Leaf surface chemicals stimulating oviposition by *Pieris rapae* (Lepidoptera: Pieridae) on cabbage

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**Summary**

The chemical stimulation of oviposition by *Pieris rapae* on cabbage was investigated by leaf washing and extraction. Isolation of the stimulant by various chromatographic techniques was monitored by a bioassay using *Sinapis alba* as a surrogate host plant. Cold water, chloroform, or chloroform followed by cold water washes failed to release the stimulant from leaf surfaces. Boiling water or chloroform followed by methanol was required. The most active stimulatory compound was identified as 3-indolylmethyl glucosinolate (glucobrassicin). Other glucosinolates were identified as sinigrin, which was only slightly active, and glucoiberin, which was completely inactive as a stimulant. The significance of the selective response of *P. rapae* and *P. brassicae* to different glucosinolates and the implications of the binding of polar allelochemicals to leaf surfaces is discussed with respect to host utilization and perception mechanisms of pierids.

**Key words**

oviposition, 3-indolylmethyl glucosinolate, sinigrin, glucoiberin, cabbage, Cruciferae, *Brassica oleracea*, Lepidoptera, Pieridae, *Pieris rapae*

**Introduction**

Host ranges of phytophagous insects are often determined to a large extent by plant chemistry (Städler 1992). Oligophagous species in particular appear to seek out plants that contain typical classes of secondary compounds, which may serve to attract the insects to their hosts or to stimulate oviposition and/or feeding. The close link between the host range of the cabbage butterflies, *Pieris rapae* and *P. brassicae*, and the presence of glucosinolates in plants has long been known (Verschaffelt 1910; Schoonhoven 1972). This relationship has served as a starting point for many studies on the chemical basis of host selection by crucifer-feeding insects (Rodman & Chew 1980; Chew 1988).

The involvement of glucosinolates and their volatile hydrolysis products (mustard oils) in host finding and acceptance has been demonstrated for many crucifer specialists (reviewed by Schoonhoven 1972; Städler 1992). However, the idea that glucosinolates are responsible for host recognition by all crucifer-feeding insects has been challenged (Nielsen 1978; Chew 1988), and behavioral studies on *Pieris rapae* using sinigrin as a representative glucosinolate have cast doubt on the role of these glycosides. The mustard oils do not appear to be attractive to *P. rapae*, and the reaction of ovipositing females to sinigrin was not typical of the response to cabbage extracts (Renwick & Radke 1983). Instead, the butterflies appeared to be confused by this compound. Studies on *P. rapae* in Australia suggest that sinigrin applied as a solution on colored paper can stimulate oviposition, but in these experiments eggs were also laid on control surfaces that lacked chemical stimulant (Traynier 1986). These results demonstrated a learning effect, in which the butterflies associated visual cues with the presence of a possible stimulant. However, no experiments were performed with a stimulatory extract of host plants to determine how important the learning phenomenon would be with a more natural, complex stimulus, on a dry substrate. Renwick & Radke (1988) have concluded that landing by *P. rapae* is guided primarily by color, and that acceptance or rejection then depends on contact chemoreception of stimulants and/or deterrents at the leaf surface. The presence of deterrents in non-host crucifers has been demonstrated (Renwick & Radke 1985), and specific oviposition deterrents from one of these unacceptable crucifers have recently been identified (Sachdev-Gupta et al. 1990). However, specific stimulants that can adequately explain host acceptance have yet to be isolated in a systematic manner.

Experiments with larvae of *P. rapae* have shown that certain glucosinolates may stimulate feeding, and larvae may orient towards the allylisothiocyanate that results from hydrolysis of sinigrin (Hovanitz & Chang 1963). These observations have led many authors to assume that adults are also likely to be guided by glucosinolates and/or their hydrolysis products. Electrophysiological studies have shown a response of tarsal receptors of *P. rapae* to certain glucosinolates, and this response was related to oviposition stimulation (Ma & Schoonhoven 1973).

Recent work on the diamondback moth, *Plutella xylostella*, has strongly suggested that glucosinolates are responsible for stimulating oviposition by this insect (Reed et al. 1989). A similar approach to *P. rapae* might determine...
whether glucosinolates other than sinigrin can explain the acceptance of potential oviposition sites by this insect.

The chemicals responsible for host recognition by ovipositing insects such as *P. rapae* must be present at the leaf surface (Städler 1986; Städler & Roessingh 1990; Renwick 1989). Thus it is reasonable to expect that washing the surface of cabbage leaves with solvents would dissolve the active compounds. The purpose of this study was to identify the most active stimulant for *P. rapae* from cabbage and to determine the conditions necessary for solvent removal of stimulatory compounds from leaf surfaces.

**Materials and methods**

**Plant Material** – Cabbage plants (*Brassica oleracea* var. Golden Acre) were grown in the greenhouse in Cornell Mix (Boodley & Sheldrake 1977) at an average temperature of 22°C with supplemental lighting provided by 400 W multivapor high intensity discharge lamps. The plants were generally 4–6 weeks old when used for leaf washes or extraction of foliage.

**Insects** – Butterflies from a colony of *P. rapae* maintained on cabbage plants, var. Golden Acre, were used for bioassays. Field-collected butterflies were added to the colony on an annual basis to reduce the risk of losing natural behavioral traits.

**Chemicals** – Samples of pure glucosinolates were obtained from Dr. Roger Fenwick at the AFRC Institute of Food Research in Norwich. Tetramethyl ammonium salts of glucosinolates were converted to the potassium salts by extraction of foliage. An ethanolic extract with hexane to yield a stimulatory aqueous extract was prepared by dipping single cabbage leaves sequentially in 400 ml each of cold water, chloroform, and again in cold water. A second batch of leaves was dipped in boiling water only. The duration of each dip was 2 s, with a 5 s pause between dips to allow excess solvent to drip off. In a second series of leaf surface washes, 2 s dips in chloroform were followed by 2 s dips in methanol, with an intermediate pause of 5 s.

**Bioassays** – The sensitivity of our previously described bioassay (Renwick & Radke 1983) for oviposition stimulants was increased considerably by the use of Sieva bean (*Phaseolus vulgaris*, var. Sieva) foliage instead of green index cards. The relatively waxy leaves of Sieva beans provide most of the natural characteristics of a host plant, without any stimulant, and apparently without any significant deterrent or repellent. Plant extracts or solutions of pure compounds were applied to both leaves of a 2-week-old plant when the first two true leaves were almost fully expanded. Test solutions or solvent alone as controls were applied in 50% methanol in water (ca. 1 ml/leaf) using a chromatographic sprayer to coat upper and lower surfaces. Caged butterflies (7 pairs) were provided with a cabbage plant to determine when they were ready to oviposit. That plant was then removed, and on the next day, the butterflies were offered a choice of one treated or one control Sieva bean plant over a period of 6 h (10:00–16:00) to test for stimulatory activity, as measured by the numbers of eggs laid. Cabbage leaf extracts or washes with various solvents were tested either as paired or washes with various solvents were tested either as paired treatments, indicated that the oviposition stimulant for *P. rapae* could not be removed with cold water (Fig. 1). Dipping in chloroform to strip off the waxes also failed to release stimulant, and a cold water wash after wax removal was also ineffective. However, dipping the leaves in boiling water clearly released the stimulatory compounds (Fig. 1). No eggs were laid on control plants offered before each test was started. In subsequent surface wash experiments, dipping in

**Leaf extracts and surface washes** – Cabbage foliage from entire plants was extracted as previously described, with hot ethanol and subsequent defatting of the ethanolic extract with hexane to yield a stimulatory aqueous extract (Renwick & Radke 1983). Leaf surface washes were prepared by dipping single cabbage leaves sequentially in 400 ml each of cold water, chloroform, and again in cold water. A second batch of leaves was dipped in boiling water only. The duration of each dip was 2 s, with a 5 s pause between dips to allow excess solvent to drip off. In a second series of leaf surface washes, 2 s dips in chloroform were followed by 2 s dips in methanol, with an intermediate pause of 5 s.

**Analysis of plant samples** – Defatted ethanolic extract (200 gram leaf equivalents [gle]) was partitioned between n-butanol and water. The post-butanol aqueous fraction was separated into 23 fractions (15 ml each) by flash chromatography (Peterka & Fenwick 1988) on a column of reverse phase octadecyl silica gel (J. T. Baker, 40 μm average particle diameter, 10 g). The column was equilibrated with 0.5% K2SO4 before applying the sample. The elution was achieved successively with 0.5% K2SO4 (fractions 1–10), H2O (fractions 11–20), 33% MeOH in H2O (fraction 21), 50% MeOH in H2O (fraction 22), and MeOH (fraction 23). On the basis of TLC pattern and HPLC after the enzymic desulfation according to the method of Minchinton et al. (1982) for glucosinolates, fractions 1–3, 4–7, 8–11, 12–16, 17–20, and 21–23 were combined. TLC was performed on precoated Whatman K6 silica gel plates (5 x 10 cm, 250 μm thickness) using the following solvent systems: (1) CHC13-MeOH-H20 (14:10:1), and (2) EtOAc-MeOH-HOAc-H20 (4:1:1:0.5). Spots were visualized by spraying the plate with a 1% solution of ceric sulfate in 2N H2SO4 followed by heating at 110°C for 15–20 min. HPLC analysis of the desulfoglucosinolates was performed on a Varian 3000 Liquid Chromatograph. A Varian Micropak C-18 column, MCH-10 (50×0.8 cm, irregular 10 μm particles) was used. Separation was achieved using the following program at a constant flow rate of 3.3 ml/min:

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<tr>
<th>Time (min)</th>
<th>% H2O</th>
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HPLC analysis of desulfated total glucosinolates of the defatted alcoholic extract was also performed as above, and individual compounds were isolated for identification. Detection by a diode array detector (Hewlett Packard model 1040A) allowed for confirmation of identities by UV spectra as well as comparison of retention times with those of authentic samples.

**Results**

Bioassays of cabbage leaf washes, offering a choice of 4 treatments, indicated that the oviposition stimulant for *P. rapae* could not be removed with cold water (Fig. 1). Dipping in chloroform to strip off the waxes also failed to release stimulant, and a cold water wash after wax removal was also ineffective. However, dipping the leaves in boiling water clearly released the stimulatory compounds (Fig. 1). No eggs were laid on control plants offered before each test was started. In subsequent surface wash experiments, dipping in