

Oviposition stimulants and deterrents control acceptance of *Alliaria petiolata* by *Pieris rapae* and *P. napi oleracea*

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Summary. Differential acceptance of garlic mustard, *Alliaria petiolata* by *Pieris rapae* L. and *P. napi oleracea* is explained by their differential sensitivities to oviposition stimulants and deterrents in the plant. Fractions containing the stimulants and deterrents were isolated by solvent partitioning between water and n-butanol and by open-column chromatography followed by HPLC. *P. napi oleracea* showed no preference when offered a choice of *A. petiolata* or cabbage, but was strongly stimulated to oviposit by post-butanol water extracts of *A. petiolata*. The most abundant glucosinolate in this extract was identified as sinigrin, which could explain the high degree of stimulatory activity. *P. rapae* preferred cabbage plants over *A. petiolata*, and the relatively low stimulatory activity was also associated with the glucosinolate-containing aqueous extract. However, this species was strongly stimulated by a fraction that contained small amounts of glucotropaeolin along with unknown compounds. Deterrents to both species were found in the butanol extract from *A. petiolata*, and *P. napi oleracea* was more sensitive than *P. rapae* to these deterrents. Some HPLC fractions from the BuOH extract were strongly deterrent to *P. napi oleracea*, but were inactive to *P. rapae*. The ecological significance of these behavioral differences between the two *Pieris* species is discussed.

Key words. oviposition – stimulants – deterrents – glucosinolates – Lepidoptera – Pieridae – *Pieris rapae* – *Pieris napi oleracea* – *Alliaria petiolata*

Introduction

In Lepidoptera, plants chosen by ovipositing adults can generally be used by larvae without incident. But sometimes adult females lay eggs on plants that larvae will not eat or on plants that do not support continued development of these larvae. For example, indigenous *Pieris napi oleracea* lay eggs on *Barbarea vulgaris*, but this naturalized mustard is lethal to the larvae (Chew 1977b, 1981). However, this butterfly has expanded its

host resources by successfully using naturalized crucifers including *Brassica rapa* and *Raphanus raphanistrum* (Chew 1981). Moreover, some naturalized plants such as *Erysimum cheiranthoides* and *Iberis amara* are readily accepted by *P. napi oleracea*, but are refused or less preferred by naturalized *P. rapae* (Huang *et al.* 1993a,b). Chew *et al.* (1989) found that although *P. rapae* larvae developed more rapidly on all naturalized crucifers they ate in the laboratory than did *P. napi oleracea*, they rejected crucifers sometimes eaten by the native caterpillars.

These observations are consistent with findings that native insects attempt to use naturalized plants that are taxonomically related or chemically similar to their native hosts. Although some of these naturalized plants elicit egg-laying or feeding, they may subsequently fail to support larval growth (Chew 1977a; Jaenike 1990). These unsuitable plants thus offer an opportunity to isolate and identify plant compounds that stimulate insect behavioral responses, quite separate from compounds that affect growth.

Oviposition and feeding stimulants and deterrents have been shown to play an important role in host recognition by phytophagous insects (*e.g.* Miller & Strickler 1984; Metcalf & Metcalf 1992; Bernays & Chapman 1994). Toxins in unsuitable plants, through aversion learning, may also change host selection behavior of insects (Bernays 1993). Differential acceptance of several potential host plants by *P. rapae* and *P. napi oleracea* have recently been explained by differences in sensitivities of these insects to oviposition stimulants and deterrents. For example, the strong deterrent effect of erysimoside and erychroside in *E. cheiranthoides* overwhelms the weak stimulation provided by glucocheirolin and glucoiberin to result in rejection of this plant by *P. rapae* (Huang *et al.* 1993a). On the other hand, the strong stimulation of the two glucosinolates to *P. napi oleracea* easily outweighs the relatively weak deterrent effect of the cardenolides, so that the plant is readily accepted (Huang *et al.* 1993a). Similar differential responses were found for these butterflies to *I. amara* (Huang *et al.* 1993b). The stimulatory activity of *B. vulgaris* was also traced to glucosinolates, which were identified as (2R)-glucobarbarin, (2S)-glucobarbarin and glucobrassicin (Huang *et al.*, 1994). Both *Pieris* species had similar responses to

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(2S)-glucobarbarin, but *P. napi oleracea* was more sensitive to the (2R)-isomer. Therefore, glucosinolates are important in determining the host ranges of *Pieris* species (Verschaffelt 1910; Chew & Renwick 1995). In addition, deterrents may be involved, and a balance between positive and negative stimuli determines whether a plant is accepted or rejected by an insect (Dethier 1982).

Garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande (= *A. officinalis* Andr.) is one of many crucifers that are naturalized and now weedy in North America. This wild plant contains a major glucosinolate (sinigrin) and a minor one (glucotropaeolin) in root material, green parts or seeds (reviewed by Kjaer 1960; Nielsen *et al.* 1979; Daxenbichler *et al.* 1991), is edible by humans, and is rich in vitamins A and C (Zennie & Ogzewalla 1977). Szentesi (1990) has examined the utilization of the two different forms of this biennial by insects and concluded that the herbivorous guilds of the rosette and stem forms are almost identical. Chew (unpubl.) found that *A. petiolata* is generally accepted by *P. rapae* for oviposition and as a larval food resource, on which the larvae grow well. However, although *P. napi oleracea* adults also oviposit and their larvae feed on *A. petiolata*, the larvae subsequently die (Bowden 1971; Courant *et al.* 1994). The factors affecting oviposition by the two insect species on *A. petiolata* are unknown. This study was designed to test whether glucosinolates can explain acceptance of the plant by both butterflies, to determine if deterrents are involved, and to compare the sensitivities of the insects to these semiochemicals.

Materials and methods

Insects and plants

P. rapae and *P. napi oleracea* butterflies for behavioral assays were obtained from colonies started from field-collected insects each summer and maintained in the laboratory at ca 22°C under fluorescent lights providing a photoperiod of L16:D8. Oviposition occurred in the greenhouse, with supplementary lighting, at ca 25°C. *P. rapae* larvae were reared on cabbage (*Brassica oleracea* L. var. Golden Acre) and *P. napi oleracea* on *Conringia orientalis* (L.) Andr. (hare's ear mustard, Cruciferae) plants. Pupae were separated by sex (Richards 1940) and kept in screen cylinders until eclosion. *Alliaria petiolata* seeds were sowed in the field in October, and in May of the following year seedlings were individually transferred into 10 cm cord pots and kept in an air-conditioned greenhouse maintained at ca 25°C until used for extraction or bioassays (in June). Thus the leaf material for the main part of this study was from first-year rosettes of this biennial plant. For comparison, stalks and cauline leaves from bolting plants as well as seed pods were collected from the field. Cabbage plants (4–6 weeks old) for extraction or bioassays were grown in the same greenhouse as the *A. petiolata* rosettes. Supplemental light was provided by 400 W multivapor high-intensity discharge lamps.

Extraction of plant materials

Fresh foliage of *A. petiolata* or cabbage was extracted in boiling ethanol for 5 min, cooled, homogenized, and filtered. The ethanolic extract was evaporated to dryness under reduced pressure and lipids were removed with n-hexane. The defatted residue was dissolved in water and the aqueous extract partitioned three times with 1-butanol. The butanol extract and the post-butanol water extract were concen-

trated under reduced pressure at ca 50°C and stored in the refrigerator.

Fractionation of stimulants

The stimulatory materials in the post-BuOH water extract were subjected to preliminary separation by open column chromatography using 45 × 2 cm reversed-phase columns packed with 30 g 55–105 µm preparative C₁₈ silica (Millipore Corporation, Milford, MA 01757). About 200 g leaf equivalents of the extracts were loaded onto each column. Twenty-five fractions (15 ml each) were collected by sequentially eluting the column with 0.5% potassium sulfate (150 ml), water (150 ml), 25% (15 ml), 33% (15 ml), 50% (15 ml) and 100% (30 ml) methanol in water. Combinations of these fractions were tested for stimulatory activity.

Preparation of desulfoglucosinolates

Desulfated samples were prepared for analysis of glucosinolates, according to the method of Minchinton *et al.* (1982). A column was packed in a 12.5 cm Pasteur pipet with 200 mg DEAE Sephadex A-25 in 0.5 M pyridine-acetate buffer. The column was conditioned with the buffer (6 ml) followed by water (6 ml). After loading the samples (5 g leaf equivalents in 0.5–1.0 ml water), the column was eluted with water (10 ml or until the eluate was colorless), and 1 ml 0.25% aqueous solution of sulfatase (Sigma Chemical Co.) was applied. The column was kept at room temperature overnight and then eluted with 1 ml water. The solutions of desulfated products were filtered for HPLC analysis.

Fractionation of deterrents

The butanol extract was separated by open-column chromatography using the same column as described above. About 160 gram leaf equivalents of the extracts were loaded onto each column. The column was sequentially eluted with 90 ml each of water, 25%, 60%, and 100% CH₃CN in water, and four fractions (90 ml each) were collected. The collected fractions were used in bioassays to test for deterrent activity.

HPLC. System 1: The desulfated products of the open-column chromatography of the post-BuOH water extract were analyzed by HPLC on a reversed-phase C₁₈ column (25 × 0.46 cm) using a solvent gradient program as follows: 0% CH₃CN in water from 0 to 2 min, 10% at 10 min, 30% at 30 min, and 100% from 35 to 40 min. The flow rate was maintained at 1 ml/min. A diode array detector (Hewlett Packard model 1040A) was used to monitor the eluate at 230 nm. System 2: The most active deterrent fractions from the open-column chromatography of the BuOH extract were separated by HPLC using a water-acetonitrile gradient. A semipreparative reversed-phase C₁₈ column (50 cm × 8 mm) was used and the flow rate was maintained at 3.3 ml/min. The solvent ratio increased linearly from water to 10% CH₃CN at 2 min, 15% CH₃CN at 10 min, 20% CH₃CN at 30 min, 35% CH₃CN at 40 min, and 100% CH₃CN at 50 min. The eluate was monitored at 254 nm.

Bioassays

Oviposition bioassays were conducted in screen cages (48 × 48 × 48 cm) in a greenhouse as described by Renwick & Radke (1988). Eight pairs of newly emerged butterflies were transferred to each cage in the greenhouse. Each cage was supplied with a vial of 10% sucrose solution containing yellow food coloring and a cotton wick to facilitate feeding. During the pre-oviposition period, a cabbage plant was placed in each cage. When more than 50 eggs in one day were observed, the plant was removed and the butterflies were used for testing the next day. Treated and control plants were placed in opposite corners of the cage. Positions of plants were alternated in each cage to control for possible position effects. Bioassays were started at 0930 hr and the eggs laid were counted at 1530 hr. Both *Pieris* species were tested at the same time to minimize the possible effects of differences between plant batches, intensity of sunlight and other factors on oviposition behavior. Three different assay systems were necessary to measure whole plant preference, stimulant activity or deterrent activity. Oviposition preferences were determined by

providing the butterflies with a choice of cabbage or *A. petiolata* plants. Deterrent activity was monitored by applying test solution on cabbage plants (ca 5 weeks old, with a canopy span of ca 16 cm) grown individually in 10 cm cord pots. Stimulatory effects were tested using neutral (stimulants were not present) bean (*Phaseolis vulgaris* var. Sieva) plants as the oviposition substrate. The bean plants used for bioassays were presented as single plants at the two-leaf stage (ca 4 weeks old, with a canopy span of ca 14 cm) in plastic pots (6.25 × 6.25 cm). Treated plants were sprayed with samples dissolved in a methanol:water combination (70% MeOH for stimulant and 90% MeOH for deterrent assays). For stimulant assays, control bean plants were sprayed with cabbage post-butanol water extract (as a standard of stimulant). An extract from the same batch of cabbage plants was used in all stimulant bioassays. In deterrent assays, control cabbage plants were sprayed with solvent (90% MeOH) alone. The solutions were applied in a fine mist with a chromatographic sprayer to both upper and lower leaf surfaces.

Design and analysis

A replicate consisted of one cage with eight pairs of butterflies and four to eight replications were performed for each bioassay. Preferences between intact plants (Cabbage vs. *Alliaria*) and stimulant activities (of post-BuOH water extract) were presented by expressing the relative numbers of eggs on each choice as an oviposition preference index (OPI) or oviposition stimulant index (OSI):

$$\text{OPI} = 100 (\text{Alliaria} - \text{Cabbage}) / (\text{Alliaria} + \text{Cabbage})$$

$$\text{OSI} = 100 (\text{Treated} - \text{Control}) / (\text{Treated} + \text{Control})$$

A positive OPI means that the test plant (*Alliaria*) is preferred over the cabbage plant, whereas a negative OPI indicates that cabbage is preferred. Similarly, a positive OSI means that the test material is more stimulatory than the post-butanol extract of cabbage, whereas a negative OSI indicates that the stimulatory activity is weaker than that of the cabbage extract.

Deterrent activities were compared by calculating an oviposition deterrent index from the number of eggs laid on treated and control plants:

$$\text{ODI} = 100 (\text{Control} - \text{Treated}) / (\text{Control} + \text{Treated})$$

A positive ODI indicates deterrent activity, whereas a negative ODI would mean that the test material is stimulatory. Because of the variable number of eggs laid in each cage, proportions were used to evaluate activity. The proportion of eggs laid on the treated plant compared to the total on both treated and control plant was calculated, and the proportions were subjected to arcsine square root transformation. Differences between treatments and controls were analyzed using a one-sample *t* test on the transformed data, under the null hypothesis that the total number of eggs was distributed evenly over treated and control plants. A Waller-Duncan *K*-ratio test on the transformed data was used to assess significance of differences among treatments.

Results

Differential acceptance of *A. petiolata* by *P. rapae* and *P. napi oleracea* was observed in oviposition assays with cabbage plants as controls (Fig. 1). *P. rapae* laid significantly more eggs on cabbage than on *A. petiolata*, giving a mean OPI of -42.5 ± 9.6 . By contrast, *P. napi oleracea* females did not show a preference (mean OPI = -6.0 ± 11.1). One sampled *t*-tests on the transformed egg counts showed that *P. napi* females did not significantly distinguish between the plants ($t = 0.54$, $P > 0.05$) whereas *P. rapae* did ($t = 3.49$, $P < 0.05$).

When offered a choice of bean plants treated with the post-BuOH water extracts of *A. petiolata* or cab-

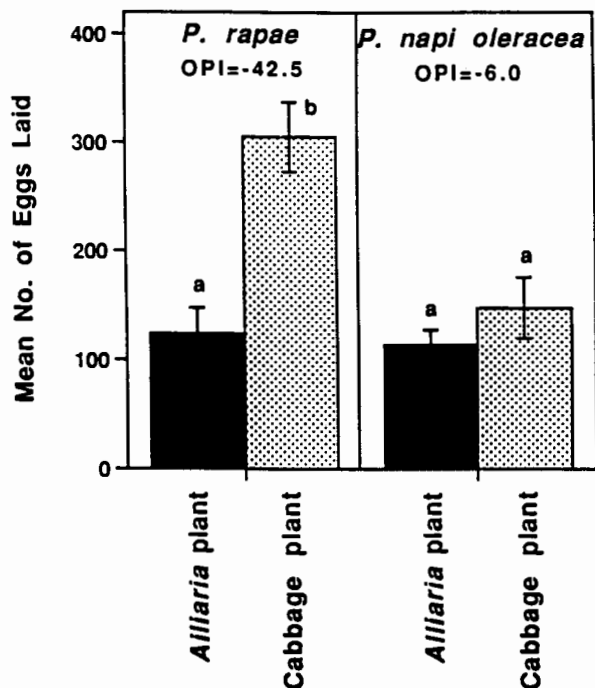


Fig. 1 Oviposition by *P. rapae* and *P. napi oleracea* on a choice of *A. petiolata* (test) or cabbage plants (control). Replicated eight times. A replication consisted of one bioassay cage with eight pairs of butterflies. Means (\pm SE) with the same letters in each series are not significantly different according to a one-sample *t* test ($P < 0.05$), performed on the transformed proportions of eggs laid on test plants, under the null hypothesis that eggs were distributed evenly over control and test plants. Mean oviposition preference indexes (OPIs) are shown. $\text{OPI} = 100 (\text{Test} - \text{Control}) / (\text{Test} + \text{Control})$

bage foliage, ovipositing *P. rapae* and *P. napi oleracea* were stimulated to differing degrees (Fig. 2). *P. rapae* laid more eggs on plants treated with the extract from cabbage than on those treated with *A. petiolata* extract (mean OSI = -17.8), although the difference between the two treatments was not significant ($t = 1.81$, $P > 0.05$). By contrast, the post-BuOH water extract of *A. petiolata* was much more stimulatory to *P. napi oleracea* than was the cabbage extract (between treatment and control $t = 2.78$, $P < 0.05$), and a mean OSI of 33.8 was obtained (Fig. 2).

Because glucosinolates had been shown to be responsible for the stimulatory effects of the post-BuOH water extracts from several plant species on oviposition by *P. rapae* and *P. napi oleracea* (Sachdev-Gupta *et al.* 1992; Huang *et al.* 1993a,b, 1994), glucosinolates in similar extracts from *A. petiolata* were analyzed by HPLC (System 1) of their desulfation products. One prominent and several other minor compounds were detected (Fig. 3A). The prominent glucosinolate was identified as sinigrin, and one of the minor ones as glucotropaeolin, based on comparison of their retention times on HPLC and their UV spectra with those of the desulfated product of authentic samples (sinigrin from Sigma Chemical Co.; glucotropaeolin from G. R. Fenwick), and on a previous finding that these glucosinolates exist in *A. petiolata* plants (Nielsen *et al.* 1979;

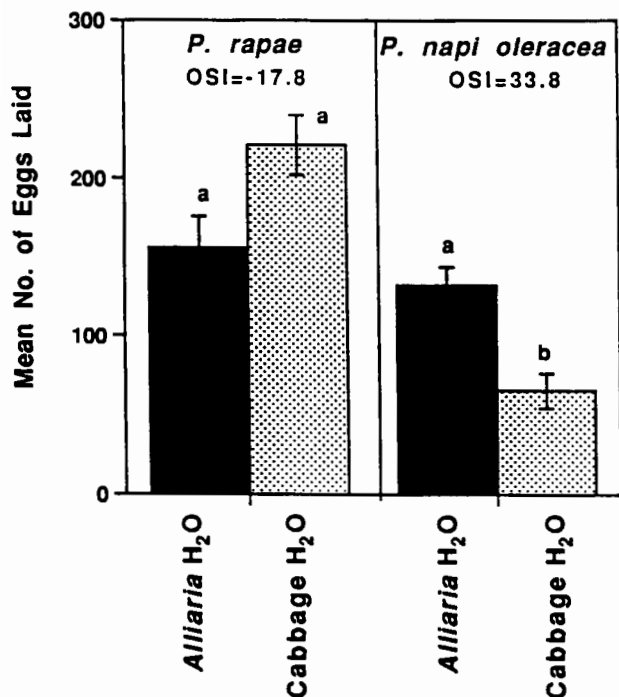


Fig. 2 Oviposition by *P. rapae* and *P. napi oleracea* on a choice of bean plants treated with post-butanol water extracts from *A. petiolata* or cabbage (control). Five gram leaf equivalents of each extract were used for each replication. Replicated eight times. A replication consisted of one bioassay cage with eight pairs of butterflies. Means (\pm SE) with the same letters in each series are not significantly different according to a one-sample *t* test ($P < 0.05$), performed on the transformed proportions of eggs laid on test plants, under the null hypothesis that eggs were distributed evenly over control and treated plants. Mean oviposition stimulant indexes (OSIs) are shown. $OSI = 100 \text{ (Treated - Control) / (Treated + Control)}$

reviewed by Kjaer 1960). The other compounds in the desulfated sample (Fig. 3A) are yet to be identified, but their retention times on HPLC along with their UV spectra indicated that they might be derived from aromatic glucosinolates. Analysis of stems with cauline leaves and of seed pods gave similar glucosinolate profiles, with sinigrin as the major constituent. *P. rapae* and *P. napi oleracea* responded differentially to these glucosinolates in oviposition assays where four combinations of the open-column fractions from the Post-BuOH water extract of *A. petiolata* were compared with the cabbage extract (Fig. 4). The combination of fractions 19–25, containing the minor glucosinolates (Fig. 3C), was most stimulatory to *P. rapae*. Significant differences were found between this combination and all the other combinations. This combination was also more stimulatory to *P. rapae* than was the control (cabbage extract), even though the original post-BuOH water extract (before open-column chromatography separation) was at best no more stimulatory than the cabbage extract (Fig. 2). These results indicate that the stimulatory effect of those compounds in open-column fractions 19–25 is inhibited by other component(s) in the original extract. Similar numbers of eggs were laid by *P. rapae* on bean plants treated with the sinigrin-containing combination (fractions 5–10, Fig. 3B) or with the cabbage extract (Fig. 4). No glucosinolate was detected in combinations containing fractions 1–4 or 11–18, and plants treated with these combinations received significantly fewer eggs when compared with plants treated with the cabbage extract. However, the sinigrin-containing combination of fractions 5–10 was most stimulatory to *P. napi oleracea*, and significant

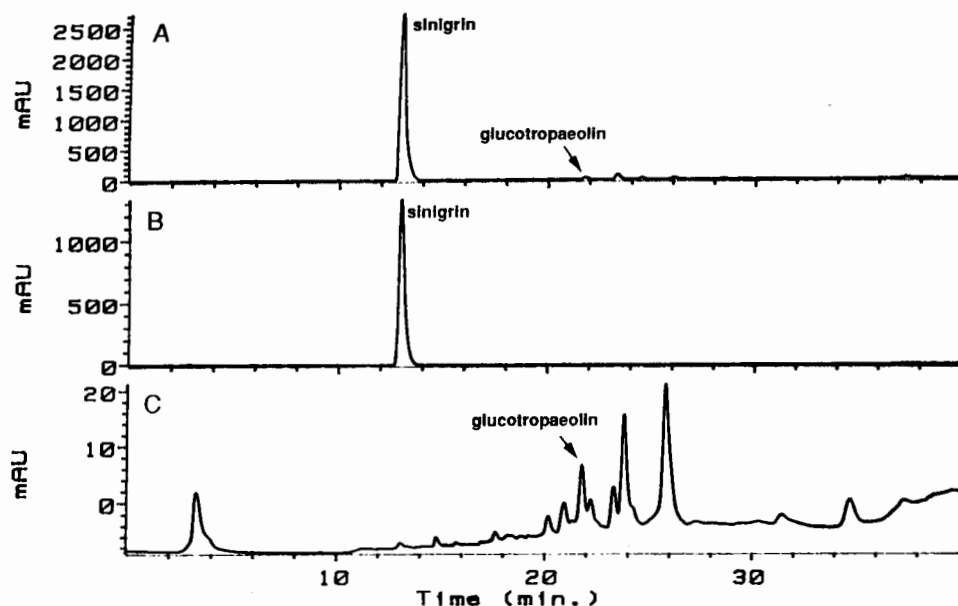


Fig. 3 HPLC separation of desulfoglucosinolates from the post-butanol water extract (A), open-column fractions 7 (B) and 25 (C) of *A. petiolata*. UV monitoring at 230 nm

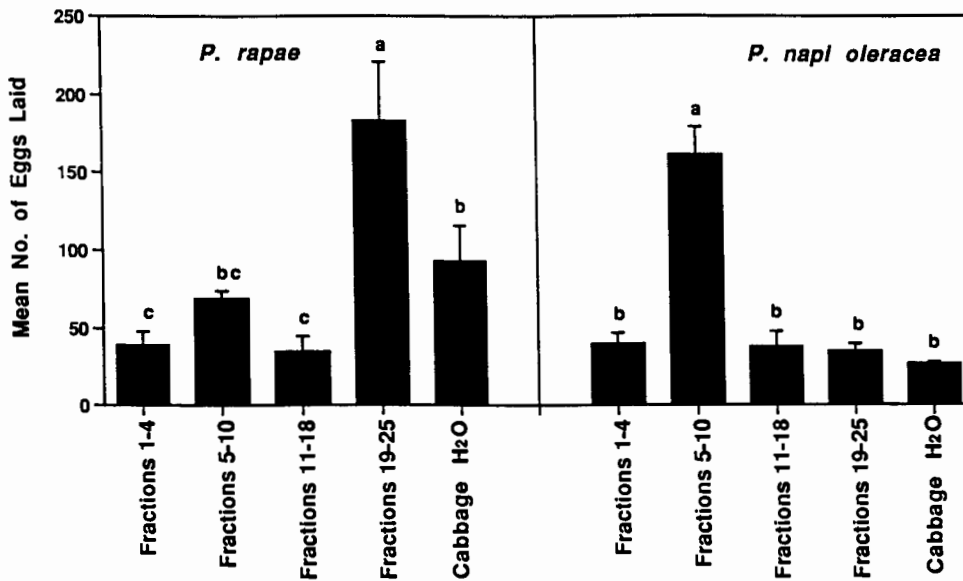


Fig. 4 Oviposition by *P. rapae* and *P. napi oleracea* on a choice of bean plants treated with open-column fractions 1-4 (non-glucosinolates), 5-10 (containing sinigrin), 11-18 (non-glucosinolates), 19-25 (containing glucotropaeolin and other unknown glucosinolates) from post-BuOH water extract of *A. petiolata*, with post-butanol water extract from cabbage as controls. Three gram leaf equivalents were used for each replication, replicated four times. A replication consisted of one bioassay cage with eight pairs of butterflies. Means (\pm SE) with the same letters in each series (insect species) are not significantly different according to a Waller-Duncan *K*-ratio *t* test ($K = 100$)

differences were found between this and other combinations or the control (cabbage extract) (Fig. 4). The numbers of eggs laid on bean plants treated with fractions 1-4, 11-18, 19-25 or cabbage extract were small, and no differences were found among these treatments.

When the BuOH extract of *A. petiolata* was applied to cabbage plants, it was deterrent to both *P. rapae* and *P. napi oleracea* in oviposition assays (Fig. 5). However, the deterrent effect was much stronger on *P. napi oleracea* (mean oviposition deterrent index, ODI = 65.7) than on *P. rapae* (mean ODI = 23.3).

Significantly fewer eggs were laid by *P. rapae* on cabbage plants treated with open-column fraction II (ODI = 39.1; between treatment and control $t = 7.05$, $P < 0.05$) and open-column fraction III (ODI = 33.3; $t = 6.69$, $P < 0.05$) from the BuOH extract of *A. petiolata* than on control plants (Fig. 6). Fractions I (ODI = 0.6; $t = 0.07$, $P > 0.05$) and IV (ODI = 19.3; $t = 2.09$, $P > 0.05$) were not active as deterrents to *P. rapae* when compared with the controls. Significant differences were found between fractions II or III and Fraction I (Fig. 6). No deterrent activity to *P. napi oleracea* was detected for fractions I (ODI = -11.5; $t = 1.41$, $P > 0.05$) and IV (ODI = -5.4; $t = 0.31$, $P > 0.05$), but fractions II (ODI = 46.1; $t = 5.20$, $P < 0.05$) and III (ODI = 40.9; $t = 7.70$, $P < 0.05$) were strongly deterrent to this species (Fig. 6). ODIs for Fractions II and III were significantly higher than those for fractions I and IV. Therefore, fractions II and III were subjected to further separation by HPLC.

Fraction II was separated into three fractions (II1, II2 and II3) by HPLC (system 2; Fig. 7A), and the resulting fractions were tested for their deterrent activity. As shown in Fig. 8, fraction II1 was deterrent to *P. rapae* (ODI = 32.7; between treatment and control $t = 11.5$; $P < 0.05$), and fraction II2 and II3 were inactive. Fraction II1 was also the most active of these fractions to *P. napi oleracea*, and a significant difference

was obtained between this treatment and the control (ODI = 48.8; $t = 4.84$, $P < 0.05$). Although ODIs of 20.8 and 17.2 were obtained for fractions II2 and II3, respectively, for *P. napi oleracea*, the differences between these fractions and the controls were not significant (Fig. 8). After fraction III1 was further separated into three sub-fractions (III1, III2 and III3; Fig. 7A), fraction III1 was deterrent to both *P. rapae* (ODI = 36.9; $t = 2.92$, $P < 0.05$) and *P. napi oleracea* (ODI = 44.9; $t = 5.00$, $P < 0.05$). However, a significant difference was found in the response of *P. rapae* and *P. napi oleracea* to fraction III3. While *P. napi oleracea* was strongly deterred by this fraction (ODI = 46.6; $t = 5.56$, $P < 0.05$), *P. rapae* was unaffected (ODI = -6.3; $t = 0.59$, $P > 0.05$) (Fig. 8).

Open-column fraction III contained less polar compounds when compared with fraction II, but some overlap occurred (Fig. 7A,B). HPLC fractions III1 and III2 (Fig. 7B) from this fraction were not significantly deterrent to *P. rapae*, although an ODI of 19.2 was obtained for fraction III1 ($t = 1.44$; $P > 0.05$; Fig. 8). In contrast, both fraction III1 and III2 were strongly deterrent to *P. napi oleracea*, giving ODIs of 49.3 ($t = 3.36$, $P < 0.05$) and 38.7 ($t = 3.79$, $P < 0.05$), respectively. The major compounds in fraction III1 and II3 had the same retention times (Fig. 7A,B) and UV spectra, but fraction III1 was more active than was fraction II3 (Fig. 8). This may be explained by the fact that this compound was present at a much higher concentration in fraction III1 than in II3 (Fig. 7A,B).

Discussion

P. rapae was stimulated to oviposit on bean plants to a similar extent ($P > 0.05$) by the post-BuOH water extracts from *A. petiolata* or cabbage. This indicates that *A. petiolata* contains strong oviposition stimulants to

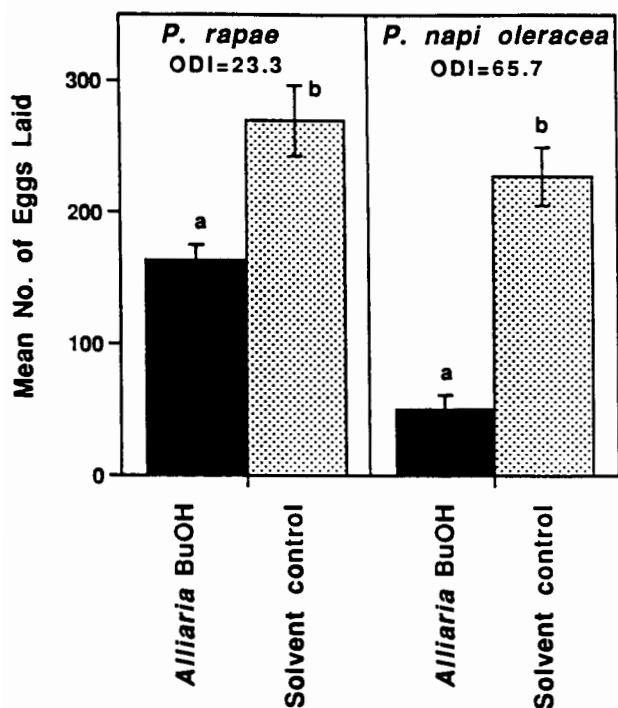


Fig. 5 Oviposition by *P. rapae* and *P. napi oleracea* on a choice of cabbage plants treated with BuOH extract from *A. petiolata* or with solvent (90% MeOH) alone (control). Five gram leaf equivalents of each extract were used for each replication. Replicated eight times. A replication consisted of one bioassay cage with eight pairs of butterflies. Means (\pm SE) with the same letters in each series are not significantly different according to a one-sample *t* test ($P < 0.05$), performed on the transformed proportions of eggs laid on test plants, under the null hypothesis that eggs were distributed evenly over control and treated plants. Mean oviposition deterrent indexes (ODIs) are shown. $ODI = 100 \text{ (Control - Treated) / (Control + Treated)}$

the butterfly. However, the same plant contains deterrents to this species, as indicated by the activity of the BuOH extract. These results explain why *A. petiolata* was less preferred by *P. rapae* in choice tests with cabbage. In the case of *p. napi oleracea*, the post-BuOH

water extract from *A. petiolata* was more stimulatory than the cabbage extract. But *A. petiolata* plants were not preferred over cabbage by this species in a choice test. This can be explained by the fact that the stimulatory effect was counteracted by strong deterrents that were found in the BuOH extract. Our results demonstrate that acceptance of oviposition substrates by female *Pieris* butterflies can be viewed as a result of a net balance between negative and positive chemical inputs to the ovipositing females (reviewed by Renwick & Chew 1994). A highly stimulatory effect on an insect herbivore can be counteracted by a strong deterrent effect of different compounds in the same plant, thus potentially providing plants with a mechanism to deter insects by modulating the relative concentrations of compounds with positive and negative effects on insect behavior. Further, differential sensitivity to a range of compounds has been demonstrated in *Pieris* species (Huang & Renwick 1994a,b). Differential acceptance of plant species by different *Pieris* species can thus be accounted for by the effects of (a) differential distribution of active compounds among plants, (b) differential sensitivity to these compounds in plants (Huang & Renwick 1993; Huang *et al.* 1993a,b, 1994), or (c) their joint effects.

In the present study, fractions containing aromatic glucosinolates were more stimulatory to *P. rapae* than was the cabbage extract. However, the original (before open-column chromatography) post-BuOH water extract of *A. petiolata* was not more active than the cabbage extract. It is therefore suggested that other components in the original extract have an inhibitory effect on the activity of the aromatic glucosinolates. Very high concentrations of sinigrin may actually suppress the activity of other stimulants (Huang & Renwick 1994a).

Glucosinolates have been shown to stimulate oviposition by *Pieris* butterflies (*e.g.* Rodman & Chew 1980; Renwick & Radke 1983; Traynier 1984, 1986; Chew 1988; Renwick *et al.* 1992; Sachdev-Gupta *et al.* 1992; van Loon *et al.* 1992; Chew & Renwick 1995). Al-

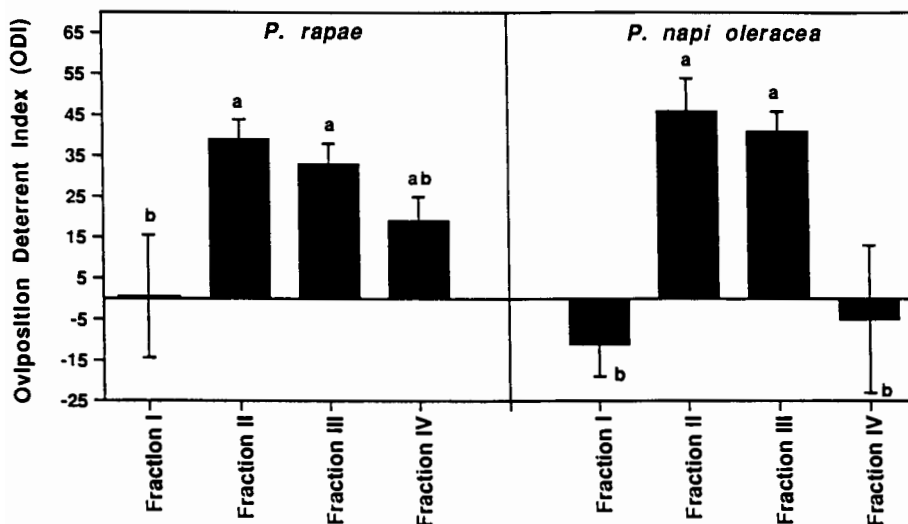


Fig. 6 Oviposition deterrent index (ODI) of open-column fractions I–IV from the BuOH extract of *A. petiolata* for *P. rapae* and *P. napi oleracea* on cabbage plants. Five gram leaf equivalents of the extract were used for each replication. Control plants were treated with solvent (90% MeOH) alone. Replicated four times. A replication consisted of one bioassay cage with eight pairs of butterflies. ODIs (\pm SE) with the same letters in each series (insect species) are not significantly different according to a Waller-Duncan *K*-ratio *t* test ($K = 100$). $ODI = 100 \text{ (Control - Treated) / (Control + Treated)}$

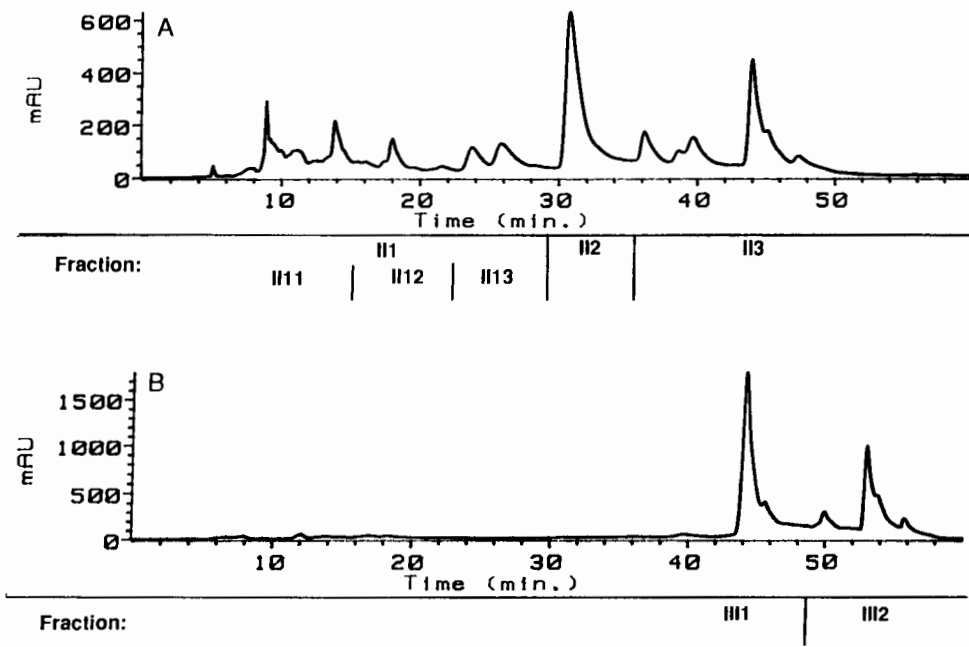


Fig. 7 HPLC separation of open-column fractions II (A) and III (B) from the BuOH extract of *A. petiolata*. UV monitoring at 254 nm

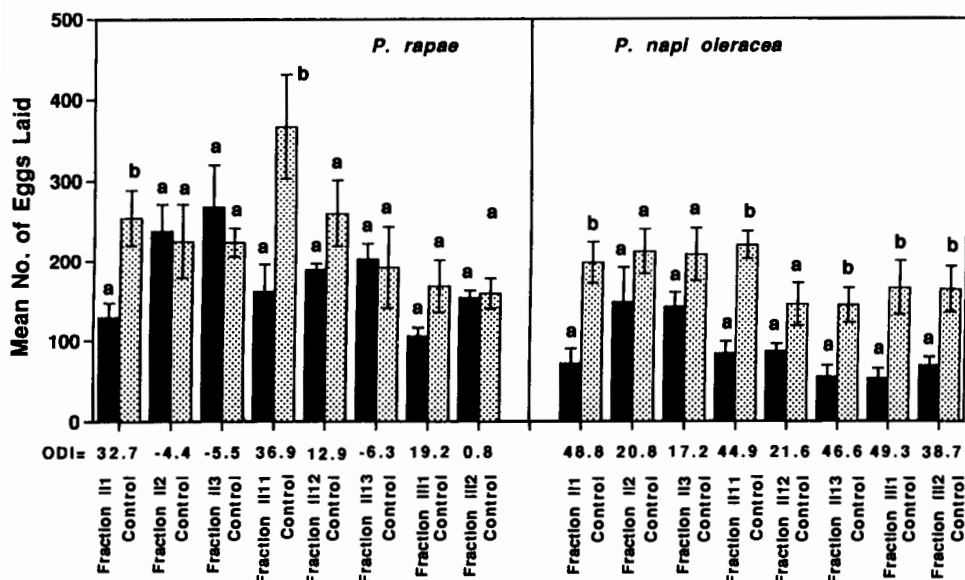


Fig. 8 Oviposition by *P. rapae* and *P. napi oleracea* on cabbage plants treated with HPLC fractions (as shown in Fig. 7) of the open column fractions from *Alliaria petiolata* BuOH extract or with solvent (90% MeOH) alone (control). Five gram leaf equivalents of each fraction were used for each replication. Replicated four times. A replication consisted of one bioassay cage with eight pairs of butterflies. Means (\pm SE) with the same letters in each series are not significantly different according to a one-sample *t* test (*P*, 0.05), performed on the transformed proportions of eggs laid on test plants, under the null hypothesis that eggs were distributed evenly over control and treated plants. Mean oviposition indexes (ODIs) are shown. ODI = 100 (Control - Treated)/(Control + Treated)

though glucosinolates are reported to exist in root material, green parts or seeds of *A. petiolata* (reviewed by Kjaer 1960; Daxenbichler *et al.* 1991) and the stimulant effect of sinigrin in this plant on feeding by *Ceutorhynchus constrictus* was examined by Nielsen *et al.* (1989), no research has previously aimed to investigate the role of glucosinolates in selection of this species by *Pieris* species. In the present study, sinigrin and glucotropaeolin were found in foliage of *A. petiolata*, thus confirming the results of previous studies (reviewed by Kjaer 1960). Gluconasturtiin was also detected in root material of *A. petiolata* in a study using paper chromatography (reviewed by Kjaer 1960), and the retention time of a compound in the desulfated post-BuOH

water extract from *A. petiolata* foliage (25.95 min; Fig. 3A,C) was identical to that of a desulfated sample of gluconasturtiin isolated from watercress (Huang & Renwick 1994a). However, the UV spectrum of this compound was significantly different from that of gluconasturtiin. We therefore conclude that gluconasturtiin was not detectable in our samples. The glucosinolates found in foliage extracts appear to be responsible for stimulating *P. rapae* and *P. napi oleracea* to lay eggs on *A. petiolata*. Fractions containing glucosinolates were consistently most active in oviposition assays. However, significant differences were found between the two *Pieris* species in response to different classes of glucosinolates. *P. napi oleracea* was stimulated more by

the fractions containing sinigrin than by any other fractions from *A. petiolata* or by cabbage extract. However, *P. rapae* was much more sensitive to fractions containing glucotropaeolin and other unknown compounds. This differential sensitivity means that acceptance of a plant by ovipositing females of *P. rapae* and *P. napi oleracea* occurs for different reasons. In the case of *P. rapae*, the relatively small quantity of glucotropaeolin and other unknown compounds appears to be responsible for acceptance of *A. petiolata*. By contrast, *P. napi oleracea* appears to respond to the relatively large quantity of sinigrin, an aliphatic glucosinolate. These results are consistent with previous findings that *P. napi oleracea* is more sensitive to aliphatic glucosinolates such as sinigrin than to aromatic ones including glucotropaeolin, whereas the reverse is true for *P. rapae* (Huang *et al.* 1993a,b; Huang & Renwick 1994a). This differential sensitivity appears to extend to the unidentified compounds with deterrent effects in *A. petiolata*. These results emphasize the fact that similar behavioral responses in *Pieris* (and perhaps other insects) are not always reliable indicators of similar plant chemistry or of similar response to specific plant compounds.

P. napi in Europe utilizes *A. petiolata* as a highly suitable hostplant (Bowden 1971; Porter 1994). This plant is naturalized in North America, and we have demonstrated that the basis for its attractiveness to ovipositing females of indigenous North American *P. napi oleracea* (Courant *et al.* 1994) is primarily the glucosinolate sinigrin. This glucosinolate is also present in relatively high concentrations in *P. napi*'s indigenous hostplant, *Dentaria diphylla* and *D. laciniata* (Feeny & Rosenberry 1982). It is likely that *P. napi oleracea* females confuse the native *Dentaria* and the naturalized *Alliaria* at this time, because they grow in similar habitats, and both contain relatively high concentrations of a glucosinolate that is highly stimulatory. In another case, Rodman & Chew (1980) suggested that similar glucosinolate profiles were the basis for maladaptive oviposition behavior by *P. napi macdunnoughii* in a montane plant community in Colorado, USA.

Providing there is sufficient genetic variation for appropriate components of oviposition behavior in *Pieris* butterflies, natural selection would be expected to operate in two directions, either to eliminate acceptance of *Alliaria* by ovipositing *P. napi oleracea* (Chew 1977a) or to increase adaptation to this plant by larvae (Chew 1977a; Courant *et al.* 1994). Whether sufficient genetic variation in oviposition behavior exists is not certain. More than 20 years after Chew's study (1975) of maladaptive oviposition behavior in *P. napi macdunnoughii*, butterflies in this population still place a large proportion of their eggs on *Thlaspi arvense*, on which their larvae die (Carol Boggs, pers. comm.). Because *Alliaria* and the indigenous host *Dentaria* both share sinigrin in their glucosinolate profiles, we predict that if oviposition acceptance behavior is eliminated in *P. napi oleracea*, this will be due to increased sensitivity to deterrent compounds rather than decreased sensitivity to stimulant compounds such as sinigrin.

P. napi oleracea has generally been less sensitive than *P. rapae* to oviposition deterrents in previous cases where the two species were compared under the same conditions. For example, erysimoside and erychroside from *E. cheiranthoides* were more deterrent to *P. rapae* (Huang *et al.* 1993a). *P. napi oleracea* was insensitive to 2-O- β -D-glucosyl cucurbitacin E and 2-O- β -D-glycosyl cucurbitacin I which acted as strong deterrents to *P. rapae* (Huang *et al.* 1993b). In a study where 18 cardenolides were tested for their oviposition deterrent activities, *P. rapae* was strongly deterred by some compounds such as K-strophanthin- β and oleandrin, but *P. napi oleracea* was not affected by these compounds (Huang & Renwick 1994b). In the present study, however, *P. napi oleracea* was much more sensitive than *P. rapae* to the deterrents in *A. petiolata*. The oviposition deterrent index (ODI) of the BuOH extract from *A. petiolata* was much higher for *P. napi oleracea* than for *P. rapae*. Higher ODIs for *P. napi oleracea* than for *P. rapae* were obtained for all the HPLC fractions separated from open-column fractions II and III. While HPLC fraction III1 from fraction III was significantly deterrent to both *P. napi oleracea* and *P. rapae*, fraction III3, which contained two major peaks, was active only for *P. napi oleracea*. A similar difference was found between the two *Pieris* species in response to HPLC fractions III1 and III2 from open-column fraction III. There was no significant difference in response of *P. rapae* to fractions III1 or III2 and the controls, whereas *P. napi oleracea* was strongly deterred by these fractions. The fact that *P. napi oleracea* is more sensitive than *P. rapae* to deterrents in *A. petiolata* appears to be contrary to a general pattern of insensitivity to deterrents in introduced plants (Huang *et al.* 1993a,b).

Although the majority of insects can depend on plant chemistry to guide their selection of the most suitable site for development and survival of their progeny, plant chemistry may lead to 'mistakes' (Jermy & Szentesi 1978; Rodman & Chew 1980; Renwick 1989). Reported 'mistakes' by females laying eggs on plants that do not support larval development have been traced to chemical similarity of the plants to actual hosts (Chew 1977a; Rodman & Chew 1980; Renwick 1989; Jaenike 1990). This idea is further supported by our finding that *P. napi oleracea* is strongly stimulated to oviposit on the imported crucifer, *A. petiolata*, which is not suitable for larval survival, but which contains specific glucosinolates characteristic of native cruciferous host plants. On the other hand, the stronger effects of oviposition deterrents from *A. petiolata* on *P. napi oleracea* than on *P. rapae* would suggest there is substantial chemical basis for any natural selection to resolve discrepancies between larval feeding behavior, larval survival and growth, and adult egg-laying behavior (Chew *et al.* 1989; Courant *et al.* 1994).

Acknowledgements

We thank Kathy Jo Champion for technical assistance. This research was funded in part by NSF grant Nos. BSR-9107322 and BSR-9108987, and the Arabis Fund.

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