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Exploration and assessment of the oviposition substrate by the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae)

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Abstract. Oviposition behaviour of *Delia radicum* is not only influenced by host plant quality but also by the quality of the substrate in which the plant grows. Direct behavioural observations showed that the females partition their visits to a host plant (cauliflower) into ovipositional bouts separated by exploration of the host plant surface. Ovipositional bouts were further partitioned into acts of egg deposition separated by exploration of the substrate. While the mean number of ovipositional bouts per visit (2.6), and eggs laid per egg deposition event (1.4) were stable, the mean number of egg deposition events per ovipositional bout significantly varied (from 2.1 to 7.3) with the quality of the substrate and the physiological state of the female (egg load). Ovipositing females adjusted the final number of eggs laid around the plant during the behavioural stage of substrate exploration. Additional experiments using plant surrogates treated with methanolic extract of *Brassica* leaves mounted in different substrates showed that: (a) the presence of living *Brassica*, *Hordeum* or *Allium* roots in a substrate enhances the number of eggs laid into this substrate, but females do not discriminate between the different plants; (b) females avoid both wet and dry substrates and prefer the substrates with a dry surface and moist particles directly accessible at a depth of about 5 mm; (c) substrates rich in organic matter are preferred to sand; (d) olfactory perçeption of volatile chemicals from the substrate must at least partially be responsible for the differences in oviposition in various substrates.

INTRODUCTION

The chain of activities of a herbivorous insect foraging for a suitable host plant can be viewed as a sequence of behaviours including finding, examining and consuming (sensu Miller & Strickler, 1984). All of these behaviours have been studied in some detail in the cabbage root fly, *Delia radicum* (L.) (Diptera: Anthomyiidae) (reviewed by Nottingham, 1988; Roessingh et al., 1992). The finding behaviour is dominated by the optomotor-guided anemotaxis elicited by olfactory signals which emanate from host plants of the Cruciferae family (Finch & Skinner, 1982; Nottingham, 1988) and, closer to the plant, also by its visual characteristics (Prokopy et al., 1983). Non-volatile, host plant-specific chemicals play a major role during examination of the plant surface (Traynier, 1967; Roessingh et al., 1992; 1997; Baur et al., 1996a, 1998; Hurter et al., 1999). However, acceptance is also significantly influenced by other plant characteristics such as plant height, stem presence, leaf form, size, colour, venation and surface structure (Zohren, 1968; Roessingh & Städler, 1990; Košťál, 1993a; Degen & Städler, 1997) and also by the plant's surroundings (Košťál & Finch, 1994). The behavioural sequence performed by females during plant examination (landing on leaf, walking on leaf, descending the stem, circling the stem base) has been described in detail by Zohren (1968) and further quantified by Hopkins (1994). Städler & Schöni (1990) found that the same behavioural sequence is performed by females on a plastic model plant coated with paraffin wax and sprayed with ethanolic extract of cabbage leaves.

The female cabbage root fly normally oviposits into the soil-substrate adjacent to the host plant. It has been shown that the decision to accept a plant as host is reached mainly during examination of the plant's leaves and stem (Zohren, 1968; Städler & Schöni, 1990; Hopkins, 1994). Egg deposition behaviour, though receiving much less attention than plant examination, has been previously described and it was observed that the females explore the soil surface with their ovipositor extended (Zohren, 1968). It was found that females do evaluate some traits of the substrate, prefering to lay eggs into sand with a structure loose enough to allow for easy penetration with their ovipositors. They also prefer specific moisture conditions and shaded areas (Traynier, 1967; Zohren, 1968; Havukkala, 1982). Furthermore, Baur et al. (1996b, c) found that sensory cues originating from the substrate, including the plant roots, allow the ovipositing cabbage root flies to discriminate plants with undamaged roots from those with roots damaged by conspecific larvae. Thus, oviposition substrate and sensory cues not originating from the aerial plant parts might considerably influence egg deposition behaviour and modify the final output of the host-plant foraging process, the number of eggs laid by an individual cabbage root fly female around the stem-base of the plant. Nevertheless, the behaviour of cabbage root fly females when on the substrate surrounding the plant stem was, according to Zohren (1968), diffi-

cult to quantify exactly and for this reason studies have remained incomplete.

In the present study, we assessed the substrate-related egg deposition behaviour of cabbage root fly females under laboratory conditions. The behavioural mechanism by which females adjust the number of deposited eggs to the quality of the substrate is described. Plant surrogates treated with methanolic surface extracts of host-plant leaves and mounted on various modified substrates were used in dual-choice oviposition assays to characterize the effect of different features of the substrate such as the presence of host or non-host roots, organic matter, substrate moisture and to distinguish between olfactory and contact cues.

MATERIAL AND METHODS

Experimental insects

Delia radicum flies originating from field-collected larvae (Tägerwilen, Switzerland, 1994) were reared according to Finch & Coaker (1969). The insect culture was maintained and all behavioural observations and oviposition assays were performed (in 1995) in an incubator with photoperiod 16L : 8D (light from 03:00 h to 19:00 h), temperature 23 ± 2°C (L) and 20 ± 1°C (D), and an 80 ± 10% r.h.

Experimental plants

Cauliflowers (*Brassica oleracea* var. *botrytis*) of c.v. CC-Cross [susceptible to cabbage root fly attack and used in earlier studies (Roessingh et al., 1992; Baur et al., 1996c)], were grown in a greenhouse in 400 ml plastic pots filled with a substrate composed of 30% compost; 60% commercial substrates (35% Floratorf® and 25% Floraton®); 10% Perlit® and fertilizer with slow release of nutrients (Tardit®). Plants with nine true leaves were used for experiments. In one experiment, barley (*Hordeum sativum*, cv. Flika, 5–7 days old) and leek (*Allium porrum*, cv. Zefa plus, 3 months old) plants were also used.

Composition of substrates

The substrate in the pot was divided into two layers: (1) a top layer into which the flies oviposited and, (2) a bottom layer which filled most of the pot volume (Fig. 1). Both layers were variously modified.

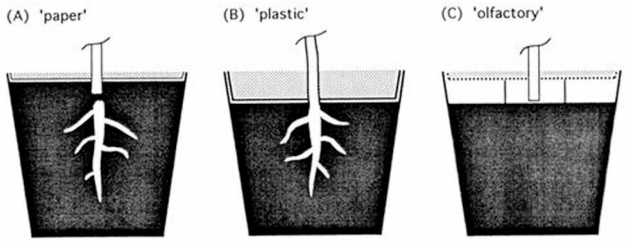

Fig. 1. The oviposition top layer (dotted area) in each pot (containing either the plant or plant surrogate) was arranged in three different set-ups: (A) "paper" – moist filter paper sheet covered with a layer (< 0.5 cm deep) of dry, sterilized sand; (B) "plastic" – two plastic foils covered with a layer (2 cm deep) of dry, sterilized sand; (C) "olfactory" – plastic wire mesh (0.5 mm) situated 2 cm above the bottom layer and covered with a layer (< 0.5 cm deep) of dry, sterilized sand. The bottom layer (black area) was formed (depending on experiment) by either watered growth substrate with or without roots of three different plants, or sterilized sand which was either watered or not. Further details are in the text.

Three different set-ups of the top layer were used for experiments (Fig. 1); these will be referred to as "paper" (A), "plastic" (B) and "olfactory" (C):

(A) "paper" – the bottom and top layers were separated by one sheet of moist filter paper. A thin (< 0.5 cm) layer of a dry, sterilized sand (limestone, particles 1–5 mm) was used as the top layer. In this set-up the filter paper was continuously supplied with moisture from the bottom layer and thus stayed moist throughout the assay while the sand particles forming the top layer remained dry. The females could directly touch (by tarsi, proboscis or ovipositor) the moist filter paper between the sand particles.

(B) "plastic" – the bottom and top layers were separated by two layers of thick polyethylene plastic foil tightly surrounding the stem. A thick (2 cm) layer of dry, sterilized sand was used as the top layer. In this set-up, the females could directly touch only dry sand while the penetration of volatile cues from the substrate bottom layer was prevented/minimized by the plastic foil.

(C) "olfactory" – the bottom and top layers were separated by a 2 cm-wide empty space. A plastic wire mesh (hole diam. 0.5 mm) was positioned (supported by a plastic ring) 2 cm above the bottom layer. A thin layer of dry, sterilized sand was used as the top layer. In this set-up the females were allowed to touch directly only dry sand, but perception of any volatiles released from the bottom layer of the substrate was possible.

Ten different modifications of the substrate (combinations of various top and bottom layers) were used in dual-choice oviposition assays. These are briefly summarized in Table 1 but some more details follow below:

(1), (2), (3) The plants [(1), one cauliflower; (2), densely grown barley; or (3), two leeks, respectively] were cut and the above-ground plant parts were removed. The remaining root-substrate complexes formed the bottom layer in the pot. The top layer was arranged as for "paper".

(4) The same substrate as that used for growing plants.

(5), (6), (7) The bottom layer was formed by sterilized sand, either watered (5), (6) or not (7). The sand used as top layer in (6) was dipped into water before it was filled into a pot. Such a set-up assured that not only the filter paper, but also the top layer of sand stayed wet throughout the assay [(contrast with (5)].

(8), (9), (10) The top layer was always arranged as for "olfactory". The bottom layer contained: (8) roots of one cauliflower plant; (9) growth substrate; or (10) sterilized sand, respectively.

Bottom layers of all the substrates except (7) were watered close to the maximum sorption capacity of the substrate prior to the experiments. Watering, together with high relative humidity of the air in the climate controlled chamber and standardized top layer set-up, minimized potential differences in water evaporation rates from the different substrates.

Plant surrogates

The design by Roessingh & Städler (1990) was used. The surrogates were made of green paper with vertical folds (projection area 45 cm^2) coated with a thin layer of paraffin wax and sprayed with 2 gle (gram leaf equivalents) of the methanolic surface extract of kale leaves prepared according to the method of Städler & Roessingh (1991).

Behavioural observations

Females of two physiological categories were used: 1) naive flies, 5 to 6 days old, without previous contact with a host plant, 2) experienced flies 7 to 14 days old, continuously exposed to a cauliflower plant before the trials. Females in the former category most likely carried a first complete load of mature eggs in

TABLE 1. List and brief description of the substrates used for the oviposition dual-choice assays with *Delia radicum*. Surrogate plants treated with methanolic surface extract of the *Brassica* leaves were mounted on top of each substrate.

No.	Name	Bottom layer			Top layer	Note
		Material	Presence of roots	Watering		
(1)	*Brassica* root	growth substrate	+	+	"paper"	
(2)	*Hordeum* roots	growth substrate	+	+	"paper"	
(3)	*Allium* roots	growth substrate	+	+	"paper"	
(4)	organic substrate	growth substrate	–	+	"paper"	
(5)	moist sand	sterilized sand	–	+	"paper"	
(6)	wet sand	sterilized sand	–	+	"paper"	wet top layer
(7)	dry sand	sterilized sand	–	–	"paper"	
(8)	*Brassica* root	growth substrate	+	+	"olfactory"	
(9)	organic substrate volatiles	growth substrate	–	+	"olfactory"	
(10)	sand volatiles	sterilized sand	–	+	"olfactory"	

All substrates except (7) were watered close to the maximal sorption capacity of the substrate prior to the experiment.

their ovaries and were prepared to start oviposition (Zohren, 1968; Kozhanova & Bogoslovskaya, 1983; Košťál, 1993b). Those in the latter category had probably already laid some of their eggs on the cauliflower plant (Košťál, 1993b; see also Fig. 1B for developmental profile of oviposition rate on cauliflower).

Twenty-five females, marked on five thorax positions using five different colours ("Bee marks"), were introduced into an empty acrylic plastic cage (50 × 50 × 50 cm) 30 min before the start of observation. The observational session always started at 07:30 h, when a plant (cauliflower) was introduced into the cage, and ended at 09:30 h. To minimize any plant effects, only three cauliflower plants of similar age, size and architecture were subsequently used to collect all behavioural data. The behaviour of individual females was recorded without disturbance from the moment they landed on the plant until they spontaneously flew away from the plant and landed on the cage wall. Particular attention was paid to the female's behaviour close to or directly on the substrate surface where four typical behavioural stages, first described by Zohren (1968), were distinguished:

(1) stem base circling – female, heading down, makes more or less complete circles around the stem base;

(2) substrate run – simple, relatively straight and rapid movement across the substrate;

(3) substrate exploration – slow and curved movement frequently interrupted by a short stop lasting a few seconds when ovipositor is inserted into a crevice;

(4) egg deposition – motionless for a few minutes with ovipositor inserted into a crevice.

The duration of each act of egg deposition was recorded using stop-watches. When the female left the plant after having finished ovipositing, the sand from around the plant stem was transferred to a plastic beaker, the eggs were separated by flotation and counted. The data come from different individuals, since no female was used more then once. During a single 2h-long observational session, only a few (3–6) complete behavioural sequences could be collected because (a) only a low proportion of females landed on the plant, (b) the duration of single complete sequence was too long (10–40 min approximately) and (c) only one single female was observed at a time; the other females which landed on the plant and were about to start their preoviposition behaviour were removed out from the cage and excluded from further experiments in order to avoid interactions between flies. The data collected during 24 observational sessions were pooled to yield 81 complete behavioural sequences including egg deposition.

Oviposition assays

A no-choice assay was performed in two cages (50 × 50 × 50 cm) made of acrylic plastic. Twenty five pairs of one-day old flies were placed into each cage. On the third day after emergence, at 16:00 h, one plant with either the "paper" or "plastic" substrate was introduced into each cage and females were allowed to oviposit for the next 24 h. Every day, at 16:00, the eggs were collected, counted after which the substrate set-up was renewed. The assays were terminated after day 11, before females began to die. The experiment was repeated with new flies and the same plants but with a switch in the substrate set-ups (i.e. the plant that had a "plastic" in the first run, received a "paper" in the second run and vice versa).

Dual-choice assays were performed in large (70 × 70 × 70 cm) metal wire cages (Städler & Schöni, 1990) that contained approximately 300 of 7–14 day-old (emerged during one week) cabbage root flies of both sexes. Prior to the assay, the flies were continuously exposed to a cauliflower plant. One dual-choice assay was performed with real plants. Six cauliflower plants were selected to obtain three pairs of plants similar in size and form. First plant of each pair was assigned to the "paper" and second plant to the "plastic" substrate. All six plants were arranged in hexagon on the bottom of the cage and females of *D. radicum* were allowed to oviposit for 22–23 h, after which the plants were removed, the eggs counted and the plants returned to the cage with a renewed substrate set-ups. The complete experiment lasted for four consecutive days (eggs counted daily) and the set-ups of the substrates within each pair were alternated after two days ("paper" changed to "plastic" and vice versa) to minimize any influence exerted by the plant.

In addition, oviposition rates on plant surrogates attached to various modified substrates (listed in Table 1) were compared in the dual-choice assays. Two or three replicates (depending on the assay) of the treatments to be tested were placed in a square or hexagon on the bottom of the cage. Females were allowed to oviposit for 22–23 h and then the treatments were removed and the eggs counted. A cauliflower plant was introduced into the cage for 1–2 h between the end of an assay and the installation of a new set of treatments (positions were alternated in a systematic manner). Depending on the assay, the experiments took three or four consecutive days and data for a total of 8 to 12 replicates were thus collected.

RESULTS

Fig. 2 illustrates the effect of two different substrates on oviposition by the cabbage root fly. In the choice assay (Fig. 2A), the females laid significantly greater numbers

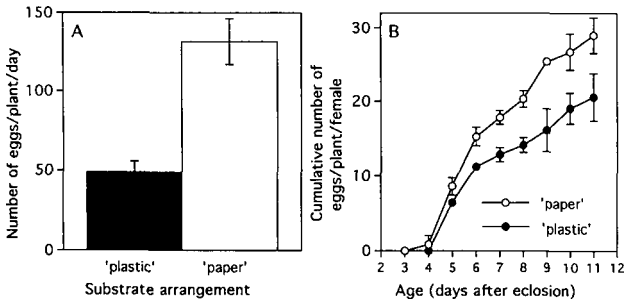

Fig. 2. Oviposition of *Delia radicum* females on the two substrates with different top layer set-ups, "plastic" and "paper" (see Material and methods for explanation) either in a dual-choice (A) or no-choice (B) assay. Each pot with particular substrate set-up (treatment) contained one cauliflower plant. Either three pairs of plants with two different substrates (A) or single plants with "plastic" or "paper" substrate (B) were exposed to groups of ovipositing females: ca 150 females per cage for 4 subsequent days in (A), and 25 females per cage for 11 consecutive days (repeated twice with different females). Vertical bars show variation of the mean: SEM in (A) and SD in (B) Differences between the numbers of eggs laid in the two substrates were significant in both choice ($P < 0.01$) and no-choice ($P < 0.05$) assays [Wilcoxon signed rank tests for: (A) mean numbers of eggs laid per replication per treatment and, (B) mean daily oviposition rates per treatment, grouped by days after eclosion].

of eggs on the "paper" than on the "plastic" substrates. This preference was less pronounced but was still significant in the no-choice assay (Fig. 2B) where the daily increases in the number of eggs laid by 25 females to two different treatments were compared. Since the same cauliflower plants were alternately used with the "plastic" and "paper" substrates, the resulting differences in oviposition must be entirely due to features of the substrates, namely the differences in moisture and/or the accessibility of olfactory and contact chemical cues.

Behavioural observations

Observations of ovipositing individual cabbage root fly females yielded data that give an indication of the behavioural mechanisms that underlie the differences in egg numbers oviposited in the two different substrates, "paper" and "plastic". Not all females that touched the substrate were directly involved in a search for a suitable oviposition site. Some females landed on the substrate and, without touching the plant, rested or moved around (substrate run) for some time and then flew away. Other females landed on the plant surface, remained motionless for some time and then jumped on the substrate, performed a substrate run and left it again. These females, showing no apparent pre-ovipositional activity, were regarded as not performing the host plant selection and consequently were not included in the statistical analysis (Table 2).

Stem base circling and substrate exploration behaviours were taken as clear indications that the female was searching for a suitable oviposition site. All females that finally oviposited first performed substrate exploration and stem base circling. Not all behavioural sequences containing the stages of substrate exploration and stem base circling ended with oviposition, however. In some cases, the stage of stem base circling was expressed as a mere stop at the base of a stem heading down, but usually the females made a more or less complete circle(s) around the stem. During circling, females would stop and almost push their heads into the sand, remaining in that position for a few seconds. Similarly, during substrate exploration, females would stop and push their heads into the pits or crevices. As we observed no proboscis probing during these stops, we suspected that this behaviour might be related with olfactory perception of cues emanating from the root-substrate complex.

The behavioural sequences in 400 females were recorded in total. Only 129 of the 400 cases included contact with the substrate ("substrate run"), only 110 cases included the "stem base circling" (and those were used for statistical analysis in Table 2) and only 81 cases included oviposition.

More than 90% of experienced females that reached the stem base circling stage continued to explore the substrate and finally laid eggs on the "paper" substrate (Table 2). In contrast, significantly fewer experienced females (only 50% of those performing stem base circling), continued to oviposit on the "plastic" substrate (Table 2). Most importantly, 10 of the 28 experienced females that began exploring the "plastic" substrate finally left the otherwise

TABLE 2. Behaviour of *Delia radicum* females in contact with the substrate in which the cauliflower plant grows. Substrate top layer was arranged as either "plastic" or "paper". Females were either naive (no previous oviposition on or contact with plant) or experienced (previous oviposition on plant).

Category of females	Step in behavioural sequence	Numbers of females observed performing the different behaviours on the two substrates				Statistical significance[2]
		"Plastic"		"Paper"		
		Counts	%[1]	Counts	%[1]	
Naive	Stem base circling	41	100	17	100	
	Substrate exploration	33	80.5	15	88.2	0.707 (ns)
	Oviposition	33	80.5	15	88.2	0.707 (ns)
Experienced	Stem base circling	36	100	16	100	
	Substrate exploration	28	77.8	15	93.8	0.245 (ns)
	Oviposition	18	50.0	15	93.8	0.004 (**)

[1] Behaviour frequencies in percentage of the females engaged in stem base circling (100%).

[2] Results of Fisher's exact test of 2 (columns) × 2 (rows) tables: columns, the two substrates ("plastic" and "paper"); rows, the responding (exploring, ovipositing, resp.) and the non-responding (leaving) females (ns, $P > 0.05$; **, $P < 0.01$).

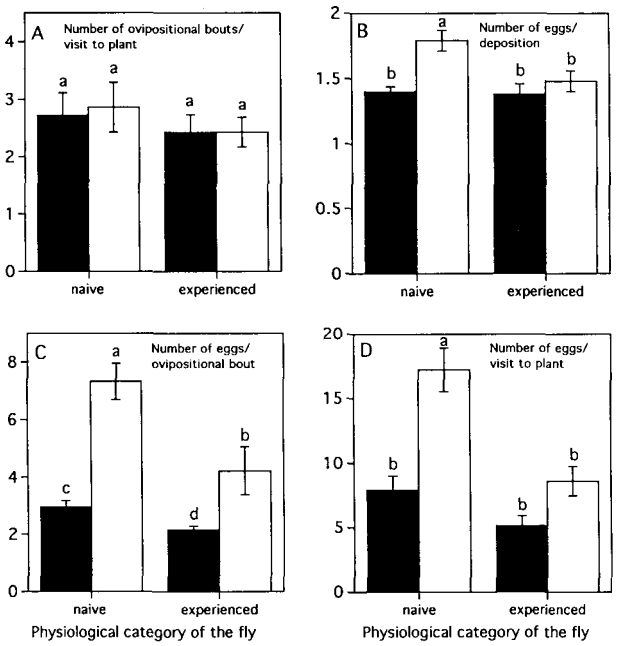

Fig. 3. Oviposition of naive (no previous oviposition on or contact with plant) and experienced (previous oviposition on plant) *Delia radicum* females in two substrate set-ups: "plastic" (black columns) or "paper" (white columns). See Material and methods for explanation of the terms used. Each column represents a mean ± SEM response obtained from data analysis of 15–33 individual ovipositing females whose preoviposition behaviour is summarized in Table 2. Different letters above the columns indicate that the means are significantly different ($P < 0.05$, Kruskal-Wallis followed by Mann-Whitney U-test).

suitable host plant without ovipositing. These females performed long and intensive substrate exploration, including numerous subsurface ovipositor probings but, apparently, the stimuli present in the "plastic" substrate were not sufficient to elicit egg deposition behaviour. A similar tendency was only slightly suggested for naive females (88.2% vs. 80.5%) but this was not statistically significant (Table 2).

By measuring the time spent by individual females at the egg deposition stage and relating this to the number of eggs counted after departure from the plant, we found that the time needed for deposition of one egg is constant. This time was very close to 1 min (1 min 3 sec ± 8 sec, mean ± SD, N = 81). We could use this result to estimate the numbers of eggs laid during individual acts of egg deposition and ovipositional bouts (description follows), assuming that each minute spent by a female in the egg deposition posture resulted in one egg being laid. During egg deposition, the female remained sitting still with the ovipositor inserted into the substrate crevice and was not sensitive to disturbance, even by direct touch. Individual acts of egg deposition were always preceded and separated by substrate exploration. Each ovipositional bout consisted of one or more acts of egg deposition. Individual ovipositional bouts were separated by return to the plant surface. On return, females would re-explore the plant's surface qualities by more or less intensive proboscis probing, running and spiral flights.

The analysis of ovipositing female behaviour showed that the number of ovipositional bouts performed by one female during each visit to a plant (2.6 in average) depended neither on the physiological state of the fly nor on the substrate (Fig. 3A). The number of eggs laid during one act of egg deposition was also relatively constant (1.4 in average); females always deposited one or two eggs irrespective of their physiological state and the substrate (Fig 3B). On the other hand, the numbers of eggs laid during one ovipositional bout significantly differed in relation to substrate qualities (Fig. 3C). Thus, the differences in the numbers of egg depositions within one bout were almost exclusively responsible for the differences in the numbers of eggs laid (Fig. 3D). Naive females laid twice as many eggs on the "paper" as on the "plastic" substrate and the same trend, though not statistically significant ($P = 0.054$, Mann Whitney U test), was seen in the experienced females. Naive females laid more eggs than experienced females both per one ovipositional bout (Fig. 3C) and per one visit to plant (though this difference was not significant on "plastic" substrate; Fig. 3D).

Dual-choice assays with plant surrogates

Delia radicum females did not significantly prefer the substrate with *Brassica* roots to the two non-host root-substrate complexes: *Hordeum* [(1) vs. (2)] and *Allium* [(1) vs. (3)] (Table 3a). *Brassica* roots were significantly preferred to the organic substrate without roots [(1) vs. (4)] and the organic substrate received significantly more eggs than moist sand [(4) vs. (5)] (Table 3b).

The moisture content of the substrate had a clear effect on the number of eggs laid. Both wet and dry substrates were avoided [(5) vs. (6)], [(5) vs. (7)] (Table 3c). Females preferred to lay eggs into substrate that had a dry surface but contained at the same time wet particles not deeper than 0.5 cm (i.e. directly accessible) (called moist sand in this study).

Volatile chemical stimuli originating from the substrate were at least partly responsible for the observed differences in oviposition. This was clear from the assays [(8) vs. (9)] and [(9) vs. (10)] (Table 3d) in which the females were allowed to directly touch only dry sand and the treatments differed in the volatiles released from the various bottom layers spatially separated from the top sand layer (Fig. 1C). The treatment using *Brassica* roots (8) was preferred to the organic substrate (9) and the latter was preferred to the sand treatment (10) (Table 3d).

DISCUSSION

Earlier studies of host plant acceptance by the cabbage root fly concentrated mainly on the chemical (Traynier, 1967; Städler & Schöni, 1990; Baur et al., 1996a, 1998; Roessingh et al., 1997) and physical (Prokopy et al., 1983; Roessingh & Städler, 1990; Košťál, 1993a; Degen & Städler, 1997) properties of the aerial plant parts. It was concluded that the decision about whether to start with oviposition or not is mainly taken by females during their exploration of the plant surface (Zohren, 1968; Städler & Schöni, 1990). Hopkins (1994) showed that the likelihood that a female, after having reached the stage of

TABLE 3. Oviposition of *Delia radicum* females in dual-choice assays; comparison between different substrates supplied in pots with a plant surrogate sprayed with methanolic host-plant extract.

Treatment[1]	Mean ± SEM % of eggs/pot/day[2,3]	No. of replic.	Total no. of eggs counted in experiment
a) Host vs. non-host roots			
(1) *Brassica* roots vs. (2) *Hordeum* roots	46.0 ± 6.6 vs. 54.0 ± 5.9 ns	9	2,271
(1) *Brassica* roots vs. (3) *Allium* roots	58.4 ± 9.3 vs. 41.6 ± 6.6 ns	12	2,159
b) Roots vs. organic substrate vs. sand			
(1) *Brassica* roots vs. (4) Organic substrate	72.8 ± 7.2 vs. 27.2 + 2.8***	9	4,617
(4) Organic substrate vs. (5) Moist sand	87.5 ± 4.4 vs. 12.5 ± 2.7***	8	6,006
c) Substrate moisture			
(5) Moist sand vs. (6) Dry sand	87.1 ± 12.9 vs. 12.9 ± 3.7***	8	2,293
(5) Moist sand vs. (7) Wet sand	90.0 ± 6.6 vs. 10.0 ± 1.8***	8	748
d) Volatile cues			
(8) *Brassica* roots vs. (9) Organic substrate	71.8 ± 6.7 vs. 28.3 ± 4.8***	9	813
(9) Organic substrate vs. (10) Sand	75.4 ± 4.0 vs. 24.6 ± 1.7***	9	1,468

[1] Letters preceding treatment names refer to detailed descriptions in Material and methods.
[2] Numbers of eggs were recalculated to percentages to allow easier comparisons between various experiments.
[3] Mann-Whitney U test was used to test for significant differences between the compared treatments (ns, $P > 0.05$; ***, $P < 0.001$).

stem base circling or substrate exploration, will go on to lay eggs is 0.92 or 0.94, respectively. In fact, our values for the "paper" substrate confirms this. But when we offered the females two substrates differing in quality, clear differences appeared in ovipositional rates on these substrates. The "plastic" substrate allowed the females to touch only dry sand, while the penetration of contact or volatile signals from the root substrate was prevented or minimized by the plastic cover-foils. The "paper" substrate was designed to allow direct perception of moisture and chemical signals from the substrate. In a choice situation, females clearly discriminated between these two substrates and preferred the "paper" substrate for oviposition. More importantly, the difference in ovipositional rates was apparent also in a no-choice situation and was further documented by the observation of the ovipositional behaviour of individual females.

The lower numbers of eggs laid on the "plastic" substrate could be explained by considering two potential mechanisms: (1) females interrupted the behavioural sequence leading to oviposition after intensive substrate exploration before any egg was laid; (2) females started oviposition but laid fewer eggs. Although the two mechanisms may represent just two extreme ends of a continuum we will discuss them separately for the sake of clarity. (1) The first mechanism occurred only with ovipositionally experienced females. Experienced females had laid a certain proportion of their eggs on cauliflower plants prior to the trial. Thus the greater selectiveness (with respect to substrate quality) of the experienced females might be due to the presence of an incomplete egg load in their ovaries which might increase their selectiveness as has been shown for *Delia radicum* (Zohren, 1968, Košťál, 1993b) and other insects (Fitt, 1986; Minkenberg et al., 1992). (2) The second mechanism was clearly noted in naive females but was less pronounced in experienced females. While similar proportions of females started laying eggs on both "plastic" and "paper" substrates, the females stopped oviposition earlier on the "plastic" substrate, perhaps owing to an enhanced selectiveness gained gradually during oviposition and a decrease of their actual egg load. Detailed analysis of the behavioural sequence performed on the substrate during oviposition revealed that the females repeatedly re-explore the substrate between individual egg deposition events (each yielding invariably 1–2 eggs) and either continue to the next deposition (continue the ovipositional bout) or return to the plant to re-explore its surface (end the ovipositional bout) or leave the substrate and plant (ending the visit to the plant). While the numbers of ovipositional bouts remained stable throughout the different substrates and physiological states, the number of eggs laid during one bout varied significantly and, as a result, so did the total number of eggs laid around the plant stem. Thus, during the substrate exploration stage which separates individual acts of egg deposition, the females assess the substrate qualities and adjust the number of eggs laid accordingly, and also with respect to their current physiological state (egg load).

We attempted to characterize the factors which influence substrate quality and, subsequently, oviposition by the cabbage root fly. Physical structure of the substrate has been earlier shown to play a clear role in oviposition. Females tended to lay more eggs into substrates which allowed penetration by their ovipositor (Traynier, 1967; Zohren, 1968; Havukkala, 1982). Similar results have been reported for the related seedcorn fly, *D. platura* (Barlow, 1965), and the onion fly, *D. antiqua* (Mowry et al., 1989).

Females strongly preferred moist sandy substrates with a dry surface and wet particles at not deeper than 0.5 cm (called moist sand in our study) to the wet (also wet on the surface) and completely dry sands. Zohren (1968) found a weak preference for dry rather than moist substrate in the ovipositing cabbage root fly females. His arrangement of the "moist" treatment was, however, different from the one presented here, having moist sand also in the surface layer (thus resembling the "wet sand"

treatment here), which most likely explains the differences between his and our results.

Delia radicum females laid almost three times as many eggs in response to the growth substrate containing *Brassica* roots than to the growth substrate without roots and seem thus to be able to perceive the presence of plant roots in a substrate and respond positively to it. However, the females did not prefer *Brassica* roots to two non-host root-substrate complexes, barley and leek. It remains to be clarified whether non-specific stimuli affect the cabbage root fly's response to the root-substrate complex.

Cabbage root fly females laid almost eight times as many eggs in organic growth substrate than in moist sand. The organic substrate harboured abundant populations of organisms (Colembolla, mites, dipteran larvae, earthworms and microorganisms). Microorganisms associated with host plant decomposition are known to considerably influence both host plant acceptance and larval survival in related species, *D. platura* (Eckenrode et al., 1975) and *D. antiqua* (Ellis et al., 1979; Hausmann & Miller, 1989). Doane & Chapman (1964a, b) have shown that *D. radicum* also transmits various bacteria associated with the decay of healthy tissues of *Brassica* roots. The authors failed, however, to find any positive effect of these bacteria on larval survival or development. On the other hand, Ellis et al. (1982) have shown that *D. radicum* females laid three or four times more eggs around radish seedlings grown from untreated seeds than from seeds treated with various sterilizing agents to eliminate or reduce microorganisms.

We attempted to exclude the possibility that the results of the above mentioned experiments with roots from different plants and with different substrates (organic substrate, sand) might have been influenced by different moisture contents in the different treatments. Prior to the experiment, all substrates were watered close to their maximum sorption capacity. Relative humidity was kept high (> 80%) which would minimize potential differences in evaporation rates from the different substrates. The arrangement of the oviposition top layers were similar in all the treatments ("paper"), which excluded any effect of different physical structure and further minimized potential differences in moisture contents and evaporation rates.

Volatile chemical signals originating from the substrate were found to considerably influence the number of eggs laid by the cabbage root flies. In fact, when the perception of contact chemicals was eliminated and only the volatiles released by different substrates could have been perceived by ovipositing females, the differences in oviposition rates to volatiles of *Brassica* roots vs. organic substrate or to organic substrate vs. sand were clear cut and similar to when the treatments included the contact chemicals (compare results in Table 3b and 3d). Although these results prove that the volatile chemicals originating from the substrate influence oviposition, we cannot estimate the relative influence of volatile and contact chemicals. This would require the identification of the chemical nature of the compounds responsible for the behavioural changes. Volatile chemicals can be perceived by the antennae or the labial palpi when females stop for short intervals (up to a few seconds) with their head close to or directly inserted into the substrate, a behavioural pattern identified in this study as typical for either the stem base circling or the substrate exploration stages.

In a parallel study, Baur et al. (1996b, c) found that plants damaged by cabbage root fly larval feeding are preferred to undamaged plants and that the major stimuli responsible for that preference apparently originate from the root-substrate complex. Thus, the root-substrate complex (including microorganisms) seems to have a strong influence on the final output of the host-plant foraging process in the cabbage root fly. This should not be surprising in an insect that deposits its eggs into the substrate and whose ancestors probably used decaying plant material as the main medium for larval development (Griffiths, 1991).

In conclusion, the results confirm that the number of eggs laid around host plants by the cabbage root fly is considerably influenced by the physical and chemical nature of the substrate in which the plant grows. The whole behavioural sequence performed by the female foraging for an oviposition site, first on the plant and then on the substrate around its stem, may be regarded as consisting of a series of three behavioural sub-sequences differing in their hierarchical level (ordered from the highest to the lowest): (1) plant exploration – assessment of the suitability of the plant as detailed in earlier studies (Zohren, 1968; Städler & Schöni, 1990; Hopkins, 1994); (2) substrate exploration – assessment of the suitability of the substrate for egg laying as described in this paper; (3) egg deposition – deterministic behaviour producing invariably 1 or 2 eggs per deposition. The completion/disruption of one sub-sequence leads either to the start of a following sub-sequence or to the return to a previous one or to the departure from the plant, and thus produces variation in the number of eggs laid by individual females, depending on their physiological state (egg load) and the "quality" of both the plant and the substrate surrounding its stem.

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REFERENCES

Barlow C.A. 1965: Stimulation of oviposition in the seed-corn maggot fly, Hylemya cilicrura (Rond.) (Diptera: Anthomyiidae). *Entomol. Exp. Appl.* **8**: 83–85.

Baur R., Birch A.N.E., Hopkins R.J., Griffiths D.W., Simmonds M.S.J. & Städler E. 1996a: Oviposition and chemosensory stimulation of the root flies Delia radicum and D. floralis in response to plants and leaf surface extracts from resistant and susceptible Brassica genotypes. *Entomol. Exp. Appl.* **78**: 61–75.

Baur R., Košťál V. & Städler E. 1996b: Root damage by conspecific larvae induces preference for oviposition in cabbage root flies (Delia radicum, Anthomyiidae). *Entomol. Exp. Appl.* **80**: 224–227.

BAUR R., KOŠŤÁL V., PATRIAN B. & STÄDLER E. 1996c: Preference for plants damaged by conspecific larvae in ovipositing cabbage root flies: influence of stimuli from leaf surface and roots. *Entomol. Exp. Appl.* **81**: 353–364.

BAUR R., STÄDLER E., MONDE K. & TAKASUGI M. 1998: Phytoalexins from Brassica (Cruciferae) as oviposition stimulants for the cabbage root fly, Delia radicum. *Chemoecology* **8**: 163–168.

DEGEN T. & STÄDLER E. 1997: Foliar form, colour and surface characteristics influence oviposition behaviour of the carrot fly. *Entomol. Exp. Appl.* **83**: 99–112.

DOANE J.F. & CHAPMAN R.K. 1964a: The relation of the cabbage maggot, Hylemyia brassicae (Bouche), to decay in some cruciferous crops. *Entomol. Exp. Appl.* **7**: 1–8.

DOANE J.F. & CHAPMAN R.K. 1964b: Development of the cabbage maggot Hylemyia brassicae (Bouche) on aseptic and decaying rutabaga tissue. *Entomol. Exp. Appl.* **7**: 115–119.

ECKENRODE C.J., HARMAN G.E. & WEBB D.R. 1975: Seed-borne microorganisms stimulate seedcorn maggot egg laying. *Nature (London)* **256**: 487–489.

ELLIS P.R., ECKENRODE C.J. & HARMAN G.E. 1979: Influence of onion cultivars and their microbial colonizers on resistance to onion maggot. *J. Econ. Entomol.* **72**: 512–515.

ELLIS P.R., TAYLOR J.D. & LITTLEJOHN I.H. 1982: The role of microorganisms colonising radish seedlings in the oviposition behaviour of cabbage root fly, Delia radicum. In Visser J.H. & Minks A.K. (eds): *Proc. 5th Int. Symp. Insect-Plant Relationships*. Pudoc, Wageningen, pp. 131–137.

FINCH S. & COAKER T.H. 1969: A method for the continuous rearing of the cabbage root fly Erioischia brassicae (Bouche) and some observations on its biology. *Bull. Entomol. Res.* **58**: 619–627.

FINCH S. & SKINNER G. 1982: Upwind flight by the cabbage root fly, Delia radicum. *Physiol. Entomol.* **7**: 387–399.

FITT G.P. 1986: The influence of a shortage of hosts on the specificity of oviposition behaviour in species of Dacus (Diptera, Tephritidae). *Physiol. Entomol.* **11**: 133–143.

GRIFFITHS G.C.D. 1991: *Flies of the Nearctic Region. Vol. VIII., Cyclorrhapha II. (Schizophora: Calyptratae), Part 2, Anthomyiidae.* E. Schweizerbart'sche Verlagsbuchhandlung (Naegele u. Obermiller), Stuttgart, pp. 954–968.

HAUSMANN S.M. & MILLER J.R. 1989: Production of onion fly attractants and ovipositional stimulants by bacterial isolates cultured on onion. *J. Chem. Ecol.* **15**: 905–916.

HAVUKKALA I. 1982: Deterring oviposition of the cabbage root fly, Delia radicum (Diptera: Anthomyiidae), by non-chemical methods. *Ann. Entomol. Fenn.* **40**: 9–15.

HOPKINS R.J. 1994: *Cabbage and Turnip Root Flies on Resistant and Susceptible Brassicas: Host Selection and Chemical Interactions.* PhD Thesis, University of Edinburgh.

HURTER J., RAMP T., PATRIAN B., STÄDLER E., ROESSINGH P., BAUR R., DE JONG R., NIELSEN J.K., WINKLER T., RICHTER W.J., MÜLLER D. & ERNST B. 1999: Oviposition stimulants for the cabbage root fly: isolation from cabbage leaves. *Phytochemistry* **51**: 377–382.

KOŠŤÁL V. 1993a: Physical and chemical factors influencing landing and oviposition by the cabbage root fly on host-plant models. *Entomol. Exp. Appl.* **66**: 109–118.

KOŠŤÁL V. 1993b: Oogenesis and oviposition in the cabbage root fly, Delia radicum (Diptera: Anthomyiidae), influenced by food quality, mating and host plant availability. *Eur. J. Entomol.* **90**: 137–147.

KOŠŤÁL V. & FINCH S. 1994: Influence of background on host-plant selection and subsequent oviposition by the cabbage root fly (Delia radicum). *Entomol. Exp. Appl.* **70**: 153–163.

KOZHANOVA N.I. & BOGOSLOVSKAYA T.S. 1983: Oogenesis in two species of cabbage flies, Delia brassicae and D. floralis. *Tsitologiya* **25**: 1252–1258 (in Russian).

MILLER J.R. & STRICKLER K.S. 1984: Finding and accepting host plants. In Bell W.J. & Carde R.J. (eds): *Chemical Ecology of Insects*. Chapman & Hall, London, pp. 127–157.

MINKENBERG O.P.J.M., TATAR M. & ROSENHEIM J.A. 1992: Egg load as a major source of variability in insects foraging for oviposition behavior. *Oikos* **65**: 81–84.

MOWRY T.M., KELLER J.E. & MILLER J.R. 1989: Oviposition of Delia antiqua (Diptera: Anthomyiidae) as influenced by substrate holes and particle size. *Ann. Entomol. Soc. Am.* **82**: 126–131.

NOTTINGHAM S.F. 1988: Host-plant finding for oviposition by adult cabbage root fly, Delia radicum. *J. Insect Physiol.* **34**: 227–234.

PROKOPY R.J., COLLIER R.H. & FINCH S. 1983: Leaf colour used by cabbage root flies to distinguish among host plants. *Science (London)* **221**: 190–192.

ROESSINGH P. & STÄDLER E. 1990: Foliar form, colour and surface characteristics influence oviposition behaviour in the cabbage root fly Delia radicum. *Entomol. Exp. Appl.* **57**: 93–100.

ROESSINGH P., STÄDLER E., FENWICK G.R., LEWIS J.A., NIELSEN J.K., HURTER J. & RAMP T. 1992: Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. *Entomol. Exp. Appl.* **65**: 267–282.

ROESSINGH P., STÄDLER E., BAUR R., HURTER J. & RAMP T. 1997: Tarsal chemoreceptors and oviposition behaviour of the cabbage root fly (Delia radicum) sensitive to fractions and new compounds of host-leaf surface extracts. *Physiol. Entomol.* **22**: 140–148.

STÄDLER E. & ROESSINGH P. 1991: Perception of surface chemicals by feeding and ovipositing insects. In Szentesi A. & Jermy T. (eds): *Proc. 7th Int. Symp. Insect-Plant Relationships. Symposia Biologica Hungarica* **39**: 71–86.

STÄDLER E. & SCHÖNI R. 1990: Oviposition behavior of the cabbage root fly, Delia radicum (L.), influenced by host plant extracts. *J. Insect Behav.* **3**: 195–209.

TRAYNIER R.M.M. 1967: Stimulation of oviposition by the cabbage root fly, Erioischia brassicae. *Entomol. Exp. Appl.* **10**: 401–412.

ZOHREN E. 1968: Laboruntersuchungen zu Massenanzucht, Lebensweise, Eiablage und Eiablageverhalten der Kohlfliege, Chortophila brassicae Bouche (Diptera, Anthomyiidae). *Z. Angew. Entomol.* **62**: 139–188.

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