Acta Zoologica Academiae Scientiarum Hungaricae 48 (Suppl. 1), pp. 265–280, 2002

SENSORY BASIS OF HOST-PLANT SELECTION: IN SEARCH OF THE "FINGERPRINTS" RELATED TO OVIPOSITION OF THE CABBAGE ROOT FLY

STÄDLER, E., BAUR, R. and R. DE JONG*

Eidg. Forschungsanstalt, Schloss 334, Postfach 185, CH-8820 Wädenswil, Switzerland
E-mail: Erich.Staedler@faw.admin.ch / Erich.Staedler@unibas.ch
**Present address: c/o L. Mattiacci, Via Gualtarella – S. Sisto 4/t, I-06132 Perugia, Italy*

The oviposition preference of the cabbage root fly, *Delia radicum* (Diptera, Anthomyiidae), was studied using leaf surface extracts of 24 different plant species that covered the whole span of preference rankings. The oviposition data were related to the content of the extract fractions containing either the glucosinolates or CIF ("cabbage identification factor", 1,2-dihydro-3-thia-4,10,10b-triaza-cyclopenta[.a.]fluorene-1-carboxylic acid). We observed a significant correlation between oviposition preference and the leaf surface content of benzyl and indolyl glucosinolates, substances that belong to the most active stimulants in oviposition assays, and in electrophysiological recordings from the tarsal $D_{4,3}$-sensilla. However, there was not a significant correlation between the extract fraction containing CIF and the recorded neural activity in the tarsal C_5-sensillum containing the CIF sensitive neuron. When this lack of correlation was investigated it was revealed that the leaf surfaces of two unacceptable host plants, *Capsella bursa-pastoris* and *Tropaeolum majus*, contain inhibitory compounds. Our data strongly support the hypothesis put forward by T. JERMY that "fingerprints" (specific mixtures of stimulatory and inhibitory plant compounds) mediate host-plant selection.

Key words: Cruciferae, leaf surface, glucosinolates, 1,2-dihydro-3-thia-4,10,10b-triaza-cyclopenta[.a.]fluorene-1-carboxylic acid (CIF1), tarsal contact chemoreceptor sensilla, inhibitors

INTRODUCTION

In his discussion of the evolution of insect/host-plant relationships JERMY (1984) hypothesised that the main role of secondary plant substances in insect/host relationships is to form the 'fingerprint', the specific pattern or biochemical profile by which the insect recognises the plants. This statement is based on the postulate that "Host plant specificity in phytophagous insects is determined mainly by the botanical distribution of plant substances …" (JERMY 1983). In the same paper, JERMY also stresses the importance of plant compounds that inhibit feeding or oviposition.

In recent years we were able to isolate and identify compounds from the leaf surface of *Brassica oleracea*, one of the major cultivated host plants that elicit oviposition by the cabbage root fly, *Delia radicum* (ROESSINGH *et al*. 1992, HURTER *et al*. 1999, DE JONG *et al*. 2000). Further, BAUR *et al*. (1996) found that

the content of the so-called CIF compounds ("cabbage identification factor"; 1,2-dihydro-3-thia-4,10,10b-triaza-cyclopenta[.a.]fluorene-1-carboxylic acid; Fig. 1) in four *Brassica* species is related to the oviposition preference of the cabbage root fly. However, *Brassica* species are not the only host plants for this fly. As shown by FINCH and ACKLEY (1977) many different wild crucifers and related plants are attacked. In endeavouring to correlate the observed preference of the fly with the presence or absence of quantifiable oviposition stimulants we had an opportunity to test JERMY's hypotheses concerning the role of secondary plant metabolites in host-plant selection of herbivorous insects.

MATERIALS AND METHODS

Oviposition behaviour

Insects: These tests were performed using *Delia radicum* from our continuous laboratory culture (restarted with field-collected maggots in 1996) and surrogate leaves treated with leaf surface extracts of the selected plants, as previously described by ROESSINGH *et al.* (1992). In each cage (70×70×70 cm) about 100 mature female flies and an equal number of males were kept at 21°C, 80% r.h., and LD 16:8h. The flies had access to a source of water, 10% sugar water on filter paper and a mixture of raw cane sugar, yeast hydrolysate, and water (4:1:1) applied on absorbent tissue strips.

Oviposition choice assay: The choice assays were performed in three separate, partially overlapping sets of 12 extract surrogate leaves and each set was replicated at least 7 times. After counting the eggs, the position of each treatment was re-randomised within the cages. *Brassica oleracea* convar. *botrytis* "CC-Cross" at two concentrations, 1.25 gle (gram leaf equivalent) and 0.125 gle and a control (methanol) were included as standards in all 3 sets. All the other extracts were applied at only one concentration of 1 gle. In all three sets of extracts bioassayed, one preferred host plant, one poor host plant and a non-host plant (*Allium porrum*) were included. This procedure resulted in 7–25 individual egg counts per plant species. For each treatment, the percentage of the total number of eggs laid on the date of counting was calculated; these percentages were averaged and correlated with other measurements using the non-parametric Spearman rank correlation test (corrected for ties if necessary).

No-choice oviposition assay: We utilised the same acrylic cages (50×50×50 cm) that were used by KOSTÁL *et al*. (2000). The surrogates, extracts, water, and food source were the same as used in the choice experiments and in the rearing cages. One surrogate plant was installed per cage and the eggs were counted daily from day 5 to 12 following emergence. Leaf surface extracts of *Brassica oleracea* (CC cross), *Capsella bursa-pastoris*, *Iberis amara, Raphanus raphanistrum*, *Sisymbrium officinale*, and *Tropaeolum majus* were tested in 3 independent repetitions with 35, 24 and 30 females and about 20 males per cage. The daily counts were divided by the number of live females and averaged. The average number of eggs were correlated to the other measures using the Spearman rank correlation test.

Inhibitory (deterrency) assays: The inhibitory effect of leaf surface extracts was tested in an oviposition choice test with three types of treatment applied on surrogate leaves: leaf extract (1.25 gle/leaf), pure sinigrin (Roth, Karlsruhe) at 1 µmol/leaf (397 µg/leaf), and a mixture of the extract and

sinigrin with each at the same concentration as in the single treatments (1.25 gle extract and 1 µmol sinigrin). Each treatment was repeated three times per cage (total of 9 surrogate leaves). After counting the eggs on four consecutive days the position of the leaves was re-randomised within the cage. The egg counts of each day were, as with the preference tests, converted into percentages and averaged. The extract treatment was compared with pure sinigrin or the mixture using the Mann-Whitney test.

Electrophysiology

Tip recordings from the tarsal C_5- sensillum of female flies were obtained using the same technique and set up as described recently by DE JONG *et al.* (2000). All the nerve impulses (spikes) recorded were counted in the first second after contact of the recording electrode with the tip of the sensillum using our spike train analysis software (STA). No attempt was made to discriminate between different spikes because we recorded mostly spikes of one shape. We investigated a total of 26 sensilla, from which we excluded those six preparations that gave < 40 spikes in the first second of stimulation with 10 ng/ml CIF1. The number of spikes recorded from each C_5-sensilla is expressed as percent of the response to 10 ng/ml CIF 1 (= 100 %) for each of the 20 sensilla.

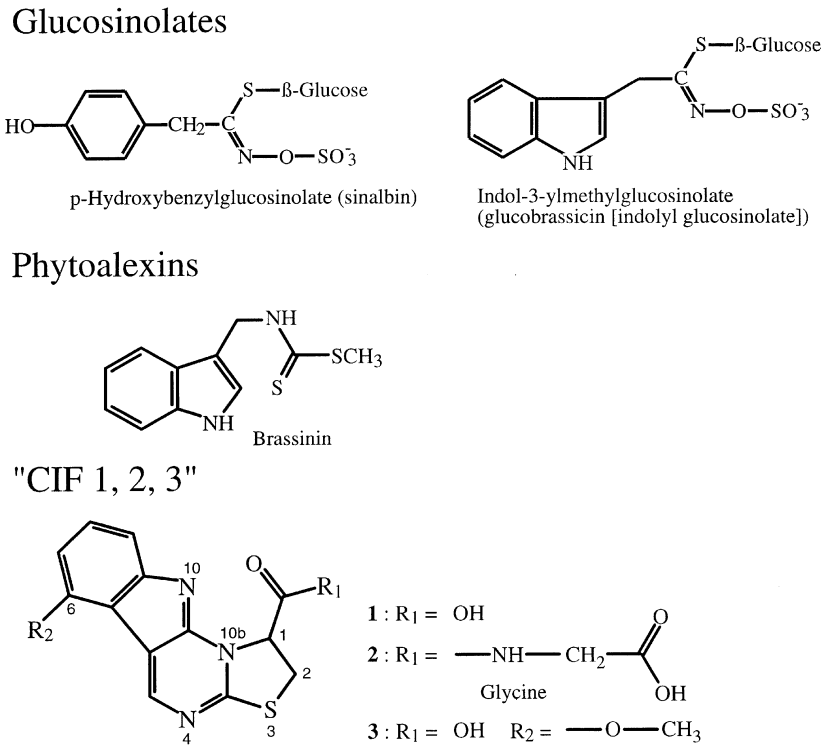

Fig. 1. Chemical formulas of a benzyl and an indolyl glucosinolate, a crucifer phytoalexin, and CIF 1, 2, 3

Plants

All the seeds of the wild plants (Capparidaceae, Cruciferae, Resedaceae, Tropaeolaceae) were obtained from the botanical garden of the University of Zürich. The seeds of cultivated plants were from the Federal Research Station Wädenswil: *Allium porrum* (convar. "ZEFA") and Zürich – Reckenholz: *Brassica napus* (var. "Eurol", winter cultivar with seeds low in erucic acid and glucosinolates) and *Brassica rapa* (convar. "Hanko"). *Brassica oleracea* var. botrytis (convar. "CC-Cross") was purchased from a local seed distributor.

Larval performance

We attempted to relate our oviposition data with the performance of the cabbage root fly larvae on the roots of the plants tested. To this end we used the published data of FINCH and ACKLEY (1977). These investigators inoculated 83 species of Cruciferae with cabbage root fly eggs in a glasshouse to determine which species could support the larvae. For each plant the number of pupae was recorded and we used these values as a measure of larval performance.

Chemical extraction and analysis

We used the same extraction procedure as described by STÄDLER and ROESSINGH (1991) and BAUR *et al*. (1996) to obtain wax-free methanolic leaf-surface extracts. These extracts were used in all the oviposition assays. The glucosinolate fraction of the extracts was separated from the fraction containing the CIF (DE JONG *et al*. 2000) compounds using an ion exchange chromatographic separation technique at atmospheric pressure. This method was developed and tested by BAUR *et al*. (1996) using *Brassica* genotypes. The glucosinolates were analysed qualitatively and quantitatively recently by GRIFFITHS *et al*. (2001). These analytical data are used in the present paper for the correlation with oviposition and sensory data.

RESULTS

Oviposition choice assays

The ranking in oviposition preference is presented in Figure 2 and summarised in Table1. The data show dramatic differences between the different plant extracts. *Sisymbrium officinale*, a wild crucifer, was the most preferred. *Brassica rapa* (kale-rape), a cultivated host plant, was only third but still in the most attractive group. These oviposition preferences correlated well (Spearman rank correlation Rho=0.644, p=0.020; Table 1) with the data on larval performance (number of pupae produced by the inoculated eggs) in the same plant species (FINCH & ACKLEY 1977). Thus, the females showed an overall preference for extracts from plants, which supported good development of the larvae on the roots. It appeared moreover that for the flies the plant extracts used were truly representative of the leaves of the plants tested.

Table 1. Summary of choice assays in response to plant extracts

	Family	Mean % eggs[1]	% CIF[2]	% pupae[3]
Readily accepted hosts				
Sisymbrium officinale	Cruciferae	74.0(8)	68.3	20
Barbarea vulgaris	Cruciferae	44.0(7)	38.5	32
Brassica rapa silvestris 'Hanko'	Cruciferae	33.0(7)	80.6	26
Lepidium campestre	Cruciferae	20.1(10)	20.9	–
Raphanus raphanistrum	Cruciferae	11.0(18)	51.1	32
Lepidium sativum	Cruciferae	8.1(10)	17.6	–
Sinapis arvensis	Cruciferae	7.8(18)	45.5	9
Brassica oleracea botrytis 'CC-Cross'	Cruciferae	7.1(25)	–	38
Brassica napus 'Eurol'	Cruciferae	6.5(7)	95.8	33
Cochlearia officinalis	Cruciferae	6.1(18)	34.8	32
Poor crucifer hosts				
Isatis tinctoria	Cruciferae	4.4(10)	37.2	0
Alyssum saxatile 'Gold Dust'	Cruciferae	4.0(7)	22.3	0
Iberis amara	Cruciferae	2.9(18)	–	0
Erysimum cheiranthoides	Cruciferae	2.2(10)	–	0
Brassica oleracea acephala 'Fribor'	Cruciferae	1.7(7)	–	–
Rorippa silvestris	Cruciferae	1.3(8)	47.1	–
Thlaspi arvense	Cruciferae	1.3(7)	68.8	–
Rorippa islandica	Cruciferae	0.9(8)	63.3	–
Crucifer non-host				
Capsella bursa-pastoris	Cruciferae	0.4(17)	34.5	0
Readily accepted non-crucifer hosts				
Cleome spinosa	Capparidaceae	5.9(7)	105.0	–
Poor non-crucifer hosts				
Reseda luteola	Resedaceae	2.0(8)	40.6	6
Non-crucifer non-hosts				
Tropaeolum majus	Tropaeolaceae	0.1(7)	32.9	–
Allium porrum	Liliaceae	0.1(8)	20.3	–
MeOH	–	0.3(25)	5.7	–

[1]The number of repetitions are in parentheses

[2]Mean CIF spikes (N= 20) stimulated by CIF fraction of plant extracts in percentage of 10 ng CIF1 / ml

[3]Mean pupal production angular transformation of % of eggs producing pupae (FINCH & ACKLEY 1977)

GRIFFITHS *et al*. (2001) has compared oviposition preference with analytical data on the glucosinolate fraction of the leaf surface extracts of the 19 plants shown in Figure 2. The amounts of 28 individual glucosinolates were determined, clustered according to the functional groups of the side chains (Fig. 1), and correlated with the mean percent oviposition preference. For the combined content of benzyl and indolyl glucosinolates, this correlation was clearly significant (Fig. 2: Rho=0.520; p=0.023), but not for the aliphatic glucosinolates (p=0.9) nor the glucosinolates with an additional sulphur molecule (methylthio; sulphonyl (p=0.13).

Contrary to our expectations, these oviposition data appeared to be unrelated to responses of the chemosensory neuron sensitive to CIF. This is illustrated in Figure 2 which shows that spike activity did not significantly correlate with oviposition preference (Rho=0.23, p=0.3). The high CIF activity of *Brassica napus*,

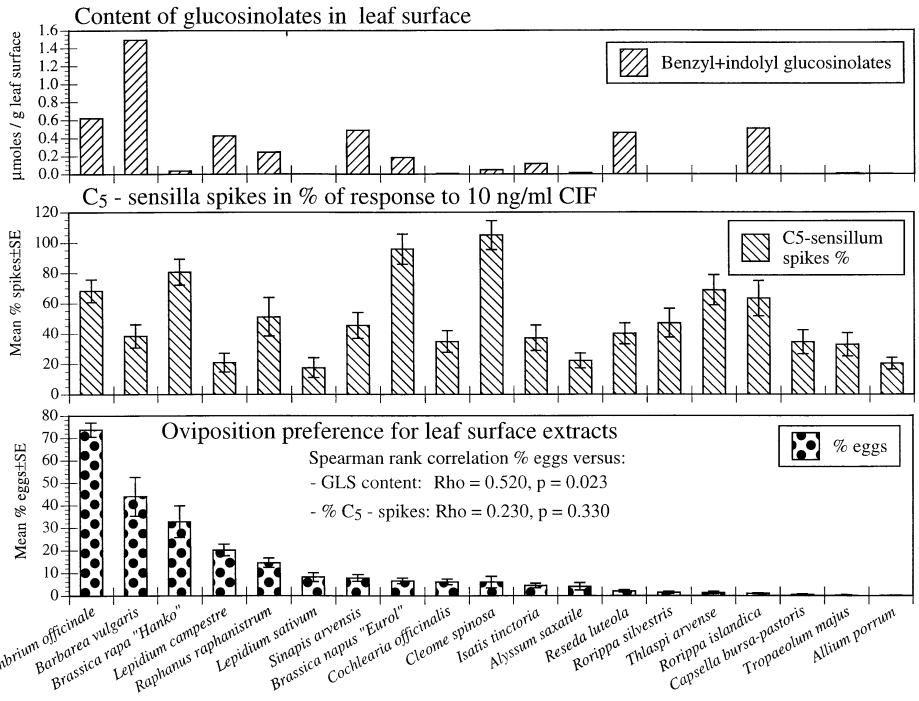

Fig. 2. Oviposition choice experiments related to analytical data of leaf surface extracts of 19 plants of the Capparidaceae, Cruciferae, Resedaceae, Tropaeolaceae and Liliaceae. The contents of the glucosinolates are derived from the data of GRIFFITHS *et al*. (2001). The spikes recorded from the C_5 sensilla are relative to (% of) the response to 10 ng/ml CIF 1 of the same sensilla. Oviposition preference is presented as % of total egg counts on 12 surrogate plants

Cleome spinosa, *Thlaspi arvense*, and *Rorippa islandica,* combined with their low oviposition preference value, were largely responsible for the low correlation. These plants were, by chance, excluded by the FINCH and ACKLEY's study (1977) (Table 1) on larval success, and therefore it is not surprising that their data correlated significantly with the CIF chemosensory response for the 12 plants that overlapped the two studies (Rho=0.615, p=0.043; Table 1).

Oviposition no-choice assays

Choice experiments can be problematic because the ranking is based on the selection of plants offered. We therefore performed no-choice experiments for several plants spanning the total range of stimulatory effectiveness in the choice

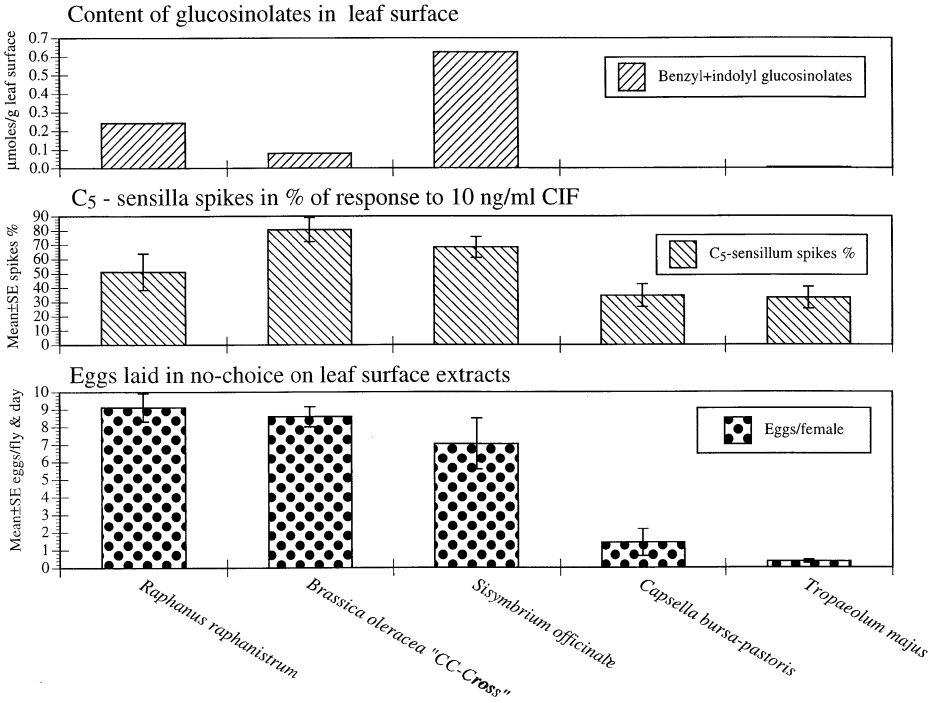

Fig. 3. Oviposition no-choice experiments related to analytical data of leaf surface extracts of and sensory responses to 5 selected plants. The analytical glucosinolate data for *B. oleracea* were derived from the data of ROESSINGH *et al*. (1992). The spikes were recorded from C_5 sensilla in response to the indicated plants, except that the data shown for *Brassica oleracea* CC-Cross were derived from those of *Brassica napus* as we had no data from the former. (These two plants are according to the results of DE JONG *et al*. 2000 equally attractive for the flies and both contain substantial amounts of CIF)

experiments. The data confirm in principle the choice experiment in the sense that the highly preferred plant extracts yielded many more eggs per female than the rarely chosen plant extracts (Fig. 3). Remarkable about the daily egg counts was the uniformity of the data. We observed no obvious difference in the ranking of egg production between the different days up to day 12 when the experiment ended. The wild crucifer *Sisymbrium officinale* was again a very stimulatory plant extract, but it was not significantly different from the other preferred plants, wild radish (*Raphanus raphanistrum*) and cauliflower (*Brassica oleracea*). Neither shepherd's purse (*Capsella bursa-pastoris*) nor *Tropaeolum majus*, two relatively unacceptable plants, were very stimulatory in the no-choice situation.

The comparison between the number of eggs, the plant content of glucosinolates, and "CIF spikes" shows clearly that the three most stimulatory plants contained more glucosinolates and stimulated more spikes in the C_5-sensillum than average. The Spearman rank correlations between the eggs per female and the CIF spike activity was relatively high (Rho=0.700, p=0.1615). The same was true for the benzyl- plus indolyl-glucosinolates (Rho=0.600, p=0.2301), but due to the smaller number of plants (N=5) tested, and a not perfect fit in the ranking, these correlation tests were not significant.

Inhibitory compounds

The data in Figure 2 and 3 show that the extracts of some plants that were not preferred (e.g., *Rorippa islandica*) did contain measurable amounts of glucosinolates or stimulated the receptor neurons in the C_5-sensillum. Thus it is surprising that they did not stimulate oviposition. A possible explanation for the weak effect of the total extract might be the occurrence of inhibitory (repellent or deterrent in the sense of DETHIER *et al*. 1960) compounds. To determine if this was the case, extracts were mixed with sinigrin, a commercially available glucosinolate that acts as a moderate stimulant for the cabbage root fly (ROESSINGH *et al*. 1992). The mixture was given in a choice experiment with pure sinigrin and the extract alone.

In the tests of two plants (*Capsella* and *Tropaeolum*) sinigrin alone was more stimulating than the extract mixed with sinigrin. The results in Figure 4 clearly demonstrate that these extracts contained one or several compounds that inhibited the stimulatory effect of the glucosinolate sinigrin. The females preferred, as expected, the mixtures to the extract alone in both plants, although in the case of *Tropaeolum* not significantly. In the case of *Erysimum cheiranthoides* (not shown in Figure 2, due to lack of analytical data) and *Rorippa islandica* no significant signs of an inhibitory effect of the extract were noted. The extract of *Iberis amara* (also not shown in Fig. 2) was in the oviposition choice experiments about as ac-

tive as *Brassica oleracea* at a tenth of its normal concentration (0.1 gle). In the experiment giving rise to Figure 4, the *Iberis* extract was significantly more stimulatory than a) the mixture with sinigrin and b) sinigrin alone. The finding b) might be the result of stimulatory activity of an additional compound(s) in the extract. The reason why the mixture with sinigrin a) was less stimulatory than the extract remains unexplained.

DISCUSSION

Comparison with field data

The ranking of plant extracts in oviposition choice was similar to the developmental data (pupae produced around the roots) of FINCH and ACKLEY (1977). Of course, there were some exceptions and this is not surprising because plant susceptibility or the attractiveness for the cabbage root fly depends on many factors, such as the plant varieties used, the plant growth conditions and age, and probably even on the genetics of the flies used. In our study all these conditions were different from those of FINCH and ACKLEY (1977). One surprising finding was that the species *Sisymbrium officinale* that yielded the most attractive extract in our study

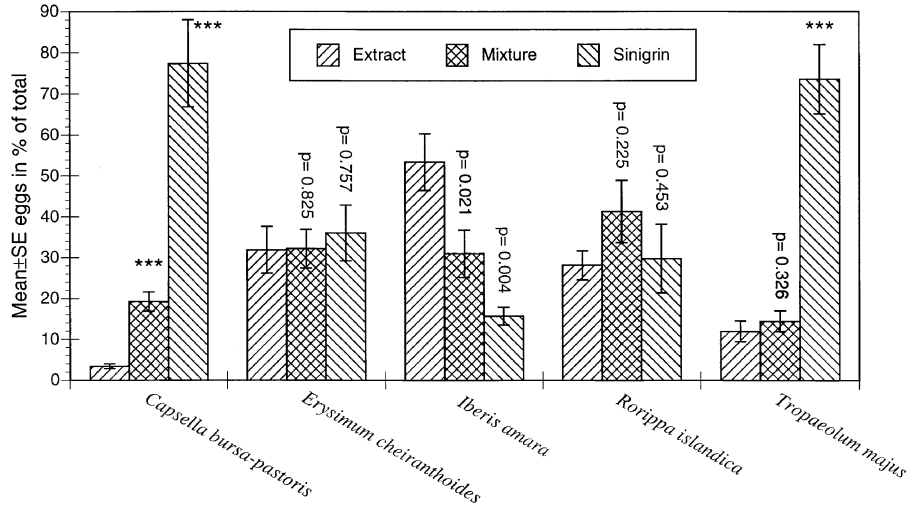

Fig. 4. Oviposition preference for surrogate leaves treated with either leaf surface extract (1.25gle), pure 1 µmol of sinigrin or a mixture of the extract and the sinigrin solution with each at the same concentration as in the single treatments. The p values shown were derived from the Mann-Whitney test for the difference to the extract treatment. *** p = 0.001

was clearly not the best host plant in the comparison of FINCH and ACKLEY (1977). Oil seed rape (*Brassica napus*) was in our choice tests an acceptable, although not one of the best host plants. There is the possibility that this was due to the type of cultivar chosen ("Eurol") a "double low" cultivar containing very little glucosinolate in the pods. The field studies of SKINNER and FINCH (1988) and of DOSDALL *et al*. (2000) confirm that oilseed rape (both *B. napus* and *B. rapa*) is susceptible to infestations by the cabbage root fly and can be damaged severely. Also in agreement with our results are the oviposition preference data obtained by DOANE and CHAPMAN (1962) in the field. These authors compared *Brassica napus* (*napobrassica*) (rutabaga), *B. rapa* (turnip), *B. nigra* (black mustard), *B. oleracea* (cauliflower), and *Raphanus sativus* (radish) and reported that *Brassica napus* and *B. rapa* consistently received the most eggs when counted on five dates during the growth period.

Another interesting comparison can be drawn from the work by NAIR *et al*. (1973), who studied oviposition and development of the cabbage root fly on 13 cruciferous weeds. These data were only partially derived from the same species that we used in our experiments. It is remarkable however, that the wild crucifer *Barbarea vulgaris*, in agreement with our study, was also very attractive and yielded many pupae. It was also more attractive than the rutabaga tested. Thus it seems safe to conclude that the surrogate treated with extracts, as used in our experiments, produced preferences very similar to those elicited by real plants. This would in turn validate the results of the comparable investigation of *Brassica* genotypes carried out by BAUR *et al*. (1996).

Comparison choice versus no-choice

Plants or extracts offered in a choice assay influence the relative preference (for detailed discussion of design problems see SINGER 1986). We tried therefore to take this into account by using multiple sets with plants varying in attractiveness based on the published data on the fly's performance (production of pupae by FINCH & ACKLEY 1977; plant resistance and oviposition by DOANE & CHAPMAN 1962). Our results show that no-choice experiments can supplement multiple choice tests very effectively but can not replace them because in an "emergency" the fly might oviposit on non-attractive plants ZOHREN (1968).

Glucosinolates

Although glucosinolates are less stimulatory than the CIFs of comparable doses (ROESSINGH *et al*. 1992, 1997), the present results show that this class of

compounds seems to have a significant influence on the host-plant choice of *Delia radicum*. In our study only the content of benzyl and indolyl GLS (Fig. 1) correlated significantly with oviposition choice. These same compounds are also the most active in eliciting oviposition behaviour as well as in electrophysiological assays (tarsal D4,3-sensilla) (ROESSINGH *et al*. 1992).

Some glucosinolate studies did not find a correlation with oviposition. For example, NAIR *et al*. (1976) tested six cruciferous plant species and found that the total glucosinolate concentrations in the leaves did not correlate with the oviposition response of cabbage root flies. The authors suggested that the presence or absence of plant inhibitors might explain the lack of correlation between glucosinolates and oviposition. It is not clear whether there was no correlation due to the role of inhibitors or because the authors determined only the total content of glucosinolates and did not consider the different functional groups. In our view the latter reason may well be relevant since we also found a much less significant correlation between the total glucosinolates than between specific groups of glucosinolates and oviposition preference. Thus at least for *Delia radicum*, it is necessary to differentiate individual glucosinolates, as we have recently reported (GRIFFITHS *et al*. 2001).

ELLIS *et al*. (1980) analysed the relationship between egg-laying and the amount of specific glucosinolates in radish (*Raphanus sativus*) populations with variable resistance to the maggots. Oviposition was significantly correlated with total amounts of 4-methylthio-3-butenyl isothiocyanate and 1-cyano-4-methylthio-3-butene (the hydrolysis products of glucoerucin respectively glucodehydroerucin) when tested on 6 dates after sowing. These compounds are methylthio glucosinolates that were in our data set only loosely correlated (Rho=0.340; p=0.1285) with oviposition preference. This does not, however contradict our results first of all because the authors compared genotypes within a plant species, *Raphanus sativus*, at different ages whereas we investigated differences between species and genera. Secondly, our choice of plants included only wild radish, *Raphanus raphinistrum*, and no cultivated varieties, as studied by ELLIS *et al.* (1980).

Glucosinolate concentrations can be manipulated by the selection of varieties. For example, special breeding programs produce pods and seeds of oilseed rape cultivars that vary strongly in glucosinolate content. It is quite possible that the relatively low preference for the variety "Eurol" of *Brassica napus* was caused by its low glucosinolate content of its leaf surface. But this need not be so: FIELDSEND and MILFORD (1994) found that other "double low" oilseed rape cultivars have low glucosinolate contents mainly in the floral tissue and pods,

whereas the leaves can have glucosinolate contents as high as the "single low" cultivars.

As reviewed recently by MOYES *et al*. (2000) and NIELSEN *et al.* (2001) correlations between glucosinolate contents and herbivore preference have been found also in some other insect and mollusc species. MOYES *et al*. (2000) examined the patterns of herbivory and the glucosinolate profiles of individual wild *Brassica oleracea* plants of different populations and habitats of the Dorset coast. A range of glucosinolate profiles were determined and the data were related to the proportion of damage by different herbivores. In the case of one specialist herbivore, *Selania leplastriana* (Tortricidae, Olethreutinae), the attacked plants contained significantly higher levels of 2-hydroxy-3-butenylglucosinolate and 3-indolylmethylglucosinolate than the uninfested plants. In relation to our study, it is remarkable that the preference of this moth species was also related to the indolyl glucosinolates. But, MOYES *et al*. (2000) found no significant influence of the different glucosinolates on the choice of the other herbivores observed (*Pieris* spp., slugs, snails, flea beetles, aphids).

NIELSEN *et al.* (2001) compared wild with transgenic *Arabidopsis thaliana* plants that, due to the introduced gene, contained sinalbin, which is not found in this plant in nature but is highly stimulatory when presented alone. Despite the four-fold increase in content of this glucosinolate, the tested flea beetles (*Phyllotreta* spp.) did not discriminate between transgenic and wild-type plants. In contrast to our study the effect of the glucosinolates was studied in plants of the same species. It might be that changes in stimulus concentration are less important for the intra-species than the inter-species and inter-genera discrimination.

CIF

In view of our earlier results (BAUR *et al*. 1996) with *Brassica* genotypes we expected a significant correlation between the oviposition choice data and the CIF content, estimated with the electrophysiological recordings from the C_5-sensillum. Several reasons can be put forward to account for the lack of a clear correlation: 1) The fractionation of plant extracts, other than those of *Brassica* might have been incomplete. Thus some CIF may have partitioned differently and not been tested. Also, some of the glucosinolates may have separated with the CIF fraction. Since the C_5-sensillum contains a chemoreceptor neuron that was shown to be sensitive to glucosinolates (ROESSINGH *et al*. 1997), CIF estimations may consequently have been too high. 2) Other compounds possibly stimulate the CIF sensitive neuron or another neuron with similarly shaped nerve impulses. Signs of this are the relatively high spike counts in responses to non-crucifers, such as *Allium porrum*

that most likely do not contain CIF, but elicited a spike activity (per 1st second, mean±SE: 21±4.4) that was significantly higher than in the control (KCl 10 mM: 5±1.8). This indicates that in the leek extract, and probably also in extracts from other plants, unidentified compounds are present that stimulate receptor cells in the C_5-sensillum. 3) Other substances not yet identified, volatile (DE JONG & STÄDLER 1999) or non-volatile compounds, could have affected the oviposition behaviour as well. Finally 4) the CIF fraction should have contained no glucosinolates, but certainly it did include many other plant compounds that could interact with the four receptor neurons that are present in the C_5-sensillum (ISIDORO *et al*. 1994). These interactions could lead to an increase or reduction of the spike activity of the stimulated cell(s). Several authors have observed such interactions between compounds acting in the same sensillum. SCHOONHOVEN and JERMY (1977) discovered a negative interaction of a secondary plant metabolite (strychnine) on a sucrose sensitive receptor neuron. Positive interactions have been observed less frequently, but they also exist: DETHIER and KUCH (1971) found that the contact chemoreceptor neurons of phytophagous caterpillars show signs of synergism nearly as often as inhibition. Thus, it seems likely that the response of the mostly active CIF receptor neuron to the CIF fraction of the plant extracts could have been influenced in different ways by other compounds present in the extract. In conclusion, our recordings from the C_5-sensillum of the cabbage root fly have to be interpreted with caution. The obvious resolution to these uncertainties would be a chemical analysis of the CIF fraction of each plant extract. Although this would in principle be possible using HPLC-MS, according to the unpublished results of GRIFFITHS *et al*., the method is not reliably repeatable across plant species due to technical difficulties caused by interactions with unknown components of the plant extracts tested.

Inhibitors

As concluded from the experiments summarised in Figure 4 *Capsella bursa-pastoris* and *Tropaeolum majus* do indeed contain compounds inhibiting oviposition. This would explain why these plants are not very stimulatory in the oviposition assays despite their relatively high CIF activity. The finding is yet another example of inhibitory compounds from both host and non-host plants that can reduce oviposition or feeding responses. We have not yet identified the compounds but as the studies by RENWICK and colleagues show, different crucifers may contain inhibitory compounds that can affect crucifer specialist insects like *Pieris rapae* (RENWICK 1996). Identical or similar compounds might also be involved in the cabbage root fly – plant relationship. The general importance of in-

hibitors in host-plant selection has long ago been postulated by JERMY (1966, 1983, 1984) and our study adds further weight to his conclusion.

Patterns of compounds

The unexpected stimulatory effect of *Iberis amara* extracts (Fig. 4) is in need of an explanation. The presence of additional stimulants in this extract might account for the fact that the mixture of extract and sinigrin was more stimulating than sinigrin alone. But it is difficult to understand why the extract alone is significantly more stimulatory than the mixture with sinigrin. One explanation might be that the stimulatory component in the extract was active as part of a pattern and that adding sinigrin might change this pattern so that it became less attractive. An example suggestive of an insect able to discriminate between different glucosinolates is the adult small white butterfly, *Pieris rapae* which has two separate receptor neurons distinct in their sensitivity to specific glucosinolates (STÄDLER *et al*. 1995). The combined activity of the two neurons produces a pattern that correlates with the observed behavioural response of the butterflies to individual glucosinolates. Perhaps adding a glucosinolate that is not highly stimulatory, such as sinigrin, could change this response pattern sufficiently to reduce the behavioural response to a plant extract. This would also be in line with the conclusion of NIELSEN *et al.* (2001) that special emphasis should be given on the effect of variations in glucosinolate profiles as well as on other plant factors that modulate insect responses.

The pattern recognition hypothesis may also apply to the cabbage root fly. A variety of phytochemicals are cues for the fly to find and oviposit on a host plant. We know that compounds of different volatility (DE JONG & STÄDLER 1999) and chemical identity, such as the glucosinolates, the CIFs and the phytoalexins (Fig. 1) (BAUR *et al*. 1998), are active stimulants. In the present study, the three preferred hosts tested in the no-choice assay contain greater amounts of both CIF and indolyl glucosinolates, suggesting that multiple chemical stimuli are important in eliciting accurate oviposition. We conclude, therefore, that the results of our study on host-plant selection by the cabbage root fly underscores the conclusion reached by TIBOR JERMY that secondary plant substances form specific patterns, or 'fingerprints', which mediate the insect/host relationships of herbivorous insects (JERMY 1983, 1984).

*

Acknowledgements – We thank Mrs JEAN BERÜTER-CASSELS for corrections, and Drs FRANK HANSON and ÁRPÁD SZENTESI for improvements in the content. This research was supported by a grant # 31–52409.97 (31–65'016.01) of the Schweizerischer Nationalfonds.

REFERENCES

BAUR, R., BIRCH, A. N. E., HOPKINS, R. J., GRIFFITHS, D. W., SIMMONDS, M. S. J. & STÄDLER, E. (1996) 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., STÄDLER, E., MONDE, K. & TAKASUGI, M. (1998) Phytoalexins from Brassica (Cruciferae) as oviposition stimulants for the cabbage root fly, Delia radicum. *Chemoecol.* **8**: 163–168.

DE JONG, R., MAHER, N., PATRIAN, B., STÄDLER, E. & WINKLER, T. (2000) Rutabaga roots, a rich source of oviposition stimulants for the cabbage root fly. *Chemoecol*. **10**: 205–209.

DE JONG, R. & STÄDLER, E. (1999) The influence of odour on the oviposition behaviour of the cabbage root fly. *Chemoecol*. **9**: 151–154.

DETHIER, V. G., BARTON BROWNE, L. & SMITH, C. N. (1960) The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol*. **53**: 134–136.

DETHIER, V. G. & KUCH, J. H. (1971) Electrophysiological studies of gustation in lepidopterous larvae. I. Comparative sensitivity to sugars, amino acids, and glycosides. *Z. vergl. Physiol*. **72**: 343–363.

DOANE, J. F. & CHAPMAN, R. K. (1962) Oviposition preference of the cabbage maggot, Hylemia brassicae. *J. Econ. Entomol*. **55**: 137–138.

DOSDALL, L. M., GOOD, A., KEDDIE, B. A., EKUERE, U. & STRINGAM, G. (2000) Identification and evaluation of root maggot (Delia spp.) (Diptera: Anthomyiidae) resistance within Brassicaceae. *Crop Prot*. **19**: 247–253.

ELLIS, P. R., COLE, R. A., CRISP, P. & HARDMAN, J. A. (1980) The relationship between cabbage root fly egg laying and volatile hydrolysis products of radish. *Ann. Appl. Biol*. **95**: 283–289.

FIELDSEND, J. & MILFORD, G. F. J. (1994) Changes in glucosinolates during crop development in single- and double-low genotypes of winter oilseed rape (Brassica napus). 1. Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. *Ann. Appl. Biol*. **124**: 531–542.

FINCH, S. & ACKLEY, C. M. (1977) Cultivated and wild host plants supporting populations of the cabbage root fly. *Ann. Appl. Biol*. **85**: 13–22.

GRIFFITHS, D. W., DEIGHTON, N., BIRCH, A. N. E., PATRIAN, B., BAUR, R. & STÄDLER, E. (2001) Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. *Phytochem*. **57**: 693–700.

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. *Phytochem*. **51**: 377–382.

ISIDORO, N., SOLINAS, M., BAUR, R., ROESSINGH, P. & STÄDLER, E. (1994) Functional morphology of a tarsal sensillum of Delia radicum L. (Diptera: Anthomyiidae) sensitive to important host-plant compounds. *Int. J. Insect Morphol. Embryol*. **23**: 115–125.

JERMY, T. (1966) Feeding inhibitors and food preference in chewing phytophagous insects. *Entomol. Exp. Appl*. **9**: 1–12.

JERMY, T. (1983) Multiplicity of insect antifeedants in plants. Pp. 223–236. *In* WHITEHEAD, D. L. & BOWERS, W. S. (eds) *Natural products for innovative pest management*. Pergamon Press, Oxford.

JERMY, T. (1984) Evolution of insect/host plant relationships. *Am. Nat.* **124**: 609–630.

KOSTÁL, V., BAUR, R. & STÄDLER, E. (2000) Exploration and assessment of the oviposition substrate by the cabbage root fly, Delia radicum. *Eur. J. Entomol*. **97**: 33–40.

MOYES, C. L., COLLIN, H. A., BRITTON, G. & RAYBOULD, A. E. (2000) Glucosinolates and differential herbivory in wild populations of Brassica oleracea. *J. Chem. Ecol*. **26**: 2625–2641.

NAIR, K. S. S., MCEWEN, F. L. & ALEX, J. F. (1973) Oviposition and development of Hylemya brassicae (Bouché)(Diptera: Anthomyiidae) on cruciferous weeds. *Proc. Entomol. Soc. Ontario* **104**: 11–15.

NAIR, K. S. S., MCEWEN, F. L. & SNIECKUS, V. (1976) The relationship between glucosinolate content of cruciferous plants and oviposition preferences of Hylemya brassicae (Diptera: Anthomyiidae). *Can. Entomol.* **108**: 1031–1036.

NIELSEN, J. K., HANSEN, M. L., AGERBIRK, N., PETERSEN, B. L. & HALKIER, B. A. (2001) Responses of the flea beetles Phyllotreta nemorum and P. cruciferae to metabolically engineered Arabidopsis thaliana with altered glucosinolate profile. *Chemoecol*. **11**: 75–83.

RENWICK, J. A. A. (1996) Diversity and dynamics of crucifer defenses against adults and larvae of cabbage butterflies. Pp. 57–79. *In* ROMERO, J. T. *et al*. (eds) *Phytochemical diversity and redundancy in ecological interactions*. Plenum, New York.

ROESSINGH, P., STÄDLER, E., BAUR, R., HURTER, J. & RAMP, T. (1997) Tarsal chemoreceptors and oviposition behaviour of the cabbage root fly (Delia radicum). *Physiol. Entomol*. **22**: 140–148.

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.

SCHOONHOVEN, L. M. & JERMY, T. (1977) A behavioural and electrophysiological analysis of insect feeding deterrents. Pp. 133–146. *In* MCFARLANE, N. R. (ed.) *Crop protection agents – their biological evaluation*. Academic Press, New York.

SINGER, M. C. (1986) The definition and measurement of oviposition preference in plant-feeding insects. Pp. 65–94. *In* MILLER, J. R. & MILLER, T. A. (eds) *Insect-plant interactions*. Springer, New York.

SKINNER, G. & FINCH, S. (1988) Oilseed rape crops as a source of cabbage root fly infestations for cruciferous vegetable crops. Pp. 61–66. *In* CAVALLORO, R. & PELERENTS, C. (eds) *Progress on pest management in field vegetables*. Balkema, Rotterdam.

STÄDLER, E., RENWICK, J. A. A., RADKE, C. D. & SACHDEV-GUPTA, K. (1995) Ovipositional and sensory responses of tarsal sensilla of Pieris rapae (Lep., Pieridae) to stimulating glucosinolates and deterring cardenolides. *Physiol. Entomol*. **20**: 175–187.

STÄDLER, E. & ROESSINGH, P. (1991) Perception of surface chemicals by feeding and ovipositing insects. *In* SZENTESI, Á. & JERMY, T. (eds) *Proc. 7th int. symp. insect-plant relationships. Symp. Biol. Hung*. **39**: 71–86.

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

Received 15th July, 2001, accepted 20th December, 2001, published 14th February 2002