flies merely because of small size or inconspicuous morphology when standing within a plant assemblage ("non-apparent" plants, Feeny, 1976). It is also possible that the amounts of attractants released by the plant varies among the host species. Although the olfactory receptor cells in the antennal sensilla are highly sensitive to certain fairly host-specific volatiles such as *trans*-asarone (Guerin et al., 1983), it is questionable whether the carrot flies can precisely locate their hosts by anemotactic flights from a distance. On the one hand, the quantity of phenylpropenes required to increase trap catches in the field was probably by several orders of magnitude higher than what is naturally emitted by undamaged host plants (Städler, 1992). On the other hand, non-host odours apparently can have a masking effect and thus make oriented movements towards a source of host-odour difficult for the carrot flies (Nottingham, 1987), unless this source is very large and uniform (e.g. a carrot field). So we may assume that characteristic volatiles possibly enhance the likelihood of encounters with hosts by attracting the flies towards vegetation that comprises patches of host plants, while the landings on hosts interspersed within non-hosts probably occur more or less at random. This notion seems to be corroborated by my observations of caged flies, which did not alight more frequently on surrogate leaves treated with host-plant extracts than on surrogates treated with solvent alone (chapter 4.1). Besides, host finding may be facilitated by the low tendency of the carrot flies to disperse after emergence (Städler, 1972). The hypothesis that volatile compounds are only of subordinate importance for host-plant resistance is further confirmed by the fact that foliar vapours of the partially resistant carrot cultivar "Sytan" were more attractive than that of the susceptible cultivar "Danvers" (Guerin & Städler, 1984). In agreement, the former cultivar was repeatedly shown to exhibit higher levels of propenylbenzenes (Guerin & Städler, 1984; Städler et al., 1990; this study, chapter 5.3). Since host-plant attractants may also function as oviposition stimulants (see Table 1), a strict distinction between host finding and acceptance might be somewhat artificial.

The results of this thesis support the idea that stimulatory compounds perceived by the carrot fly when it touches the plant surface – volatiles accumulated in the boundary layer of the leaves and non-volatiles – are the key to the understanding of the different acceptabilities shown by the various hosts. Examining a wider range of host-plant species, I obtained similar results as have been earlier reported from studies of carrot plants: a) the known oviposition stimulants act together in a synergistic manner; b) alone they cannot adequately explain host choice of the carrot fly. In addition, I could present evidence for yet unidentified, more polar stimulatory compounds, which are supposed to be non-volatile and appear to be more relevant to antixenotic host-plant resistance. This is not surprising, since non-volatile oviposition stimulants are known to be of primary importance to host acceptance in other fly species as well (e.g. Baur et al., 1996). Therefore, it would have been rather an exceptional situation, when volatile compounds present in the headspace of the foliage and perceived by olfactory sensilla on the antennae were alone responsible for the oviposition preferences of the carrot fly. Indeed, the outcome of ablation experiments and of electrophysiological recordings using methanolic extracts of carrot foliage already suggested that contact chemoreceptors in the tarsal D-hairs might be involved in the perception of host-plant stimuli, too (Städler, 1977; Städler, 1982). It remains to be verified whether this response can be ascribed to the compounds that account for the stimulatory activity detected in fraction 4 of the hexane extracts (chapter 5.2).

Insects associated with cruciferous plants invariably seem to be stimulated for oviposition or feeding by glucosinolates, a group of compounds contained in members of this plant family (Städler, 1992). The glucosinolates may allow the insects to discriminate between non-crucifers, which are devoid of these allelochemics, and crucifers. However,

the acceptability of particular crucifer species to a herbivorous insect (e.g. flea beetles) generally does not depend on a particular pattern of glucosinolates, but rather on other stimulatory (e.g. flavonol glycosides) or inhibitory (e.g. cardenolides) compounds (Nielsen, 1990). It is tempting to assume an analogous situation for the carrot fly: the mixture of identified oviposition stimulants as "chemical search image" (Atema et al., 1980) enables the carrot fly to distinguish among umbelliferous and non-umbelliferous plants, while the not yet identified polar stimulants and deterrents – possibly less specific for the host-plant family – mediate the oviposition preferences for particular species within the umbellifers. The combination of propenylbenzenes (*trans*-asarone, *trans*-methylisoeugenol), furanocoumarins and C17-polyacetylenes is characteristic of the umbellifers (Städler, 1986). Yet, in all host species analysed, at least one group of these stimulants, mostly the propenylbenzenes or furanocoumarins, rarely the polyacetylenes, were only present in very small amounts – often below the detection threshold for GC-MS – and hence perhaps not perceptible to the carrot fly (chapter 5.3). This was especially intriguing in the case of garden chervil, *Anthriscus cerefolium*, which contained only moderate quantities of polyacetylenes and almost completely lacked the two other compound groups, but still was one of the most acceptable host plants. Unless the yet unidentified stimulatory compounds are typical of umbellifers as well, host recognition must rely in such cases primarily on the C17-polyacetylenes as positive host-specific stimuli. This may be feasible, as these compounds are more distinctive of the host-plant family and are still active at lower concentrations than the other stimulants (Städler & Buser, 1984). In the rare cases of non-umbellifers containing polyacetylenes of the falcarinol type (e.g. tomatoes, De Wit & Kodde, 1981), it is likely that inhibitory compounds may eventually cause avoidance by the carrot fly. Ethanolic respectively methanolic extracts of *Brassica oleracea* and *Tanacetum vulgare*, two of the non-host species tested in my oviposition assays, were shown to reduce oviposition, when sprayed onto carrot foliage (Schöni et al., 1987; Luisier, 1989). It seems justified to postulate that the occurrence of polar deterrents is widespread among non-hosts and according to my results (chapter 5.1 and chapter 5.2) maybe also among hosts. The significance of deterrents for host specificity of the carrot fly needs further verification. I often have implicitly equated umbellifers with hosts and non-umbellifers with non-hosts. However, some umbelliferous plants are "quasi" non-hosts (e.g. *Pimpinella major*) and a few non-umbelliferous plants might be acceptable for oviposition and suitable for larval development under extraordinary circumstances (e.g. *Cichorium intybus*, Van't Sant, 1961). Consequently, hosts and non-hosts do not represent two discrete entities from the perspective of the carrot fly, but rather a continuum from totally rejected to highly acceptable plants.

In most herbivorous insects strong ovipositional responses are only generated by "exquisitely blended mixtures" of host-plant allelochemics (Städler, 1992). This is nicely exemplified by the multicomponent system of oviposition stimulants for the swallowtail butterfly *Papilio xuthus*: an artificial combination of ten compounds, none of which was active by itself, was as stimulatory as the original methanolic extract prepared from leaves of the rutaceous host plant (Ohsugi et al., 1991). Comparable conditions are found in the carrot fly, as is illustrated by the survey of all semiochemicals that are known to influence behaviour or physiology of adult flies and larvae (Table 1). Along with the six oviposition stimulants characterized by Städler & Buser (1984) some closely related compounds have been demonstrated to elicit egg-laying, e.g. the furanocoumarins isopimpinellin and imperatorin, whereas other structural analogues were inactive, e.g. pimpinellin (E. Städler, unpublished). Synergistic interactions among the semiochemicals occur at several levels. A blend of *trans*-asarone and hexanal was more effective in attracting flies to traps than either compound singly (Guerin et al., 1983). Only when combined, the six identified oviposition stimulants proved as stimulatory as the crude carrot leaf extract, from which they were isolated (Städler & Buser, 1984). Furthermore, my results suggest that an unknown number of non-volatile stimulants and deterrents liable to be perceived by taste receptors supplements the many compounds perceived by olfaction, thereby making the system even more intricate. The slight stimulatory effect of apigenin-7-O-β-D-glucoside (E. Städler, unpublished) prompts me to speculate whether the stimulatory activity detected in the polar fractions of the host-plant extracts (chapter 5.2) might be attributable to flavonoids.

Table 1. Semiochemicals known to influence behaviour or physiology of carrot fly adults and larvae

Life stage	insect response	effect	identity of active compounds	compound group	sensory organs	putative relevance as factor for host-plant resistance
adult fly	host finding	attractants	hexanal, heptanal, (*E*)-2-hexenal (Guerin et al., 1983)	green leaf aldehydes	olfactory antennal sensilla (hexanal, (*E*)-2-hexenal) (Guerin & Visser, 1980)	low (Guerin & Städler, 1984)
			t-methylisoeugenol, *t*-asarone (Guerin et al., 1983)	propenylbenzenes	olfactory antennal sensilla (Guerin et al., 1983)	low (moderate?) (Guerin & Städler, 1984)
		repellents	none known			
	host acceptance	oviposition stimulants	*t*-methylisoeugenol, *t*-asarone (Berüter & Städler, 1971; Guerin et al., 1983; Städler & Buser, 1984)	propenylbenzenes	olfactory antennal sensilla (Guerin & Visser, 1980; Guerin et al., 1983; Städler & Roessingh, 1991)	moderate (Visser & de Ponti 1983; Guerin & Städler 1984; Städler et al. 1990; this study)
			4-allylanisol = estragol = methylchavicol (Städler, 1972)	allylbenzene	olfactory antennal sensilla (Guerin & Visser, 1980; Guerin et al., 1983)	low?
			anisaldehyd (Städler, 1972)		olfactory antennal sensilla (Guerin & Visser, 1980)	low?
			bergapten, xanthotoxin, isopimpinellin, imperatorin, athamantin (Städler & Buser 1984; Städler, unpublished)	furanocoumarins	olfactory antennal sensilla (Bergapten) (Städler & Roessingh, 1991)	moderate (this study)
			osthol, ostruthin (Städler & Buser 1984; Städler, unpublished)	substituted coumarins	olfactory antennal sensilla (osthol) (Städler & Roessingh, 1991)	low (moderate?) (this study)
			falcarindiol, falcarinol, falcarinone, 1,8-pentadecadiyne (Städler & Buser, 1984; Städler, unpublished)	polyacetylenes	olfactory antennal sensilla (Städler & Roessingh, 1991)	moderate (Städler et al. 1990; this study)
			oleic acid (Städler & Buser, 1984)	acids	?	?
			apigenin-7-O- -D-glucoside (Städler, unpublished)	flavonoids	(tarsal contact chemoreceptors?) (Städler, 1982)?	?
			unidentified (this study)	polar compounds	(tarsal contact chemoreceptors?) (Städler, 1982)?	high (this study)
		oviposition deterrents (repellents?)	p-anisic acid? isoeugenol? methylnonylketon? (Städler, 1972)			low?
			unidentified (this study)	polar compounds	(tarsal contact chemoreceptors?) (Städler, 1982)?	moderate? (this study)

Table 1. Continued

larva	host finding	attractants	carbon dioxide			low
			methyleugenol (Jones & Coaker, 1977; Jones & Coaker, 1979)	allylbenzene		?
			bornyl acetate, 2,4-dimethyl styrene, -ionone, -ionone, biphenyl (Ryan & Guerin, 1982)			moderate (Guerin & Ryan, 1984)
			falcarinol (Maki et al., 1989)	polyacetylene	olfactory ampullaceous sensillum (also falcarindiol, falcarindiol monoacetate) (Maki et al., 1989)	moderate
			(±)-2-methoxy-3-*sec*-butylpyrazine (Maki et al., 1989)			?
		repellents	*t*-2-nonenal (Ryan & Guerin, 1982)			low (moderate?) (Chamberlain et al., 1991)
	host acceptance	feeding stimulants	none known			
		feeding deterrents	none known			
	growth and development	role for sclerotization at ecdysis?	chlorogenic acid (Cole, 1985)			unclear (cause or effect?) (Cole et al., 1987)
		çantibioticsé, insecticidal properties	*t*-2-nonenal (Guerin & Ryan, 1980)			low (moderate?) (Chamberlain et al., 1991)

Given the enormous diversity of secondary plant compounds discovered in the Apiaceae (see the compilation of Hegnauer, 1962–1996) and the already considerable complexity of the chemical signals identified so far, one could get the impression that the search for the whole set of semiochemicals involved in this insect-plant relationship is an endless task. Yet, the number of relevant plant compounds has a natural limitation that lies in the capacity of the insect's sensory system, which is relatively simple compared to that of a vertebrate. A fly possesses some hundreds of chemosensory hairs at the tarsi, mouthparts and antennae, occasionally also at other organs (e.g. ovipositor). These sensilla belong to relatively few functional types and contain only few receptor cells (two to four in olfactory sensilla, Kaib, 1974; four in gustatory sensilla, Hanson, 1987), which are sensitive to a variable, but finite set of compounds. Thus the number and specificity of the existing receptor sites set an upper limit to the range of perceptible compounds. In spite of this paucity of receptors, the sensory system of the fly can very efficiently cope with a multitude of chemical signals (Dethier, 1971). This versatility is achieved by the sensory coding and the integration of the chemical information in the nervous system. Receptors highly specific for particular compounds ("labelled lines") and several receptors with overlapping spectra of sensitivity ("across fibre patterns") can be viewed as two extremes in a continuum of modes to code chemical signals (van Loon, 1996). At the molecular level, the key to the understanding of chemically mediated host-choice lies in the structure-activity relationships of the semiochemicals bound to the receptor proteins.

Our knowledge about chemoreception in the carrot fly is still limited. The oviposition stimulants quantified in this study are supposed to be present in the boundary layer surrounding the leaves, as they are perceived by antennal sensilla, but not by contact chemoreceptors of the tarsal sensilla (Städler & Roessingh, 1991). Nothing is known about the specificity of single cells, single sensilla or groups of sensilla. Hence the following assumptions are only inferential. The non-additive, but synergistic interaction among the oviposition stimulants implies that there should be minimally two different receptor sites for the stimulants (plus an additional one for the non-specific green leaf volatiles such as hexanal). Since none of the plants, which I tested, contained high amounts of both phenylpropenes and furanocoumarins, it is unclear whether these two compound classes also synergize in eliciting egg-laying and are bound to different receptor sites. A full factorial oviposition assay including all possible artificial combinations of pure compounds belonging to the three groups could help to solve this question. An aromatic core is common to all oviposition stimulants identified so far except the polyacetylenes. It seems plausible that the configuration of the side chains attached to this benzene ring are crucial for the binding of the molecule to the receptor. For example, the propenylbenzenes *trans*-methylisoeugenol (but not isoeugenol) and *trans*-asarone are stimulants, whereas most allylbenzenes tested so far showed no stimulatory activity. The isomer pair anethole (= propenylanisole; inactive) and estragole (= allylanisole; stimulatory) does not fit into this scheme, though (Städler, 1972), a phenomenon that needs to be validated, also because p-allylanisole elicited inconsistent electroantennogram activity relative to *trans*-methylisoeugenol in two studies (lower, Guerin & Visser, 1980; equal, Guerin et al., 1983). *Trans*-asarone evoked higher EAG responses than its *cis*-analogue, *trans*-methylisoeugenol higher than the corresponding allyl counterpart methyleugenol (Guerin et al., 1983). It is noteworthy that methyleugenol, which has no stimulatory effect in the adult fly (at the tested concentration in ± pure form), is an attractant for the larvae. Larvae and imagines apparently diverge in their sensitivities to host-plant compounds to some degree. Up to now, the C17-polyacetylenes are the only behaviourally active compounds known to be shared by adult flies and larvae (Table 1).

The profiles of the known stimulants vary widely among the diverse host-plant species (chapter 5.3). The same applies to the importance of these substances relative to the unknown polar stimulatory compounds (e.g. high in carrot, *Daucus carota*, and hemlock, *Conium maculatum*, but low in caraway, *Carum carvi*; chapter 5.2). This cautions against conclusions drawn from studies in oligophagous insects that cover solely one typical host species. For example, the pattern found in carrot is rather anomalous, insofar as the propenylbenzenes *trans*-methylisoeugenol and *trans*-asarone, notably the first identified oviposition stimulants for the carrot fly, seem to be largely restricted to this host genus. Usually behaviourally active compounds are first

isolated from a prevalent host-plant species and their ecological significance for mediating oviposition or feeding preferences is established only later on. Starting with a comparison of the activity of extracts from a variety of host plants, I have followed up the opposite approach. Within the scope of this thesis it was not possible to identify the stimulatory compounds of the polar fraction, which accounted fairly well for the differential antixenotic resistance among the host species. Yet, the foundation is laid for promising future research projects.

I did not investigate chemoreception of the carrot fly with electrophysiological techniques. Without profiting from these methods, it will be difficult to achieve further progress in the understanding of the mechanisms of host selection in this species. In view of the multiple purification steps required to isolate stimulants and deterrents, the elucidation of the modes by which these compounds are perceived may prove advantageous. Indeed, screening for active compounds often is only feasible with reasonable effort when behavioural assays can be complemented with electrophysiological recordings (e.g. the identification of the host marking pheromone of the cherry fruit fly, *Rhagoletis cerasi*, or of CIF, an oviposition stimulant for the cabbage root fly, *Delia radicum*, respectively, Städler et al., 1994; Roessingh et al., 1997). Along with the identification of the still unknown polar stimulants and deterrents, another rewarding objective could be to establish the sensitivity and specificity of antennal receptors for the identified oviposition stimulants by carrying out GC-EAGs and recording from single sensilla, combined with appropriate oviposition assays. Practically nil is our knowledge about chemical factors (allelochemics and nutrients) affecting survival and growth of the larvae (Table 1). Unfortunately, no artificial rearing medium for carrot fly larvae is available at present, which would allow to test the antibiotic effects of plant extracts and pure compounds originating from unsuitable umbellifers (e.g. *Smyrnium olusatrum, Pimpinella major*).

The significance of non-chemical plant traits for host finding and acceptance

Leaf morphology seems to be more variable among the plant taxa than other physical characters of foliage. Correspondingly, fly species associated with different plant families diverge more in their ovipositional responses to specific leaf shapes than to other non-chemical plant traits such as leaf colour (chapter 4.1, chapter 4.2). The preference of the carrot fly for compound leaves, which are typical, but by no means unique of the umbelliferous hosts, may contribute to increase the probability of "correct" host-plant recognition, particularly in cases where the available chemical information is not completely unambiguous (see previous section). To some extent, the carrot flies seem to be able to visually discriminate among leaves of different shapes before landing, which potentially allows them to roughly distinguish among broad plant categories. For example, it is conceivable that the flies enhance their efficiency in host finding by avoiding to alight on plants with narrow blades (e.g. grasses) or with other non-pinnate leaves. Such an increased discovery of host plants has been shown for some readily observable butterflies that develop search images based on visual perception of leaf shapes (e.g. Rausher, 1978). There is no evidence, that the more subtle differences in leaf morphology among the umbelliferous host plants substantially contribute to variation in acceptability. Hence non-chemical plant traits such as foliar form are of low value as selection criteria for breeding less susceptible carrot cultivars.

In conclusion, the data presented in the chapters 4.1 and 4.2 suggest that perception of leaf shape by whatever modality (mechanoreception, vision) may indeed have a certain adaptive significance in the carrot fly. However, in contrast to the influence of chemical plant properties, any direct or indirect impact of leaf morphology on larval survival and growth is hardly imaginable. That is why the preference for pinnate leaves is liable to be rather an effect than an evolutionary cause of the specialized feeding habit.

I have mostly examined the influence of several non-chemical and chemical cues separately. However, the manifold stimuli originating from the plant affect the ovipositional behaviour of the carrot fly in concert. Full factorial assays comprising different combinations of visual, tactile and chemical stimuli have been recommended to reveal possible interactions among the sensory modalities (Harris & Foster, 1995).

Comparative aspects and a tentative evolutionary outlook

Along with the carrot fly (Psilidae) umbellifer specialists can be found in two other families of the Diptera, viz. in leaf mining Tephritidae (celery fly, *Philophylla heraclei*) and Agromyzidae (several members of the large genus *Phytomyza*), in the Coleoptera (Curculionidae; Chrysomelidae) and in the Lepidoptera, which contribute the highest number of species, most prominently in the families Papilionidae and Oecophoridae. The hemimetabolous insects are relatively underrepresented in the umbellifer fauna; noteworthy are only several taxa in the Homoptera (Aphidoidea; Psylloidea) and a handful of species in the Heteroptera (Berenbaum, 1983; Berenbaum, 1990). No obvious convergence is detectable among the preference hierarchy of the carrot fly for various umbelliferous hosts (chapter 3.1 and 3.3) and the preference rankings reported for two other umbellifer-feeding insects, namely the swallowtail butterfly, *Papilio machaon* (Wiklund, 1975) and the carrot psyllid, *Trioza apicalis* (Nehlin et al., 1996; I. Valterova, G. Nehlin & A.-K. Borg-Karlson, unpublished manuscript). This not really unexpected finding indirectly indicates that host-plant selection in these species is not mediated by identical sets of semiochemicals occurring in the respective hosts. Indeed, the essential host recognition cue for *Trioza apicalis* is supposed to be a combination of monoterpene hydrocarbons (Nehlin et al., 1996), whereas the black swallowtail butterfly, *Papilio polyxenes*, closely related to *Papilio machaon*, responds to polar odor constituents, monoterpenoids with oxygenated functional groups, but neither to non-polar monoterpenoids nor to propenylbenzenes and furanocoumarins that elicit egg-laying in the carrot fly (Feeny et al., 1983; Baur et al., 1993). In addition to these volatile compounds, *trans*-chlorogenic acid and the flavonoid glycoside luteolin-7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside have been identified as contact oviposition stimulants for *Papilio polyxenes*, which are presumed to be present at the leaf surface (Feeny et al., 1988; Brooks et al., 1996). Larvae of the black swallowtail butterfly and of the carrot fly likewise are not attracted by the same allelochemicals (Dethier, 1941; Jones & Coaker, 1977; Ryan & Guerin, 1982; Maki et al., 1989). The different chemical profiles used by the above insects suggest that various umbellifer specialists may not rely on the same chemical cues for host finding and acceptance. In contrast, the glucosinolates and their volatile fission products, the isothiocyanates, are characteristic positive sign stimuli for a whole range of insects feeding on Cruciferae. Similarly, the onion fly and the leek moth, two oligophagous species on Liliaceae, were both shown to react to disulfides, which are typical of this plant family (for a survey see Städler, 1992). Still, the contention that there is no such common theme for the insects associated with umbellifers, may be somewhat premature as long as even in the two best studied cases, the carrot fly and the black swallowtail butterfly, the full set of chemical stimuli involved in the host-selection process is not yet known. The active principles in the polar fraction that stimulates egg-laying in the carrot fly (chapter 5.2) might eventually turn out to be flavonoids (but then for reasons of solubility rather aglycones than glycosides). I performed a preliminary oviposition assay that gave no evidence for a stimulatory activity of chlorogenic acid, but this result needs to be verified. Interestingly this compound has been postulated as a factor that negatively influences antibiotic resistance of carrot cultivars to attack by carrot fly larvae (Cole, 1985).

Insight into evolutionary aspects of the host associations of psilid flies is compromised by the fact that information on the host ranges and life histories of species other than the carrot fly is extremely scarce and mostly anecdotal. The family Psilidae comprises about 130 species, about 75 per cent of which live in the Holarctic region (Soós, 1984). Further species occur in the Ethiopian and in the Oriental Region (Shewell, 1965; Cogan, 1977). The family is poorly represented in South America and unknown in the Australian Region except for the carrot fly introduced in New Zealand and southern Australia. Psilid larvae are phytophagous, burrowing in roots and stems of herbaceous plants or under the bark of trees, to which they gain access through wounds. In Table 2 I attempted to compile a survey of the published host records. If some of the more dubious and badly documented records are dismissed, e.g. *Carex* for *Psila fimetaria*, *Cakile* for *Psila gracilis* and several uncorroborated hosts for *Psila rosae* (Hardman & Ellis, 1982), the following picture emerges. *Phytopsila carota*, three *Psila* species in the subgenus *Chamaepsila* and one in the subgenus *Psila* are associated with the Apiaceae (= Umbelliferae). Two species in the subgenus

Chamaepsila are affiliated with the Asteraceae (= Compositae), viz. *Psila bicolor* and *Psila nigricornis*. The latter species possibly also feeds on carrot and apparently is very closely related to *Psila rosae*, from which it is hardly distinguishable. Finally, *Psila nigromaculata* – also classified within the subgenus *Chamaepsila* – was found on a dipsacaceous plant. This putative pattern of host utilization prompts me to put forward the following hypothesis: the Umbelliferae are the ancestral host plant family of the genus *Psila* and of the closely related genus *Phytopsila* with two – possibly independently occurring – host shifts to the Asteraceae and one to the Dipsacaceae. This situation would be paralleled by the swallowtail butterflies of the *Papilio machaon* complex: several species within this group feed on Umbelliferae and occasionally on Rutaceae, while one species, *Papilio (machaon) oregonius* is monophagous on *Artemisia dracunculus* in the Compositae (Sperling & Feeny, 1995; Thompson, 1995). In contrast, an ancestral association with Asteraceae and two independent switches to Apiaceae has been regarded as most likely for leaf beetles belonging to the genus *Oreina* (Dobler et al., 1996). Members of the two families Compositae and Rutaceae consistently appear in the host lists of near-relatives of umbellifer specialists (and indeed of umbellifer specialists as well), with *Artemisia* taking a prominent position amongst them (Berenbaum, 1983; Berenbaum, 1990). For the psilid genera other than *Psila* no obvious pattern can be detected, as the recorded host plants belong to several unrelated families. Clearly, host-plant ranges and phylogenetic relationships of the psilid flies await further investigation, before any founded conclusions can be drawn.

Ever since the publications of Dethier (1941) and Ehrlich & Raven (1964) host shifts from one plant family to another are believed to be facilitated by the chemical similarity among the old and the new hosts. Attempted colonizations are likely to be more frequent on plants of similar chemistry, because shared compounds that act as feeding or oviposition stimuli predispose the insect to "mistakes" that could initiate shift of diet. Also, a greater proportion of attempted colonizations tends to be successful on chemically similar plants owing to metabolic preadaptation of the insect to cope with the new source of food. However, it is somewhat uncertain whether chemical similarity as defined by chemists is meaningful in terms of insect physiology or behaviour (Futuyma & Keese, 1992). The Compositae are considered to be phytochemically related to the Umbelliferae (Hegnauer, 1982). Phenylpropenes, C17-polyacetylenes and coumarins, though only rarely furanocoumarins (e.g. bergapten, imperatorin), have been found in Compositae, thus essentially all compound groups to which the known oviposition stimulants for the carrot fly belong (Hegnauer, 1962–1996; Städler, 1986). The host-plant recorded for *Psila nigromaculata* is a member of the Dipsacaceae, which are chemically quite distinct from the Compositae according to Hegnauer (1962–1996). Yet, it is noteworthy that chlorogenic acid regularly and bergapten, notably an oviposition stimulant for the carrot fly, occasionally occur in dipsacaceous plants (Hegnauer, 1962–1996), both compounds that are also characteristic of umbellifers. The two composite species tested in my oviposition assays, *Cichorium intybus* and *Tanacetum vulgare*, did not appear to be more acceptable than the other non-umbelliferous plants (e.g. *Ranunculus repens*, *Potentilla anserina*). However, these experiments were not designed to reveal such differences. It would be interesting to examine whether non-umbelliferous plants that more closely match the chemical and physical cues of host-plants would be preferred by ovipositing carrot flies to other, more divergent non-hosts. The following plants could be candidates for such a study. The rutaceous plants *Ruta graveolens* and *Dictamnus albus (= fraxinella)* contain phenylpropenes (e.g. methylchavicol) and furanocoumarins (e.g. Xanthotoxin, Bergapten); the composite *Artemisia (dracunculus)* contains phenylpropenes (methylchavicol; anethole), simple coumarins (e.g. herniarin; scoparone) and the polyacetylene dehydrofalcarinone (Hegnauer, 1962–1996). Moreover, unlike *Cichorium* these three plant species are distinguished by pinnate or dissected leaves resembling in shape the leaves of umbellifers. On the basis of behavioural observations, *Ruta graveolens* and *Artemisia dracunculus* previously have been classified as non-hosts (Bohlen, 1967). Araliaceae such as ivy, *Hedera helix*, in which falcarinone has been detected, are thought to be very closely related to the Umbelliferae (Hegnauer, 1962–1996), but like many other potentially acceptable plants might grow in habitats that are not frequented by carrot flies or might be unsuitable as hosts because of their growth form.

Table 2. List of host-plant records of psilid flies

fly species	infested plant organs	host family	host species (references)
Psila (*Chamaepsila*) *rosae* (Fab.), carrot fly	roots	Apiaceae	121 species[1,3] (Ellis et al., 1992)
		Asteraceae	*Cichorium endivia*[3], *Cichorium intybus*[3], *Lactuca sativa*[3] (Miles, 1956; Van't Sant, 1961)
		?Brassicaceae	?*Brassica napus*, ?*Brassica rapa* (Hennig, 1941; Hardman & Ellis, 1982)[5]
		?Chenopodiaceae	?*Beta vulgaris* (Hardman & Ellis, 1982)[5]
		?Solanaceae	?*Solanum tuberosum* (Hardman & Ellis, 1982)[5]
		?Poaceae	?*Zea mays*[(3)] (Beirne, 1971)
Psila (*Chamaepsila*) *nigricornis* Meigen, Chrysanthemum stool miner	stools, roots, crowns, new shoots	Asteraceae	*Chrysanthemum*[3], *Lactuca sativa*[3] (Glendenning, 1952; Vernon, 1962)
		?Apiaceae	?*Daucus carota*[4] (Glendenning, 1952; Hill, 1987)[5]
Psila (*Chamaepsila*) *gracilis* Meigen		Apiaceae	*Daucus*[4], *Anthriscus*[4], *Angelica*[4] (Hennig, 1941)[5]
		?Brassicaceae	?*Cakile*[4] (Hennig, 1941)[5]
Psila (*Chamaepsila*) *bicolor* Meigen	roots, root stocks	Asteraceae	*Chrysanthemum*[3] (Osborne, 1955)
Psila (*Chamaepsila*) *nigromaculata* Strobl	stems	Dipsacaceae	"*Scabiosa succisa* L."[3] (= *Succisa pratensis*) (Hennig, 1941)[5]
Psila (*Chamaepsila*) *humeralis* Zetterstedt	in flowers	Apiaceae (not exclusively)	*Heracleum sphondylium* (Sheppard, 1991)
Psila (*Chamaepsila*) *pallida* Fallén	in flowers	Apiaceae (not exclusively)	*Heracleum sphondylium* (Sheppard, 1991)
Psila (*Psila*) *fimetaria* L.	roots	Apiaceae	*Daucus carota*[(1),3], *Anthriscus sylvestris*[1], *Apium graveolens*[2], *Chaerophyllum aureum*[2] (Freuler & Fischer, 1991; S. Fischer, personal communication)
		?Cyperaceae	?*Carex* (Balachowsky & Mesnil, 1936)[5]
Phytopsila carota Iwasa, Hanada & Kajino, red carrot fly	roots	Apiaceae	*Daucus carota*[(1),3] (Iwasa et al., 1987)
Chyliza scutellata Fab. (syn. *Chyliza leptogaster* Panzer)	nut-like woody galls on stems	Rosaceae	*Physocarpus opulifolius* (=*Spiraea opulifolia*)[3] (Hennig, 1941)[5]
Chyliza vittata Meigen	galleries in roots, ("stems")	Orchidaceae	*Neottia nidus-avis*[3] (Hennig, 1941)[5]
Chyliza extenuata Rossi (syn. *Megachetum atriseta* Meigen)	thickened basal part of stems (swollen underground stem)	Orobanchaceae	*Orobanche rapum-genistae*[3], (*Orobanche hederae*) (Hennig, 1941)[5]
Chyliza leguminicolia Melander	pupae found on stems	Fabaceae	*Lupinus polyphyllus*[(3)] (Hennig, 1941)[5]
Chyliza erudita Mel.	pupae found in "pitch" (resin)	Pinaceae	*Pinus strobus*[(3)] (Hennig, 1941)[5]
Loxocera albiseta Schrank	pupae found in stems	Juncaceae	*Juncus*[(3)] (Hennig, 1941)[5]

? dubious, uncorroborated hosts
[1] oviposition in the field
[2] oviposition in insectarium
[3] larval development possible
[4] capture of flies on the plants concerned or in the corresponding cultures
[5] not original reference

Unfortunately, nothing is known about the host-plant preferences of psilids other than the carrot fly. A comparative study on the chemical and non-chemical plant cues determining host choice in different psilid species may be a very worthwhile approach that could shed light on the evolutionary forces behind host shifts in this insect group (e.g. changes in the sensitivities to particular semiochemicals). Along with the carrot fly, the closely related chrysanthemum stool miner, *Psila nigricornis*, and the more distant and considerably bigger "yellow" carrot fly, *Psila fimetaria*, might be an appropriate selection of species for such an investigation. Yet, some practical problems would have to be overcome: it may prove difficult to obtain the flies to start a laboratory culture and a rearing method would have to be developed, too.

Implications for applications

My thesis concentrated on fundamental aspects of the relationship of the carrot fly with its host-plants, yet the very fact that it dealt with an insect that damages vegetable crops suggests that some of the results may be relevant for pest control, at least in the long term. According to Lipke & Fraenkel (1956) the study of host preferences in phytophagous insects constitutes "the very heart of agricultural entomology", a notion that has been repeatedly endorsed (Kennedy, 1965; Städler, 1992). True as it may be in principle, the idea that the development of resistant crops may substantially profit from the knowledge of the resistance mechanisms has remained so far largely a promise in view of the obvious complexity of insect-plant interactions, which do not make them easily amenable to manipulation. Investigations aiming at the breeding of resistant or at least partially resistant crop varieties can proceed along several steps that may or may not necessarily depend on each other:

– Identification of the major factors that cause antixenotic or antibiotic resistance to phytophagous insects. These resistance factors may be non-chemical plant traits, but in most cases are supposed be secondary plant compounds. There has been considerable progress in this field, but still research has rarely advanced to the point where the knowledge of the resistance mechanisms can directly be applied.

– Screening for resistant genotypes. Resistance to insect herbivores can be achieved by actually using the insects as the selective agents (Berenbaum & Zangerl, 1992). Painter (1951) claimed that a knowledge of the resistance mechanisms (e.g. secondary compounds) may sometimes be of little use for attempts to breed resistant varieties. Since the analytical methods are much more sophisticated now than in those days, quantitative chemical analyses for semiochemical involved in resistance are assumed to be a valuable alternative or supplement to the traditional screening methods (e.g. Nielsen, 1990). It has also been proposed to employ electrophysiological techniques in projects monitoring antixenotic resistance (e.g. in *Brassica* crops, Baur et al., 1996).

– Investigations into the modes of inheritance of the resistance traits and location of genes or gene complexes that confer resistance. Simple Mendelian control over secondary metabolism is very likely to be the exception rather than the rule. While single genes may be responsible for the presence or absence of a compound, quantities of secondary compounds often reflect the action of many genes and are more appropriately treated by quantitative genetic methods (Berenbaum & Zangerl, 1992). Two recent examples involve genes for resistance to the Colorado potato beetle (Yencho et al., 1996) and genes controlling the accumulation of maysin, an antibiotic factor in maize silks effective against the corn earworm (Byrne et al., 1996).

– Introgressing genes into breeding lines. When there is not enough heritable variability in resistance available for selection, attempts can be made to introduce resistance genes from other plants into the crop varieties, either by traditional breeding methods (e.g. crosses with closely related resistant wild types) or with genetic engineering. The latter approach has the advantage that only desirable characters are transferred, but requires a profound knowledge of the genes controlling the resistance factor. For example, a nematode-resistance gene coding for a protein involved in the interaction between host and pathogen has been found in wild beet and might be engineered into major commercial crops (Cai et al., 1997). However, similar achievements might be more difficult

for quantitatively inherited resistance factors such as the accumulation of end-products of complex biochemical pathways.

Subjecting partially resistant carrot cultivars to recurrent selection in the field and producing inbreds in single seed programs has only led to slightly increased levels of resistance (Ellis & Hardman, 1990). The same applies to hybrids produced between cultivated carrots and *Daucus capillifolius* (Ellis et al., 1993). Screening seedlings for lower content in chlorogenic acid according to the intensity of radicle fluorescence was problematic, because it was unclear whether a high concentration of this compound causes increased susceptibility in carrots or is rather an effect of carrot fly attack (Cole et al., 1987). It is questionable whether carrot cultivars and compatible wild *Daucus* species possess the genetic potential for more than only partial resistance, which of course is valuable in itself as less insecticides need to be applied for the same protective effect. Further improvements may be achieved by the introduction of resistance genes from more distant relatives of the carrot. This might only be feasible with genetic engineering. But then the question arises what should be engineered? Since our understanding of the resistance mechanisms is still fairly rudimentary, I can only put forward some vague speculations based on the results of my thesis.

Antixenotic resistance to adult flies seems to be better understood than antixenotic and antibiotic resistance to larvae. Changing the quantities of the known oviposition stimulants does not seem to be a valid approach, as these semiochemicals do not adequately account for variation in the acceptability among the host plants (chapter 5.2). Furthermore, reduced levels of stimulatory compounds that potentially play a role in plant defense may result in higher susceptibility to fungal diseases or other pathogens. Such "trade-offs" clearly should also be kept in mind in the opposite situation, when it is intended to breed carrot cultivars with higher levels of antifungal compounds (e.g. falcarindiol) as has been suggested for example by Mercier et al. (1993). The yet unidentified stimulatory compounds correlate better with antixenotic resistance (chapter 5.2), but here again the same argument might hold true as with the known stimulants. Breeding cultivars with higher contents of oviposition deterrents intuitively appears to be a more promising approach. However, as Feeny (1992) cautions: "It would be foolish to base pest-control strategies on deterrents, whether applied externally or introduced into crops by genetic engineering, unless deterrency is reinforced with other forms of resistance, or unless agricultural practices are modified to reduce plant apparency". In large uniform carrot fields the insect's opportunity to exercise choice is limited. Behavioural responses sometimes may differ between free-choice assays as performed in this study and no-choice assays (Tingey, 1986). Antibiosis appears to be a more desirable form of resistance than antixenosis. Antixenotic and antibiotic resistance to carrot fly attack do not seem to be very tightly linked to each other (Guerin & Ryan, 1984; Maki & Ryan, 1989; this study, chapter 3.3). The coincidence of strong deterrency with antibiosis in *Pimpinella major* leaves open the possibility that these phenomena have a common underlying cause in this species, though. It remains to be seen whether the considerably higher inhibitory activity found in *Pimpinella major* is due only to quantitative or also to qualitative differences from the other host plants. The identification of the yet unknown behaviourally active compounds clearly is the next hurdle to be taken before any further conclusions can be drawn about the direction to pursue. Intraspecific variability in the host-selection behaviour of the adult flies and in the performance of the larvae also has to be considered, as information about heritable variation in these traits is important to judge the prospective success of a particular breeding strategy. While there are probably no theoretical reasons arguing against solutions of this agricultural problem that involve genetic engineering, the practical obstacles to be overcome, i.e. the enormous research efforts necessary for such an achievement, may be at present exceedingly high in consideration of the comparatively limited economic importance of an oligophagous insect injurious to only a few vegetable crops.

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Curriculum vitae

Personal Data

Name:	Thomas Degen
Date and Place of Birth:	28 May 1963, Basel
Nationality:	Swiss
Languages:	German, English, French

Education:

1970 – 1975	Primary school in Oberwil, Switzerland
1975 – 1979	Progymnasium (secondary school) in Oberwil, Switzerland
1979 – 1982	Gymnasium (highschool) in Oberwil, Switzerland
1982 – 1989	Studies in Biology (Zoology and Botany) and Chemistry (secondary subject) at the University of Basle, Switzerland. Diploma in Biology with a thesis on habitat selection and food of passerine birds during autumn migration
1991 – 1997	Postgraduate student at the University of Basle. Ph.D. thesis at the Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture in Wädenswil

Professional Experience:

1989 – 1991	Research associate at the Swiss Ornithological Station in Sempach and at the Natural History Museum in Basle (September – December 1990). Collaboration in several projects: – Telemetry project on habitat utilisation of tits in a deciduous forest – Projects on bird migration: habitat selection and food of passerines during migration; registration of nocturnal migration with tracking radar – Photographic documentation, data collection and data analysis for the book: Jenni. L. & R. Winkler, 1994. Moult and Ageing of European Passerines. Academic Press, London
1991 – 1997	Research associate at the Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture in Wädenswil. Collaboration in several projects: – Monitoring of thrips with sticky traps (July – August 1991) – Screening of different carrot breeding lines for antibiotic resistance to carrot fly, *Psila rosae* (collaborative project with a Dutch seed producing company) – Electrophysiological studies with the tephritid fruit flies *Rhagoletis pomonella* (January – April 1992; January – April 1994) and *Anastrepha ludens* (January – April 1995)

Publications:

Degen, T. & L. Jenni, 1990. Biotopnutzung von Kleinvögeln in einem Naturschutzgebiet und im umliegenden Kulturland während der Herbstzugzeit. Der Ornithologische Beobachter 87: 295–325.

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Forthcoming publications

Degen, T., G. Poppy & E. Städler. Extracting oviposition stimulants for the carrot fly from host-plant leaves. Submitted to Journal of Chemical Ecology.

Degen, T., H.-R. Buser & E. Städler. Patterns of oviposition stimulants for the carrot fly in leaves of various host plants. Submitted to Journal of Chemical Ecology.

Degen, T., E. Städler & P. R. Ellis. Host-plant susceptibility to the carrot fly, *Psila rosae*. 1. Acceptability of various host species to ovipositing females. Submitted to Annals of Applied Biology.

Degen, T., E. Städler & P. R. Ellis. Host-plant susceptibility to the carrot fly, *Psila rosae*. 2. Suitability of various host species for larval development. Submitted to Annals of Applied Biology.

Degen, T., E. Städler & P. R. Ellis. Host-plant susceptibility to the carrot fly, *Psila rosae*. 3. The role of oviposition preferences and larval performance. Submitted to Annals of Applied Biology.

During my studies I attended lectures and courses given by the following lecturers:

W. Arber, P. Baertschi, C. Baroni-Urbani, Th. Boller, B. Bruderer, E. De Robertis, P. Duelli, M. Ermini, B. Erni, T.A. Freyvogel, W.J. Gehring, U. Gisi, R.H. Gisler, C.A. Grob, R. Guggenheim, H.-Ch. Im Hof, U. Halder, H. Hecker, C.L. Hottinger, E. Kellenberger, D.B. McKey, F. Meins, J. Meyer, B. Naef, W. Pankow, U. Rahm, H. Reichert, J.B. Rosenbusch, H.F. Rowell, G. Schatz, V. Schmid, P. Schmid-Hempel, D.G. Senn, U. Séquin, H. Sigel, S.C. Stearns, W. Stingelin, H.R. Striebel, Ch. Tamm, A. van Noordwijk, H.P. von Hahn, C.M. Wall, D. Walz, A. Wiemken, H. Zoller

The End

Actors

The Good	Carl Roth
	Dilly the Kid
	Yves St.Pasternac-Sylvestre
	Peter Sly "Stallone"
	Hemlock Shores
	Weedy Hedgehog
	Verchil Gardner
	"The Great Stonebreaksby" alias Sunny Burnet
	Nel Fen
	Carl A. Wayne
	Don Apio Graveolone
	Lady Fernie
	Chick Korea
The Bad	"The Fly" alias Rosa Epsilon
The Ugly	"The Pee-counter" alias Tom Floret Dumb

Driving Mr. Dumb
"Car" Stan Hippie
Rolf Brave Heart

Mr. Dumb's Psychiatrist
Conny Clown

Scientific and Technical Advisors
Sir Popper, Guy of Rothamsted
Bob Farmr

Microwave stunts
Tom Floret Dumb himself

Massy spectral effects
John-Rod Buzzer

Layout
Chaos Production L**TD**

Screenplay
Richie Stadler

directed by
Tom Bolero
Dean Martine Rahier

produced by
De Gen Manipulation inc.

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Few animals, but many plants were harmed during this filmsy project.