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The chemical world of crucivores: lures, treats and traps

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Abstract

The host ranges of several insects that are specialists on crucifers (Brassicaceae) are closely linked to the presence of glucosinolates in these plants. These glycosides often serve as stimulants for oviposition and/or feeding, while their volatile hydrolysis products may be attractants for several species. However, many crucifers produce additional secondary compounds that act as repellents, deterrents or toxins, which protect them from these insects. The widely different responses of the various crucifer specialists to these compounds reflect different degrees of adaptation to the plant defenses. Thus native insects are often unable to survive on introduced plants, although the ubiquitous glucosinolates may trigger oviposition 'mistakes'. The success of highly invasive cruciferous weeds may be due in part to a lack of local herbivore adaptation to unique chemical constituents of these plants. However, the concentrations of secondary chemicals vary with season, environmental conditions, and geographical location. This could mean that windows of opportunity exist for utilization of introduced plants. Recent studies with garlic mustard, *Alliaria petiolata*, and wintercress, *Barbarea vulgaris*, in the USA have shown that these introduced plants are resistant to the native butterfly, *Pieris napi oleracea*. The combined effects of a flavone glycoside and a unique butenenitrile glycoside in the garlic mustard appear to be responsible for blocking feeding by this insect. *Barbarea vulgaris* is also resistant to the diamondback moth, *Plutella xylostella*, in North America and to the flea beetle, *Phyllotreta nemorum*, in Europe. Comparative studies indicate that common resistance mechanisms are involved and bioassays have been developed to elucidate the chemical nature of this resistance.

Introduction

The role of plant chemistry in shaping plant-insect relationships is now well recognized, and several examples have served to illustrate the close association of certain oligophagous insects with specific chemicals, or classes of chemicals, in their host plants (Städler, 1991; Bernays & Chapman, 1994; Schoonhoven et al., 1998). The best known example is provided by the crucifer-insect relationship, which has served as a model system for the study of chemically mediated host selection for several decades. The close association between *Pieris* butterflies and their crucifer host plants was first linked to the presence of glucosinolates in these plants through the astute observations of Verschaffelt (1910). Since that time, the key role of glucosinolates in host selection by several other crucifer specialists has been amply demonstrated (Städler, 1991).

The glucosinolates and mustard oils have been known since the 17th century to be responsible for the sharp taste of mustard seeds (Fahey et al., 2001). However, most of the chemistry and biochemistry of glucosinolates has been elucidated relatively recently, and much of our present knowledge stems from excellent work that has been performed in Denmark (e.g., Ettlinger & Kjaer, 1968; Kjaer, 1974, 1976). Many authors have adequately described the chemistry, biochemistry, and biology of the glucosinolates, and the recent review of Fahey et al. (2001) provides a comprehensive compilation of glucosinolates and isothiocyanates, with information about their occurrence in nature, structures, biological activity, and methods of chemical analysis. From the perspective of plant-insect interactions, the most important reaction of glucosinolates is their hydrolysis, in the presence of the enzyme myrosinase, to produce products that usually include isothiocyanates, which are referred to as the mustard oils. Although the intact glycosides and these volatile products appear to be largely responsible for the close association between crucifers and their specialist insect invaders, other plant compounds play a key role in the selectivity of these insects. This paper will examine the importance of glucosinolates in plant-insect relationships, but will emphasize the role of additional plant secondary compounds in the escape of many invasive crucifers from herbivory. The impact of seasonal and genetic variation in plant chemistry, and factors affecting the sensory perception of bioactive compounds by the insects are discussed. Finally, the value of new knowledge about the chemistry of crucifer-insect interactions as a basis for dealing with both agricultural and environmental problems will be addressed.

Glucosinolates as plant protection agents. The presence of glucosinolates in crucifers and related plant families is thought to serve as a first line of defense against a variety of invading organisms (Feeny, 1977). The glucosinolates and/or their isothiocyanate products are known to act as antibiotics, fungal growth inhibitors, toxins to nematodes, and feeding deterrents to caddisflies, snails and amphipods (Fahey et al., 2001; Newman et al., 1992), as well as deterrents and toxins against a wide range of generalist herbivores (Chew, 1988). In addition, glucosinolates or their isothiocyanates have been shown to exhibit allelopathic properties that allow many wild crucifers to compete successfully with established vegetation (Brown & Morra, 1997; Vaughn & Berhow, 1999).

Insect adaptation to glucosinolates. Despite the general effectiveness of glucosinolates as barriers against herbivory, many insects have adapted to these defenses, and several such species have become major pests of cole crops. In fact, those insects that have become specialists on crucifers often use the glucosinolates or isothiocyanates as positive cues for host plant recognition (Schoonhoven, 1972; Chew, 1988). The isothiocyanates may serve to attract specialist insects to their hosts, whereas the glucosinolates often trigger oviposition or feeding after an insect lands on the plant (Renwick et al., 1992; van Loon et al., 1992; Chew & Renwick, 1995) (Figure 1). Since the glucosinolates or their hydrolysis products are generally toxic to non-adapted insects, the specialists must have some mechanism for dealing with these potentially toxic compounds. This may occur by rapid excretion, hydrolysis of the glucosides, inhibition of hydrolysis, the action of protective enzymes, or by actually sequestering the glucosinolates (Schoonhoven et al., 1998).

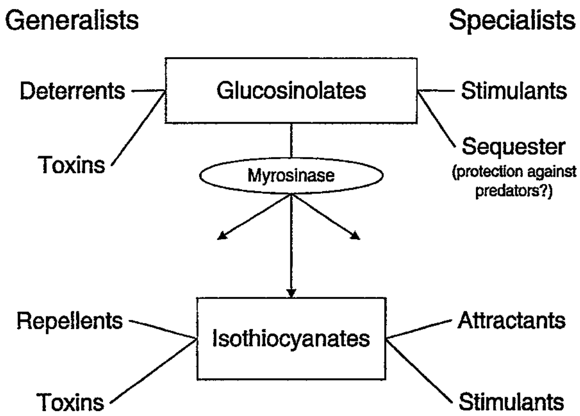

Figure 1. Comparative effects of glucosinolates and isothiocyanates on non-adapted (generalist) and adapted (specialist) insects.

Dependence on glucosinolates. The close association of crucifer specialists with glucosinolates in their hosts has developed into a type of dependence on these chemicals in many cases. Host finding may depend on the emanation of isothiocyanates, and acceptance may depend on glucosinolates that are perceived upon contact with the plant. Ovipositing *Pieris* butterflies depend on glucosinolates at the leaf surface to recognize suitable sites for their progeny to feed (van Loon et al., 1992; Renwick et al., 1992). The hatching larvae may then require glucosinolates to initiate or continue feeding. Recent studies on *P. rapae* have shown that neonate larvae may actually feed in the absence of glucosinolates, but after they gain experience with these compounds in their diet, they will refuse to feed unless glucosinolates are present (Renwick & Lopez, 1999). Other insects may depend on glucosinolates for protection from natural enemies. Sequestration of glucosinolates has been suspected, but not clearly demonstrated until recently. Müller et al. (2001) have shown that the turnip sawfly, *Athalia rosae*, can sequester various glucosinolates from its cruciferous hosts. Potential attackers are exposed to high concentrations of glucosinolates in the hemolymph through a type of reflexive bleeding that occurs when the sawfly larvae are disturbed. Recent studies have shown that ants are clearly deterred by the glucosinolate-laden hemolymph, although additional compounds may also be involved (Müller et al., 2002). Sequestration of glucosinolates has also been demonstrated for the harlequin bug, *Murgantia histrionica*, and evidence has been presented to suggest that this could result in effective protection from avian predators (Aliabadi & Whitman, 2001).

New developments and discussion

Escape of wild crucifers from herbivory. Since almost all crucifers are known to produce glucosinolates, we might expect that adapted insects would feed on all available species to some degree, depending on concentration and type of glucosinolates present. However, many crucifers are completely avoided by crucifer specialists. This has led to the idea that such plants have developed a 'second line of defense' against herbivores (Feeny, 1977). Past work in our laboratory focused on the chemistry of two prominent plants that are avoided by the cabbage butterfly, *Pieris rapae*. Avoidance of *Iberis amara* has been attributed to the presence of cucurbitacin glycosides that act as oviposition and feeding deterrents. Two glycosides were found to deter oviposition, but only one of these functioned as a feeding deterrent to the larvae (Sachdev-Gupta et al., 1990; 1993a, b; Dimock et al., 1991; Renwick, 1996). In the case of *Erysimum cheiranthoides*, cardenolides that can explain plant rejection by both butterflies and larvae have been identified. Interestingly, the most active oviposition deterrents are different from the most active feeding deterrents for the larvae, so the plant appears to have two lines of secondary defense against the different life stages of the insect (Renwick & Huang, 1994; Renwick, 1996).

Sensory aspects of host selection. Comparative studies with *Pieris rapae* and *P. napi oleracea* have shown that these related species have different sensitivities to the deterrents in *Iberis* and *Erysimum* species. *Pieris n. oleracea* is only weakly deterred by the cardenolides and cucurbitacin glycosides that are most active for *P. rapae*. These differences in behavioral responses are reflected in the electrophysiological responses of tarsal receptors of the two butterflies (Du et al., 1995; Städler et al., 1995). Different behavioral and electrophysiological responses of the two species to various glucosinolates that act as oviposition stimulants also show distinct differences in sensitivity that can explain different degrees of acceptance or rejection. The balance between stimulants and deterrents perceived by the insects apparently determines whether a plant will be accepted or rejected. However, this balance may be tipped in either direction as a result of changes in plant chemistry due to seasonal and environmental factors, or nutrition (Hugentobler & Renwick, 1995). Furthermore, recent experiments to determine the effects of dietary experience on larvae indicate that such a balance may be altered by habituation to deterrents (Huang & Renwick, 1995) or the development of dependence on specific stimulants in the host plant (Renwick & Lopez, 1999).

Alternative cues for host recognition. The chemical basis for host recognition by insects that are specialists on crucifers has always been assumed to involve the glucosinolates and isothiocyanates that are so typical of this plant family. However, recent studies using electrophysiological techniques to detect stimulatory activity have revealed the presence of additional compounds that may play an important role in host selection. Roessingh et al. (1997) found that contact chemoreceptors on tarsi of the cabbage root fly, *Delia radicum*, responded strongly to very low concentrations of compounds extracted from the surface of cabbage leaves. The active compounds, which act as potent oviposition stimulants, were subsequently identified as a complex tetracyclic carboxylic acid and its glycine conjugate (Hurter et al., 1999). The involvement of non-glucosinolates in stimulating oviposition by the turnip root fly, *Delia floralis*, was also suggested, as a result of similar extractions and electrophysiological experiments (Hopkins et al., 1997). Since the most active compound for *D. radicum* is similar in structure to phytoalexins that are produced in response to pathogen infection of cabbage plants, several known phytoalexins were tested as oviposition stimulants. Three of these compounds, methoxybrassicin, cyclobrassinin, and brassitin proved to be active (Baur et al., 1998). Comparative analyses of the glucosinolates at the surface of various crucifers and related plants were recently used to demonstrate correlations of profiles with host preferences of the cabbage root fly. However, the results of this study further suggested that additional compounds are involved in regulating oviposition by this insect (Griffiths et al., 2001). It appears likely, therefore, that many compounds in crucifers that could be used by specialist insects to recognize their host plants remain to be identified.

Host recognition by Plutella xylostella. Previous work on the diamondback moth, *Plutella xylostella*, has strongly suggested that host recognition for oviposition by this insect is dependent on glucosinolates. Reed et al. (1989) used plant extraction followed by myrosinase treatment to show that the stimulant activity was greatly reduced after hydrolysis of the glucosinolates. Indvidual glucosinolates were also active, but not to the same extent as homogenized plant tissue. The role of wax in synergizing this activity was demonstrated by Spencer (1996), who reported only limited stimulant activity of sinigrin in the absence of wax. Experiments in our laboratory also showed that glucosinolates alone have very limited activity when compared with homogenized cabbage leaves in water. These observations prompted further studies to determine whether oviposition stimulants could be extracted with less polar solvents. Consequently, soaking cabbage leaves in chloroform for 90 min provided a highly active extract. A new bioassay was then developed to routinely test extracts and to monitor the isolation of the potent stimulant that was apparently present (Hughes et al., 1997). Preliminary fractionation of extracts by silica gel chromatography provided two distinct, highly active fractions. The non-polar nature of the active material would exclude the possibility that glucosinolates could be responsible for the observed activity.

On-going research in our laboratory is focused on the identification of components of these active fractions that are involved in stimulating oviposition. One of the active fractions has yielded two compounds that have been identified as iberin and sulforaphane. These compounds have proved to be highly stimulatory on their own, but additional compounds remain to be identified (J.A.A. Renwick, unpubl.). Comparative bioassays of other isothiocyanates indicate that sulforaphane is considerably more active than any of the available aliphatic or aromatic representatives of this group (J.A.A. Renwick, unpubl.). Further separation of active fractions by open column chromatography and HPLC has resulted in the isolation of at least one active compound that is not related to the isothiocyanates. Chemical characterization of this compound is in progress.

Escape of invasive crucifers from specialist insects. The diamondback moth is a good example of a crucifer specialist that has become a major pest of brassica crops on a world-wide basis (Talekar & Shelton, 1993). However, despite the devastating effect of this insect on cultivated crop plants, many wild crucifers appear to escape attack. One such plant in North America is *Barbarea vulgaris*, which has demonstrated remarkable resistance to *P. xylostella* (Idris & Grafius, 1996). Experiments in our laboratory have shown that oviposition readily occurs on *B. vulgaris*, but the hatching larvae do not survive. The invasive nature of this weed in North America as well as Europe would indicate that it is indeed well defended against many crucivores. Elucidation of the chemistry of invasive weeds, including *B. vulgaris*, has become a major objective of our research that could have theoretical, evolutionary, as well as practical importance.

Resistance of Barbarea *species to crucifer specialists.* Recent studies on the variable resistance of *B. vulgaris* to flea beetles in Denmark have formed a basis for collaborative research on the mechanism of resistance of this plant to several crucifer specialists in North America as well as the flea beetle in Europe. Nielsen (1997a) found that different types of *B. vulgaris arcuata* differ dramatically in their resistance to the flea beetle, *Phyllotreta nemorum*. These plant populations have been described as a ‘G-type’, with glabrous leaves and a ‘P-type’, which has pubescent leaves. The P-type is susceptible to the flea beetle, whereas the G-type is generally resistant. In addition to these genetic differences in levels of defense, Nielsen (1997a) found that seasonal variation and differences between leaf type occur. Subsequent work revealed the existence of populations of the flea beetle that could deal with this plant resistance to feed successfully on the G-type of the subspecies (Nielsen, 1997b). Comparative analyses of the glucosinolates of the two types have shown that the predominant glucosinolate in foliage of the G-type is (*S*)-2-hydroxy-2-phenylethylglucosinolate (glucobarbarin), whereas the P-type contains predominantly the epimer, (*R*)-2-hydroxy-2-phenylethylglucosinolate (glucosibarin) (Jensen, 1990; Huang et al., 1994). However, a recent study of the seasonal variation in leaf glucosinolates has indicated that even such extreme differences in glucosinolate composition cannot account for differences in susceptibility to the flea beetle (Agerbirk et al., 2001).

Comparative studies to evaluate the susceptibility or resistance of *B. vulgaris vulgaris* from Ithaca, NY and the G- and P-types of *B. vulgaris arcuata* from

Table 1. Suitability of *Barbarea* accessions for selected crucifer-feeding insects (based on successful feeding on test plants)

	Pieris rapae	*Pieris napi*	*Plutella xylostella*	*Phyllotreta nemorum*
Barbarea				
vulgaris vulgaris	YES	NO	NO	NO
B. vulgaris arcuata G	YES	NO	NO	NO
B. vulgaris arcuata P	YES	YES	YES	YES
B. verna	YES	NO	NO	?[a]

[a]*B. verna* is partially acceptable for *P. nemorum* without R-genes, which are linked to resistance in some beetle populations (Nielsen, 1997b). All *Barbarea* species and types listed are acceptable to *P. nemorum* with R-genes (J. K. Nielsen, pers. comm.).

Denmark to a variety of crucifer insects indicate that the same plant species and accessions are resistant to *Pieris napi oleracea, Plutella xylostella*, and *Phyllotreta nemorum*. However, none of the tested plants was resistant to *Pieris rapae* (Table 1). These observations suggested that a common resistance mechanism against several insects exists, and, therefore, further study has focused on *P. xylostella* for isolation of a potential resistance factor. After detailed examination of the behavior of *P. xylostella* larvae on resistant foliage, a bioassay was developed to monitor isolation of the active material, which appears to be toxic and/or deterrent to all instars (N. Agerbirk, pers. comm.).

Chemical defenses of garlic mustard. Garlic mustard, *Alliaria petiolata*, is a highly invasive weed that was introduced into North America relatively recently. It appears to be spreading throughout the continent with little or no herbivory to check its advance (Nuzzo, 1993). The native butterflies, *Pieris napi oleracea* and *P. virginiensis* are known to oviposit on this plant, but the hatching larvae do not survive (F. S. Chew, pers. comm.). There is some evidence to suggest that garlic mustard is displacing much of the natural host plant (*Dentaria* sp.) of *P. virginiensis* in its woodland habitats, so that the existence of this insect species is seriously threatened (Bowden, 1971). The mechanism of resistance to larvae of the native butterflies is clearly chemical. We have found that stimulation of oviposition can be explained by high concentrations of sinigrin in the rosette leaves. The neonate larvae of *P. n. oleracea* appear to ingest small amounts of plant tissue before further feeding is inhibited. If late instars are transferred from cabbage to garlic mustard foliage, little or no feeding occurs. Two compounds responsible for this inhibition of feeding have been identified. A flavone glycoside, isovitexin-6″-β-D-glucopyranoside, deters feeding by 4th instars, whereas a novel cyanopropenyl glycoside, alliarinoside, strongly inhibits feeding by the neonates (Haribal et al., 2001; Renwick et al., 2001). It appears, therefore, that garlic mustard is protected from larvae of *P. n. oleracea* (and probably *P. virginiensis*) by two distinct chemical mechanisms. However, recent comparative studies over the course of a year have revealed considerable variation in the concentrations of these bioactive compounds with geographical location, type of foliage, and season (Haribal & Renwick, 2001). In particular, concentrations of the flavonoids in sampled plants dropped to very low levels in June and July. Such variation in chemistry could explain observed variation in survival of the insects and might suggest that windows of opportunity exist for native insects to adapt to the introduced plants.

Specialists for biological control of invasive weeds. It is clear that many invasive weeds owe their success in part to chemical defenses against local herbivory, although allelopathy may contribute by providing a competitive advantage over other vegetation (Vaughn & Berhow, 1999). In the case of garlic mustard, field observations in North America have detected little or no herbivory, although some limited damage is evident during the summer, at which time flavonoid levels are at a low point (M. Haribal, unpubl.). However, in Europe, 69 species of insects have been found feeding on *A. petiolata* (Hinz & Gerber, 1998). Most important is the weevil genus *Ceutorhynchus* with 17 species, of which four are considered to be monophagous on *A. petiolata*. A recent survey of insects associated with *A. petiolata* in southern Germany and Switzerland found 26 different species feeding on the plant (Hinz & Gerber, 1998), but most of these occurred at low levels. Studies by Nielsen in Denmark have shown that *C. constrictus* is a specialist on *A. petiolata* and further demonstrated that neutral compounds in the plant, probably flavonoids, are involved in stimulating this weevil to feed. Evidence was also presented to suggest that synergism between glucosinolates and the flavonoids occurs (Nielsen, 1991; Nielsen et al., 1989).

It is clear, therefore, that adaptation to the defenses of *A. petiolata* has occurred in Europe, where *Ceutorhynchus constrictus, C. alliariae*, and *C. roberti* have apparently developed a monophagous relationship with this plant (Nielsen et al., 1989; Hinz & Gerber, 1998). These species are currently being studied as potential biological control agents for *A. petiolata*, and information about the mechanisms of their specificity would allow for better predictions about safety of an introduction into North America (B. Blossey, pers. comm.). It will also be of interest to investigate the response of these specialists to the defensive compounds that are so effective against larvae of the indigenous pierid butterflies in the USA.

Another invasive crucifer that is relatively free of herbivory in North America is dame's rocket, *Hesperis matronalis*. Larsen et al. (1992) showed that *Ceutorhynchus inaffectatus* in Europe was monophagous on this plant, and found that three specific glucosinolates that contain apiose were powerful feeding stimulants for the weevil. Thus specific glucosinolates may play a key role in the specificity of monophagous insects that could be considered for biological control.

Conclusions

The association of specific insects with Cruciferae has long served to demonstrate the importance of chemistry in plant defense against attacking insects. Our expanding knowledge of this chemistry is leading to a better understanding of adaptation to these defenses and the eventual specialization of many insect species on plants within the genus. The key role of glucosinolates and isothiocyanates has been amply demonstrated, and the extension of host ranges to related plant families that produce these compounds can be easily explained. However, it is clear that many additional compounds can have either positive or negative effects on specialist as well as generalist insects. The selectivity of different insect species can be related to the presence of deterrents and toxins in potential host plants. The behavioral responses of insects that result in avoidance of particular plants can be related to sensory perception of deterrent compounds by the insects. Sensitivity to negative cues may be reduced as a result of habituation. Likewise, responses to positive cues for oviposition or feeding are usually innate, but may be triggered or accentuated by the development of dependence on specific compounds. The crucifer-insect system can serve as a convenient model for studying the chemical basis for specialization. Adaptation to deterrents and toxins is likely to involve exploitation of windows of opportunity when concentrations are at their lowest, along with the development of physiological systems to deal with the toxins. Adaptation of insects may also involve reduced sensitivity to deterrents or utilization of toxins for their own defense against predators.

Examination of a very limited number of wild mustards has uncovered many new compounds as well as chemicals that are known in other plant families. It is likely that study of additional invasive species will expand the list of compounds that function to protect these plants from herbivory. For example, *Bunias orientalis* in Europe is particularly invasive and appears to be relatively free of herbivory. The chemistry of this plant could provide new insight into the successful spread to the west of a cruciferous weed from Eurasia.

New information about the chemistry of crucifers in their battle against herbivores may have considerable practical value. In an agricultural setting, the use of trap crops that are particularly high in oviposition stimulants may offer a promising approach to pest management. Deterrents, repellents, and feeding inhibitors may be incorporated into crop plants in the quest for more resistant cultivars. Variation in resistance factors may be monitored to detect seasonal and environmental differences that could affect levels of herbivory. A clearer understanding of the mechanisms involved in adaptation to plant defenses will be useful in the search for insects that could be utilized for biological control of invasive weeds. Finally, the study of native insects that show some ability to adapt to introduced crucifers might provide an opportunity for us to document and follow cases of evolution in action.

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