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Sinigrin as a feeding deterrent in two crucifer-feeding, polyphagous lepidopterous species and the effects of feeding stimulant mixtures on deterrency

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SUMMARY

The glucosinolate, sinigrin (allyl- or 2-propenyl glucosinolate), present in several Cruciferae, was incorporated in varying concentrations into four different diet background mixtures to study the behavioural reactions of *Mamestra configurata* and *Trichoplusia ni*. Sinigrin concentrations were chosen to mimic normal levels in naturally occurring cruciferous plants, or to represent a plant during a particular stage in its growth cycle. One diet background mixture contained potassium chloride (KCl) and no stimulatory sugar or sugar alcohol, two backgrounds contained KCl and a single sugar or sugar alcohol (sucrose or inositol, respectively), and the fourth background contained KCl and both sugar and sugar alcohol (sucrose and inositol, respectively). Sinigrin acted primarily to reduce (deter) feeding in all backgrounds, although the effect varied with sinigrin concentration, background and species. When inositol or sucrose was included in the mixture, the deterrent effect of sinigrin was decreased in both species. When inositol and sucrose were present, suppression of the deterrent effect of sinigrin was greatest for *M. configurata*. The effects of mixtures were not predictable from a knowledge of the action of individual components. Differences observed between species may reflect different sensory capabilities.

1. INTRODUCTION

Host plant specificity and host selection in phytophagous insects are governed largely by responses to chemicals that are characteristic of certain plant taxa (Fraenkel 1969). Tissues of higher plants contain a variety of chemicals (secondary plant compounds) including alkaloids, steroids, phenolics, saponins, glucosinolates, tannins, resins, essential oils, organic acids, thought to have a defensive function (Beck & Schoonhoven 1980) and to provide the stimuli to which specialized insects respond.

Dethier *et al.* (1960) defined a deterrent as a chemical which inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit. Adaptations to overcome deterrence involve behavioural (sensory) and metabolic capabilities, by which the allomone may ultimately become a positive behavioural stimulus and be readily degraded and/or used biochemically (Beck & Schoonhoven 1980). It is thought that as polyphagous insects are able to use a wide range of host plants, they must be equipped with a broader range of metabolic detoxification capabilities. In addition, their chemosensory systems (particularly taste) must allow feeding to proceed in the face of a wide range of stimulus mixtures.

Sinigrin is usually considered a feeding stimulant for crucifer-feeding insects (Verschaffelt 1910; Thorsteinson 1953; Gupta & Thorsteinson 1960; Schoonhoven 1967; Nayar & Thorsteinson 1963; Tanton 1965; David & Gardiner 1966*a*, *b*; Ma 1969; Hicks 1974). However, work by Bodnaryk (1991) on *M. configurata* and *Phyllotreta cruciferae*, and Blom (1978*a*) on *Mamestra brassicae*, clearly demonstrated sinalbin (p-hydroxybenzyl glucosinolate) and sinigrin-based deterrence, respectively, in these crucifer feeders. It has also been shown that sinigrin acts as a feeding deterrent to non-crucifer-feeding Lepidoptera (Erickson & Feeny 1974).

It is difficult, as Ma (1972) observed, to measure the behavioural response on diets containing only a deterrent compound, as the behavioural response will result in a decrease in food intake leaving only a very small margin to show feeding-inhibiting effects. The base diet should first be made more palatable by the addition of some kind of stimulant. The present study addresses the action of sinigrin on feeding by examining its effect at a range of concentrations on food intake by larvae of two lepidopterous species that are crucifer feeders, but not exclusively so. Sinigrin, alone and in combination with phagostimulatory compounds (i-nositol and sucrose), was employed to determine the effects of phagostimuli on the dose–response dynamics of this secondary plant compound.

M. configurata and *T. ni* both have polyphagous feeding habits. *M. configurata* is native to grasslands across the Canadian prairies and into the interior of British Columbia, as well as south along the Cordilleras

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to Mexico City (Turnock 1977). It is known to feed on at least 34 plant species from 14 families (King 1928; Beirne 1971). *T. ni* is a common insect in the United States and feeds on plants from at least 20 plant species from nine families (Shorey *et al.* 1962; Beirne 1971).

2. MATERIALS AND METHODS

(*a*) *Insects and diet*

Fifth instar, 12–22 h post-moult *M. configurata* and *T. ni* larvae were obtained from an artificial diet-reared laboratory culture. Larvae of both species were reared from the fourth instar in groups of ten per 120 ml plastic container at 24 °C and 27 °C, respectively, with a L16:D8 photoperiod.

M. configurata larvae were reared on a soybean-based artificial diet (Bucher & Bracken 1976), with minor modifications by B. A. Keddie (unpublished data). *T. ni* larvae were reared on a pinto bean-based artificial diet (Tanada & Chang 1968), with minor modifications by Keddie & Volkman (1985). Larvae were deprived of food 2–4.5 h prior to the experiments. Each experimental treatment was tested on 7–19 *M. configurata* and 10–12 *T. ni* larvae.

(*b*) *Feeding behaviour*

Agar disks were prepared according to Bucher & Bracken (1976), with modifications to allow a direct comparison with electrophysiological results, as described in Shields & Mitchell (1995).

The components used in the preparation of four diet background mixtures were: agar (powder type IV, Sigma Chemical Co.); debittered brewer's yeast (Vita Health Co. Ltd); 50 mM KCl (Fisher Scientific Co.), instead of Wesson salt mix; 60 mM sucrose–saccharose (Anachemia); 100 mM meso-inositol (J. T. Baker Chemical Co.); sinigrin (United States Biochemical Corp.), or sinigrin monohydrate from horseradish (Sigma Chemical Co.), and deionized water.

The basic diet included agar, distilled water, yeast and KCl. Test mixtures were: sinigrin (various concentrations) incorporated with: (i) KCl (K); (ii) KCl/inositol (KI); (iii) KCl/sucrose (KS); or (iv) KCl/inositol/sucrose (KIS). The dietary components were kept in similar proportions and concentrations to that described by Bucher & Bracken (1976). Only one diet background mixture was prepared for each experimental session.

Sinigrin solutions were prepared in 20 ml plastic bottles (Fisher Scientific Co.) to yield 10 ml volumes at concentrations of 0, 1, 2, 4, 5, 8, 10, 15, 20 and 30 mM. The agar suspension was heated to 100 °C for 10–15 min on a magnetic mixer. A 9 ml aliquot of the heated mixture was added to each 1 ml sinigrin volume. The components were mixed thoroughly by shaking in a capped bottle. Three-millilitre plastic syringe barrels, with the tapered end cut off, were used to draw up the hot liquid agar and to extrude the cooled agar cylinders. After the hot test mixture was drawn up, the open end of the syringe was capped with aluminum foil. The filled syringe was then packed, foil side down, in crushed ice.

After the agar had gelled (at least 20 min), the solid cylinder was extruded from the syringe barrel. A multiwire cutting apparatus, described by Mitchell (1978), was used to produce test disks of a reproducible size and dry mass. Agar cylinders were cut in ascending order of sinigrin concentration and the apparatus was cleaned of agar debris between cuttings. Disks containing air bubbles or any other visible imperfections were discarded.

For feeding assays, each disk was placed on a labelled coverslip (22 mm × 22 mm, #1 thickness) which was then placed in a 60 mm × 15 mm Petri dish lined by a #1 Whatman 5.5 cm filter paper. To prevent desiccation of the disk, 500 µl aliquots of distilled water were added to the filter paper. The Petri dishes were set out in rows on a strip of moistened Kaydry table soaker paper (Kimberly-Clark), each row representing a different sinigrin concentration. Each larva (one larva per dish) was randomly selected and placed in a Petri dish. The entire group of dishes was covered with a large plastic sheet, fastened at all corners, to maintain high humidity. Seven to 19 disks from each cylinder were retained to determine the dry mass of a typical disk from that block (see entire disks, below).

Tests were terminated when about 50% of any one of the sinigrin treatment groups (including 0 mM sinigrin) had been consumed (adapted from Schoonhoven & Jermy 1977). Most sinigrin concentrations were included in each experiment and a 0 mM sinigrin control treatment was always present.

After each experiment, disks were allowed to dry at room temperature for at least 24 h. The dried agar disks were then separated from the coverslips using a sharp single-edged razor blade and the material was gently scraped off and collected on glassene weighing paper. The material was weighed on an automatic electrobalance (Cahn Model 36), with an accuracy to ± 0.001 mg.

Mean consumption (mg) for a single treatment was calculated by subtracting the mean mass of the portion remaining from test disks in the treatment group from the mean mass of a sample of entire disks cut from a cylinder from the same treatment group and held in the absence of larvae. Standard error (s.e.) of consumption was calculated by combining the standard errors of test and entire disks (s.e. consumption = $[(\text{s.e. test})^2 + (\text{s.e. entire})^2]^{\frac{1}{2}}$. Experiments for *M. configurata* and *T. ni* lasted 4.5–7.5 h and 3.5–5 h, respectively.

The effect of sinigrin on feeding in various background mixtures was analysed using the non-parametric Kruskal-Wallis one-way analysis of variance by ranks ($p \leqslant 0.05$) and Dunn's Multiple Comparison test using rank sums ($p \leqslant 0.05$). Data were analysed using Gibbons (1976), Number Cruncher Statistical Software (NCSS) (1987, Dr J. Heintz, Kaysville, Utah, U.S.A.), and Statview (1986, Abacus Concepts Inc., Berkeley, California, U.S.A.).

(*c*) *Glossary*

Diet: agar, distilled water, yeast and KCl.

Components: K (potassium chloride); KI (potassium chloride and inositol); KS (potassium chloride

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and sucrose); KIS (potassium chloride, inositol and sucrose).

Diet background mixture: diet and components (K, KI, KS, or KIS) to which sinigrin was added.

Test mixture: a diet background mixture in addition to sinigrin.

Entire disk: disks cut from the same cylinder as those being tested, but held in the absence of larvae. Used to determine the consistency (s.e.) of disks in each cylinder.

0 mM sinigrin (control) disk: disk containing the dietary background components being tested in the absence of sinigrin.

Active feeder: larva which ate $\geqslant 50\,\%$ of a test disk.

3. RESULTS

(*a*) M. configurata

The strong deterrent effect of sinigrin against only a K diet background mixture was very clear even at the lower concentrations tested, e.g. 1, 2, 4 mM sinigrin (figure 1*a*, $n = 109$). There was a proportional decrease in feeding with dose and maximum inhibition of feeding occurred as low as 1 mM sinigrin. Mean consumption of 0 mM sinigrin (K) disks was 2.3 mg, compared with 0.12 mg at 30 mM sinigrin. There were no larvae that consumed 50 % or more of test disks containing sinigrin using this diet background mixture (table 1).

The deterrent effect of sinigrin was still very prominent when sinigrin was incorporated into the KI diet background mixture; however, mean consumption of 0 mM sinigrin (KI) disks was almost double that of the K diet background mixture (4.4 mg) due to the stimulatory effect of inositol (figure 1*b*, $n = 100$). Feeding was not significantly inhibited until as low as 4 mM sinigrin. The apparent stimulatory effect on consumption at 1 mM sinigrin was not statistically significant. Fifty-five, 60, 20 and 10 % of larvae consumed 50 % or more of test disks containing 1, 2, 4 and 5 mM sinigrin, respectively (table 1).

Mean consumption of 0 mM sinigrin (KS) disks was 4.9 mg (figure 1*c*, $n = 88$). The sinigrin dose–response curve was less pronounced than those for the KI or K diet background mixtures, indicating that the deterrent effect of sinigrin, especially at lower concentrations, was less prominent when sucrose was present. Feeding inhibition was significant at 4 mM sinigrin but did not reach maximum until as low as 8 mM sinigrin. The difference in consumption at 1 and 2 mM sinigrin was not statistically significant. Eleven percent of larvae consumed 50 % or more of the disks containing 5 mM sinigrin (table 1). This concentration is only slightly higher than that typically found in older leaves of some Cruciferae (Bodnaryk 1991).

Mean consumption of 0 mM sinigrin (KIS) disks was 5.9 mg (figure 1*d*, $n = 133$). This was 1.3 and 1.2 times, respectively, that of the KI and KS diet background mixtures and slightly more than 2.5 times that of the K diet background mixture. The inositol/sucrose combination (KIS) had an additive, rather than a synergistic, effect on feeding in *M. configurata*. The apparent peaks in consumption at 1, 2 and 10 mM sinigrin were not statistically significant. Maximum

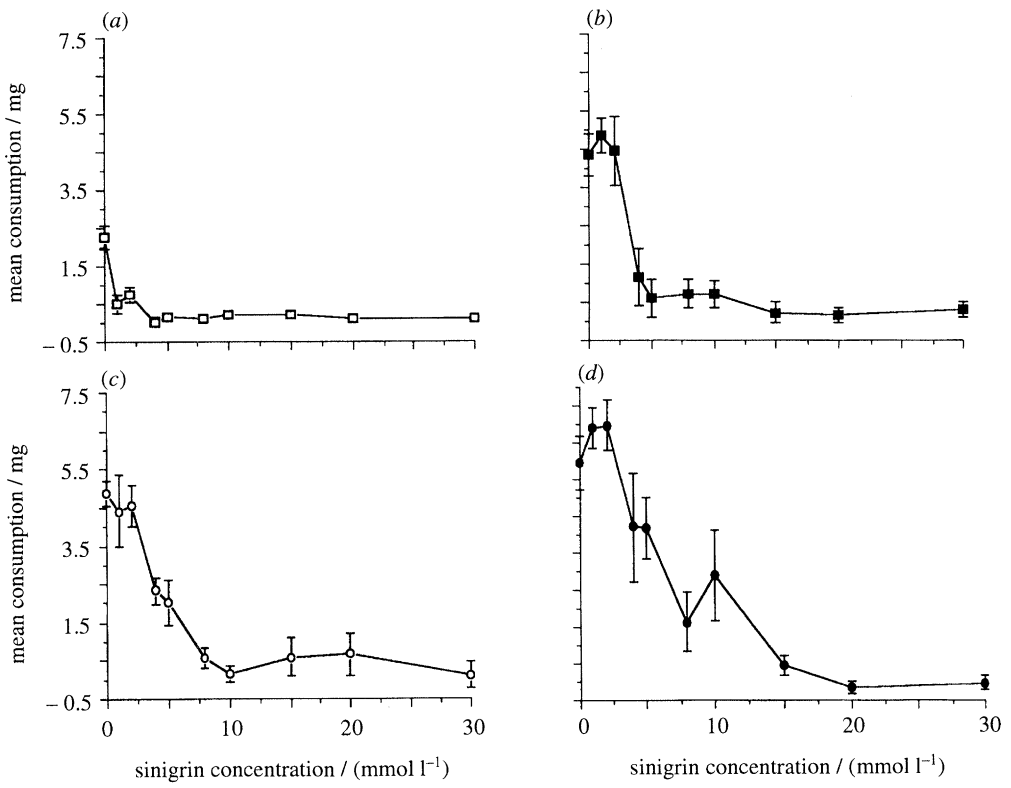

Figure 1. Mean consumption by *Mamestra configurata* larvae exposed to increasing concentrations of sinigrin in four diet background mixtures containing (*a*) potassium chloride (K), (*b*) potassium chloride/inositol (KI), (*c*) potassium chloride/sucrose (KS), (*d*) and potassium chloride/inositol/sucrose (KIS). Each point represents means for 7–19 larvae. Error bars represent the standard errors of the means.

Table 1. *Percent of larvae showing 50% or greater consumption (active feeders) at various sinigrin concentrations using four different diet backgrounds mixtures.*

(*M.c.* = *Mamestra configurata*; *T.n.* = *Trichoplusia ni*; K, KI, KS, KIS = agar backgrounds; ( ) indicates total number of larvae; 0 indicates 0 sinigrin control.)

sinigrin concentration	K		KI		KS		KIS	
(mM)	*M.c.*	*T.n.*	*M.c.*	*T.n.*	*M.c.*	*T.n.*	*M.c.*	*T.n.*
0	64 (11)	100 (12)	70 (10)	71 (14)	67 (9)	81 (16)	63 (19)	87 (15)
1	[a] (11)	42 (12)	55 (11)	62 (13)	67 (9)	80 (15)	83 (18)	73 (15)
2	[a] (12)	[a] (12)	60 (10)	86 (14)	67 (9)	80 (15)	41 (17)	93 (14)
4	[a] (11)	[a] (11)	20 (10)	29 (14)	[a] (9)	50 (10)	25 (8)	53 (15)
5	[a] (11)	[a] (12)	10 (10)	29 (14)	11 (9)	56 (16)	29 (17)	20 (15)
8	[a] (11)	[a] (11)	[a] (10)	7 (14)	[a] (10)	13 (16)	13 (16)	14 (14)
10	[a] (11)	[a] (11)	[a] (10)	8 (12)	[a] (9)	31 (16)	22 (9)	[a] (14)
15	[a] (11)	[a] (11)	[a] (10)	[a] (14)	[a] (8)	[a] (10)	[a] (10)	7 (14)
20	[a] (10)	[a] (11)	[a] (10)	[a] (14)	[a] (7)	[a] (10)	[a] (9)	[a] (14)
30	[a] (10)	[a] (11)	[a] (9)	[a] (13)	[a] (9)	[a] (10)	[a] (10)	[a] (14)

[a] Indicates consumption was less than 50%

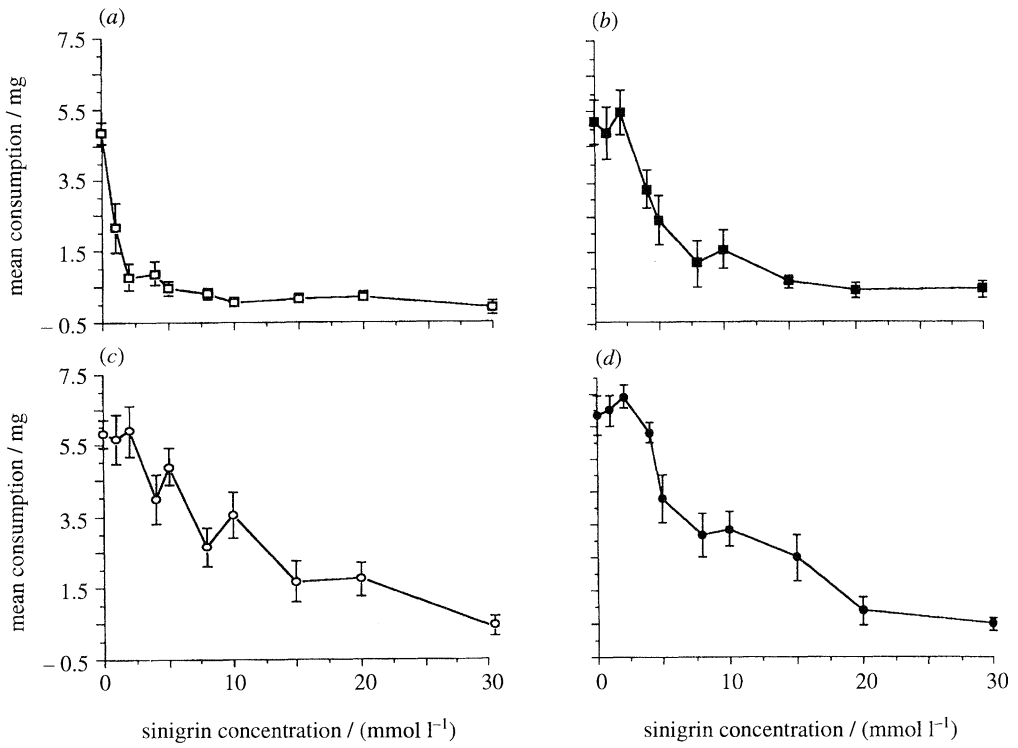

Figure 2. Mean consumption by *Trichoplusia ni* larvae exposed to increasing concentrations of sinigrin in four diet background mixtures containing (*a*) potassium chloride (K), (*b*) potassium chloride/inositol (KI), (*c*) potassium chloride/sucrose (KS), and (*d*) potassium chloride/inositol/sucrose (KIS). Each point represents means for 10–16 larvae. Error bars represent the standard errors of the means.

feeding inhibition did not occur until as low as 8 mM sinigrin. Even at 10 mM sinigrin, 22% of the larvae consumed more than 50% of the test disk during the experiment (table 1).

(*b*) T. ni

Sinigrin also had a pronounced deterrent effect on feeding when it was added to the K diet background mixture, even at low concentrations (figure 2*a*, $n = 114$). Maximum feeding inhibition occurred as low as 1 mM sinigrin. Mean consumption of 0 mM sinigrin (K) disks was 4.9 mg. Most *T. ni* larvae did not consume any of the 30 mM sinigrin impregnated disks. Forty-two percent of larvae consumed 50% or more of disks containing 1 mM sinigrin (table 1).

Mean consumption of 0 mM sinigrin (KI) disks was 5.2 mg (figure 2*b*, $n = 136$). Maximum feeding inhibition occurred as low as 8 mM. The apparent decline in consumption at 1 mM and similar increases at 2 and 10 mM sinigrin were not statistically significant. Sixty-two, 86, 29, 29, 7 and 8% of larvae consumed 50% or more of disks containing 1, 2, 4, 5, 8 and 10 mM sinigrin, respectively (table 1).

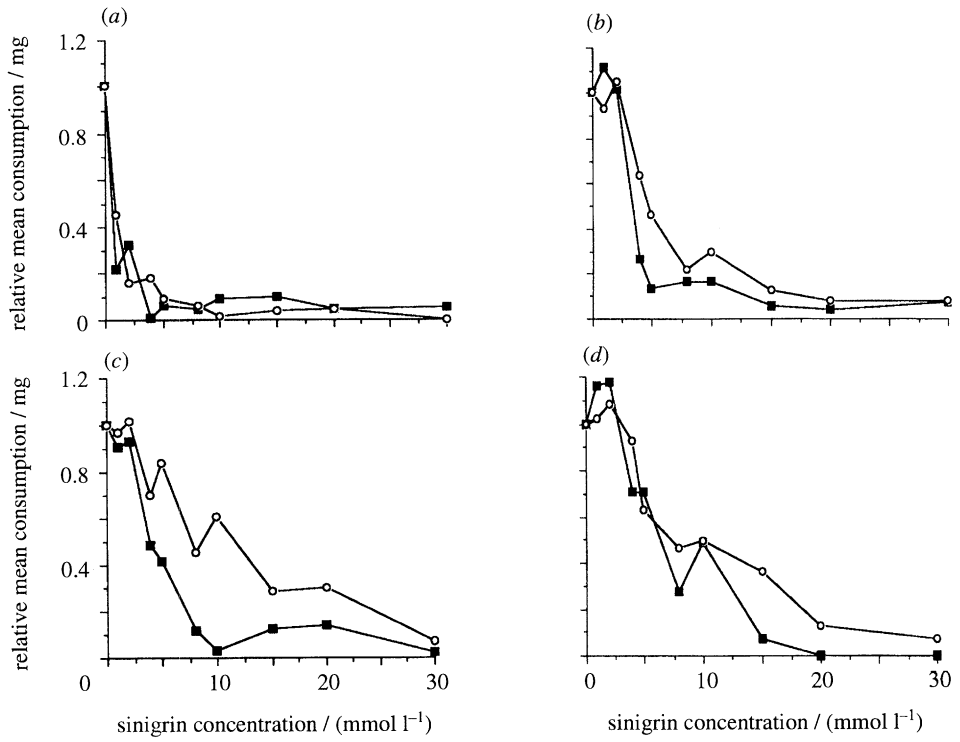

Figure 3. Relative mean consumption by *Mamestra configurata* (filled squares) and *Trichoplusia ni* (open circles) exposed to increasing concentrations of sinigrin in four diet background mixtures containing (*a*) potassium chloride (K), (*b*) potassium chloride/inositol (KI), (*c*) potassium chloride/sucrose (KS), and (*d*) potassium chloride/inositol/sucrose (KIS). Each point represents means for 7–19 larvae for *M. configurata* and 10–16 larvae for *T. ni*. Data derived from those used in figures 1 and 2. Refer to figures 1 and 2 for the standard errors of the data points.

The deterrent effect of sinigrin was less pronounced when it was incorporated into the KS diet background mixture (figure 2*c*, $n = 134$). Maximum feeding inhibition occurred as low as 15 mM sinigrin. Mean consumption of 0 mM sinigrin (KS) disks only slightly increased from that of the KI diet background mixture to 5.8 mg. The apparent slight inhibitory effect on consumption at 1 mM and similar increases at 2, 5 and 10 mM sinigrin were not statistically significant. Eighty, 80, 50, 56, 13 and 31% of larvae consumed 50% or more of disks containing 1, 2, 4, 5, 8 and 10 mM sinigrin, respectively (table 1).

Mean consumption of 0 mM sinigrin (KIS) disks was 6.8 mg (figure 2*d*, $n = 144$), indicating an additive effect of inositol and sucrose. The slight apparent peaks in consumption at 1, 2 and 10 mM sinigrin were not statistically significant. Maximum feeding inhibition occurred as low as 20 mM sinigrin. Seventy-three, 93, 53, 20, 14 and 7% of larvae consumed 50% or more of disks containing 1, 2, 4, 5, 8 and 15 mM sinigrin, respectively (table 1).

(*c*) M. configurata *compared with* T. ni

For both species, there was a general trend of increase in consumption on the various 0 mM sinigrin disks (K, KI, KS, KIS) in the order K < KI < KS < KIS, but this trend was much more pronounced in *M. configurata* (figures 1 and 2). Figure 3*a–d* summarizes the deterrent role that sinigrin played in all diet background mixtures for both species in terms of relative mean consumption. For each diet background mixture, consumption at 0 mM sinigrin was set at 1.0. With no stimulatory additives in the mixture (K diet background mixture), the negative slopes for both species were almost identical (figure 3*a*), indicating that sinigrin deterred feeding in both species to a similar degree. When inositol was added (KI) (figure 3*b*), the strong deterrent effect of sinigrin appeared to be only slightly compensated for at lower sinigrin concentrations in *M. configurata*, unlike that in *T. ni*. This difference in compensation between the two insects was more pronounced when sucrose was present (KS) (figure 3*c*). When inositol and sucrose were both present in the diet background mixture (KIS) (figure 3*d*), deterrence in *T. ni*. remained similar relative to KS, while it significantly decreased in *M. configurata* (relative to KS). Thus, with the KIS diet background mixture, the dose response curves for both species largely overlapped, as they did with the simplest background (K) (figure 3*a*).

Table 1 summarizes the results with respect to those larvae which ate 50% or more of disks (active feeders) containing some level of sinigrin. From these results, it appeared that sinigrin, at 1 mM, when added to a K diet background mixture, had no deterrent effect in 42% of *T. ni* larvae ($\geqslant 50\%$ of disk consumed). In contrast, all *M. configurata* larvae ate less than 50% of the disk at 1 mM sinigrin. With a KI diet background mixture, the deterrent effect of sinigrin appeared to be slightly masked by inositol. Large numbers of *M. configurata* larvae were able to compensate and to continue to feed normally at the lower concentrations of sinigrin (1 and 2 mM), while others continued to feed even on slightly higher levels (4 and 5 mM). A wider tolerance to sinigrin was displayed by *T. ni* larvae

when inositol was present in the diet, with a large proportion of larvae continuing to feed actively at sinigrin concentrations of 8 and 10 mM. A large number of active feeders were found with a KS diet background mixture at most sinigrin concentrations with *T. ni* larvae. With *M. configurata* larvae, few were stimulated to feed actively above 2 mM sinigrin. The KIS diet background mixture generally produced the same result for *T. ni* larvae, as it did with the KS mixture (figure 4*b*). One *T. ni* larva fed actively at 15 mM sinigrin. The KIS background produced the greatest number of feeders at all sinigrin concentrations in *M. configurata* (figure 4*a*).

4. DISCUSSION

(*a*) *Sinigrin: feeding stimulant or deterrent?*

It has been reported that sinigrin acts as a feeding stimulant for specialized crucifer-feeding insects (see references below) and general application of this conclusion has led to some confusion. Sinigrin does act as a feeding stimulant for specialized species but apparently only at relatively low concentrations and often in concert with phagostimulants such as sucrose (see below). As the present work demonstrates, when sinigrin concentrations are increased, larvae of some crucifer-feeding species can be deterred from feeding. Feeding experiments with glucosinolates have incorporated the compound into plants by passive diffusion uptake (Hicks 1974; Bodnaryk 1991), by surface application (Verschaffelt 1910; Thorsteinson 1953; Gupta & Thorsteinson 1960), or into artificial substrates, such as agar (Nayar & Thorsteinson 1963; Tanton 1965; David & Gardiner 1966*a*; Ma 1972; Blom 1978*a*, *b*; Bodnaryk 1991; McCloskey & Isman 1993). It is difficult, when using the leaf treatment methods, to determine if sinigrin is the decisive factor regulating feeding. A better approach is to isolate the glucosinolate in a neutral substance, such as a synthetic diet, to ensure that it is the only component being tested. The latter technique was used in the present study.

David & Gardiner (1966*b*) showed that sinigrin stimulated feeding when it was incorporated into an artificial diet at very low concentrations (0.0003–0.3 mM) in *Pieris brassicae* larvae. Blom (1978*a*), using the same larval species, found that a sucrose–sinigrin mixture reached a maximum stimulating effect at a lower sucrose concentration than sucrose alone and feeding subsequently decreased at higher concentrations of sucrose. This was in agreement with Ma's (1972) findings, on the same species, but Ma (1969, 1972) inferred synergism between sucrose and sinigrin, in contrast to Blom (1978*a*). Schoonhoven (1976) also found that sinigrin in combination with sucrose strongly promoted feeding in *P. brassicae* and a synergizing effect was shown. In an earlier study, Schoonhoven (1967) found that higher concentrations of sucrose (100 mM) and sinigrin (10 mM) evoked increased feeding but without synergism. Blom (1978*a*) stated that high concentrations of sinigrin are not deterrent to *P. brassicae*. Blom (1978*a*) found that a sucrose–sinigrin mixture did not have a positive effect on feeding in *M. brassicae* larvae and that sinigrin was deterrent at 10 mM in a weakly phagostimulatory test diet. Nayar & Thorsteinson (1963) observed an increase in feeding activity with *Plutella maculipennis* larvae due to the stimulatory effect attributed to the combination of sinigrin and glucose.

The present study follows from the work of Bodnaryk (1991), who investigated the effect of sinalbin on feeding by the flea beetle, *P. cruciferae*, as well as by *M. configurata*. Bodnaryk determined that sinalbin acted as a deterrent, causing a decrease in feeding in *M. configurata* in a dose-dependent manner (see figure 1 in Bodnaryk 1991). At 20 mM, the highest concentration tested, feeding was completely deterred in *M. configurata* and partially deterred in *P. cruciferae* (Bodnaryk 1991).

Bodnaryk & Palaniswamy (1990) found that sinigrin, the major glucosinolate occurring in the mustard, *Brassica juncea* cv. Cutlass, occurred at a concentration range of approximately 1–6 mM (calculated from figure 2A in Bodnaryk & Palaniswamy 1990) in cotyledons. Seedlings of *Sinapis alba* cv. Ochre contain high concentrations of sinalbin in their cotyledons (> 20 mM) and young leaves (up to 10 mM) (Bodnaryk 1991). This rapid early synthesis in young leaves may function to inhibit pest or pathogen attack. It also coincides with high amino acid levels and high rates of protein synthesis. Bodnaryk (1991) found that the sinalbin concentration fell rapidly during the first few days of growth and levelled off at about 2 mM in older tissues. This decrease was largely due to the 'dilution' of sinalbin by increasing tissue biomass. The accumulation of polyphenols and secondary thickening during this time could also aid in leaf protection (Underhill 1980). Lower concentrations (2–3 mM) of sinalbin offered little or no protection against *P. cruciferae* and may have been slightly stimulatory between 0.5–2 mM (see figure 1 in Bodnaryk 1991). These low concentrations of sinalbin in the older leaves were still found to be an effective feeding deterrent against *M. configurata*; an example of antixenosis (Bodnaryk 1991). *M. configurata* larvae that had fed on young seedlings (> 20 mM sinigrin concentration levels) were also found to have reduced levels of fitness as measured by their low survival, low body masses, and abnormal diapause compared with cohorts fed on older *S. alba* or *Brassica napus* plants; an example of antibiosis (Bodnaryk 1991).

In the present study, the relationship of the negative feeding slope for *M. configurata* in response to increased sinigrin incorporated into a weakly stimulatory (K) diet background mixture was approximately linear (figure 1*a*). When compared with the sinalbin deterrent curve (see figure 1 in Bodnaryk 1991), sinigrin appeared to be the more potent feeding deterrent for *M. configurata*, especially at the lower concentrations (i.e. 1, 2, 4, 5 mM). At 1 mM sinigrin, mean consumption was reduced to 22 % (relative mean consumption) or 0.5 mg (mean consumption), as compared with the control (2.3 mg, mean consumption). With 1 mM sinalbin, relative mean consumption remained at 95 % of the control (calculated from figure 1 in Bodnaryk 1991). *M. configurata* was completely deterred from feeding by 4 mM sinigrin, whereas it was not completely deterred until 20 mM sinalbin.

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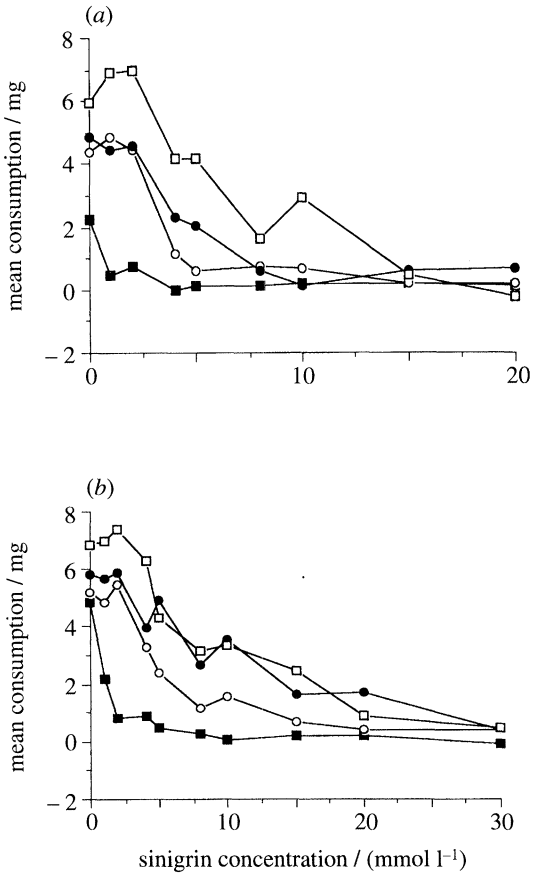

Figure 4. (*a*) Mean consumption by *Mamestra configurata* exposed to increasing concentrations of sinigrin in four diet background mixtures, as in figure 1 (filled squares, potassium chloride (K); open circles, potassium chloride/inositol (KI); filled circles, potassium chloride/sucrose (KS); open squares, potassium chloride/inositol/sucrose (KIS)). Data are the same as in figure 1 but have been rearranged for ease of comparison across backgrounds and standard error bars have been removed. (*b*) Mean consumption by *Trichoplusia ni* exposed to increasing concentrations of sinigrin in four diet background mixtures, as in figure 2. Data are the same as in figure 2 but have been rearranged for ease of comparison across backgrounds and standard error bars have been removed.

From the present study, it is apparent that sinigrin acts as a strong feeding deterrent to both *M. configurata* and *T. ni* using only a weakly stimulatory (K) diet background mixture.

(*b*) *The mode of action of sinigrin as a feeding deterrent*

It was clear in both species that the sinigrin concentration at which feeding started to decrease was higher with KI, KS and KIS versus K diet background mixtures. Interestingly with KI, KS and KIS, maximum feeding occurred at 1–2 mM sinigrin in both *M. configurata* and *T. ni*.

The KIS diet background mixture (with 0 mM sinigrin) evoked the highest feeding in *M. configurata* larvae and also rendered the deterrent the most palatable. This was apparent at several concentrations (1–8 mM sinigrin) (figure 4*a*). Increased feeding was also noted in *T. ni* with a KIS diet background mixture; however, its superior protection from the deterrent effect of sinigrin was limited to only low concentrations (1–4 mM) (figure 4*b*). At medium to high sinigrin concentrations ($\geqslant 5$, < 20 mM), feeding on this background was similar to that on the KS background (figure 4*b*). Thus, with *M. configurata*, maximum protection against the deterrent effect of sinigrin required both inositol and sucrose (KIS) while with *T. ni*, maximum protection could be achieved with sucrose (KS) alone. In other words, sucrose (KS) and inositol and sucrose (KIS) were equally effective. In both species, inositol alone (KI) afforded moderate protection.

These diet background mixture effects and the protection that stimulants afford against sinigrin-based deterrence suggest that sensory mechanisms are at least partly responsible for the observed behaviour. The relatively short feeding times used emphasizes the contribution of sensory-based mechanisms. However, feedback effects following ingestion cannot be ruled out completely. The differences between the two species, with respect to the action of inositol and sucrose in ameliorating the deterrent effect of sinigrin, suggest that several differences may exist at the level of sensory cells. An investigation of possible sensory mechanisms to explain these behavioural results is presented in the following paper.

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