ANTIFEEDANTS

INSECT ANTIFEEDANTS

Murray Isman of the University of British Columbia in Vancouver, Canada, reviews the chemistry and biological properties of insect antifeedants, and discusses their potential deployment for pest management

Introduction

The concept of using insect antifeedants as crop protectants is intuitively attractive. Pest management in agriculture, forestry and managed landscapes has often relied on toxic, broad-spectrum insecticides with negative impacts on natural enemies, pollinators and other non-target organisms. And continuous use of specific insecticides has frequently resulted in the development of resistance in the very pests targeted for population suppression.

Insect-plant chemical interactions in nature are usually very subtle. Most plant defensive chemicals *discourage* insect herbivory, either by deterring feeding and oviposition or by impairing larval growth, rather than by killing insects outright. One application of our understanding of plant defensive chemistry then, is the identification of putative deterrent substances that could be isolated in sufficient quantities or synthesized for use as crop protectants.

What exactly is an insect antifeedant? According to some authors, any substance that reduces consumption (feeding) by an insect can be considered an antifeedant (NB the terms antifeedant and feeding deterrent are used synonymously). I prefer a more restrictive definition, *i.e.* a behaviour-modifying substance that deters feeding through a direct action on peripheral sensilla (= taste organs) in insects (Isman *et al.,* 1996). This definition excludes chemicals that suppress feeding by acting on the central nervous system (following ingestion and absorption), or a substance that has sublethal toxicity to the insect. In short, an antifeedant is a substance that tastes bad to insects! Antifeedant activity is generally demonstrated through laboratory bioassays consisting of either choice or non-choice tests conducted over a short duration (Figure 1). Bioassays purporting to show antifeedant effects but extending beyond 4–6 hours should be viewed with suspicion, since reduced feeding in a long term test could easily result from post-ingestive toxicity or malaise in the exposed insects, rather than having a behavioral basis.

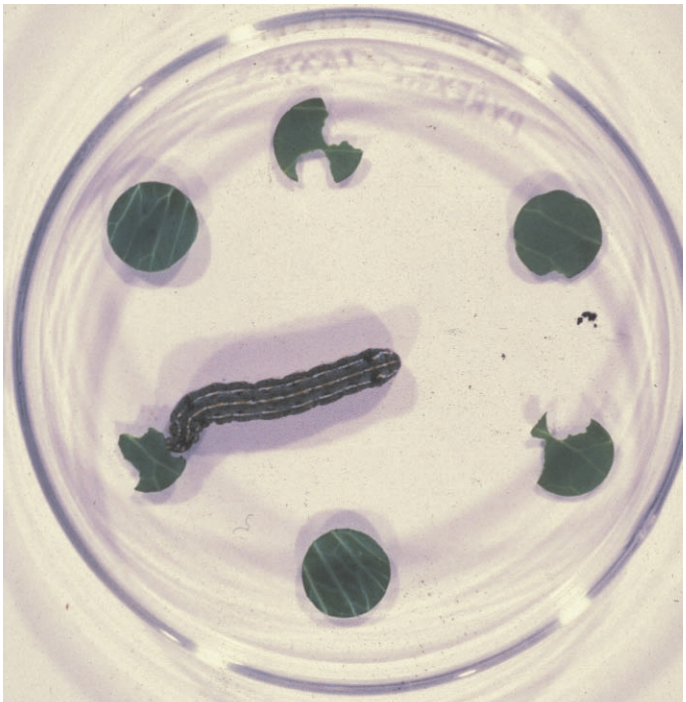

Figure 1. **Typical leaf disc choice test used to measure feeding deterrence in chewing insects such as caterpillars and beetles. Areas consumed are determined from digital photographs of the leaf discs using an image analysis computer program.**

Sources and chemistry

Terrestrial plants produce a diverse array of secondary metabolites, likely more than 100,000 unique compounds, and there is compelling evidence that at least some of these are important in the defense of plants against herbivores (Schoonhoven, 1982). It should come as no surprise then, that the vast majority of substances documented to deter feeding by insects have been isolated from plants. Antifeedants can be found amongst all the major classes of secondary metabolites – alkaloids, phenolics and terpenoids (Frazier, 1986). But it is in the last-mentioned category that the greatest number and diversity of antifeedants, and the most potent, have been found.

Chemically speaking, many well documented insect antifeedants are triterpenoids. Based on a 30-carbon skeleton, these substances often occur as glycosides (conjugated with sugars) and are often highly oxygenated. Especially well studied in this regard are the limonoids from the neem (*Azadirachta indica*, Figure 2) and chinaberry (*Melia azedarach*) trees, exemplified by azadirachtin and toosendanin (Figure 3), and limonin from *Citrus* species. Other antifeedant triterpenoids include cardenolides, steroidal saponins and withanolides. Several types of diterpenes (based on a 20-carbon skeleton) are well known as antifeedants, including the clerodanes and the abietanes.

Sesquiterpenes (15-carbon skeleton) with potent antifeedant action include the drimanes, and the sesquiterpene lactones. One particularly well-studied example is the drimane polygodial (Figure 3), which occurs in foliage of the

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Figure 2. **A neem tree (*Azadirachta indica*) showing developing fruits, source of the outstanding antifeedant azadirachtin.**

water pepper, *Polygonum hydropiper* (Figure 4). Finally, certain monoterpenes (based on a 10-carbon skeleton), major constituents of many plant "essential oils", deter insect feeding.

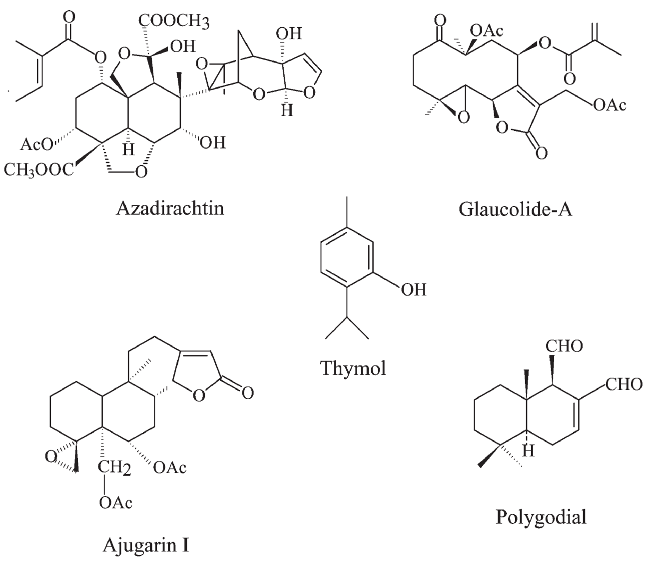

Figure 3. **Structures of some potent and well documented insect antifeedants from plants.**

Among plant phenolics, the best known antifeedants include the furanocoumarins and the neolignans. Alkaloids with well documented antifeedant effects on insects include certain indoles and the solanaceous glycoalkaloids. Specific examples of well documented antifeedants from plants are listed in Table 1; structures of some of these are shown in Figure 3.

A cursory view of the predominantly complex structures shown in Figure 2 would suggest it not possible to predict antifeedant activity based on chemical structure alone. While this may be true for two-dimensional representations, studies of quantitative structure-activity relationships (QSAR) based on three-dimensional structures have led to models of putative antifeedant binding sites in insect taste cells (Mullin *et al.* 1997). Efforts to enhance antifeedant bioactivity through analogue synthesis have seldom met with success (Blaney *et al.*, 1990), although there are some exceptions (Yamasaki and Klocke, 1989).

In addition to plant secondary metabolites, some synthetic pesticides have been reported to have insect feeding deterrent activities. Synthetic pyrethroids, while highly potent contact insecticides, can effectively deter feeding of specific insects at doses or concentrations below that causing any mortality (Hajjar and Ford, 1990). Triphenyltin acetate, used as a fungicide, algicide and molluscicide, had shown potential for use as a crop protectant against lepidopteran pests through antifeedant action, but this pesticide has fallen from favour owing to environmental concerns (Perry *et al.*, 1998). The inorganic fungicide Bordeaux Mixture, is also reputed to deter certain insect pests. Whether these pesticides act specifically on the gustatory sensilla of insects, thereby meeting the criterion for an antifeedant as defined herein, remains to be determined.

A recently introduced insecticide with a unique mode-of-action is pymetrozine. Effective against a number of homopteran pests, this substance specifically interferes with the feeding process by blocking salivary flow required by these insects, ultimately leading to starvation (Fuog *et al.*, 1998).

For most antifeedants, the modes-of-action are directed at the taste cells. A typical gustatory sensillum in an insect contains receptors selective for deterrents and others for

Figure 4. **A water pepper plant (*Polygonum hydropiper*), source of the antifeedant polygodial. Photo courtesy of IACR-Rothamsted.**

Table 1. Some examples of potent insect antifeedants isolated from terrestrial plants.

Chemical type	Compound	Plant source
Monoterpene	Thymol	*Thymus vulgaris* (Lamiaceae)
Sesquiterpene lactone (germacranolide type)	Glaucolide A	*Vernonia* species (Asteraceae)
Sesquiterpene (drimane type)	Polygodial	*Polygonum hydropiper* (Polygonaceae)
Diterpene (abietane type)	Abietic acid	*Pinus* species (Pinaceae)
Diterpene (clerodane type)	Ajugarin I	*Ajuga remota* (Lamiaceae)
Triterpene (limonoid type)	Azadirachtin	*Azadirachta indica* (Meliaceae)
Triterpene (cardenolide type)	Digitoxin	*Digitalis purpurea* (Scrophulariaceae)
Triterpene (ergostane type)	Withanolide E	*Withania somnifera* (Solanaceae)
Triterpene (spirostane type)	Aginosid	*Allium porrum* (Liliaceae)
Alkaloid (indole type)	Strychnine	*Strychnos nuxvomica* (Loganiaceae)
Alkaloid (steroidal glycoside)	Tomatine	*Lycopersicon esculentum* (Solanaceae)
Phenolic (furnanocoumarin)	Xanthotoxin (= 8-methoxy psoralen)	*Pastinaca sativa* (Apiaceae)
Phenolic (lignan)	Podophyllotoxin	*Podohyllum peltatum* (Berberidaceae)
Phenolic (benzoate ester)	Methyl salicylate	*Gaultheria procumbens* (Ericaceae)

stimulants (such as sugars and amino acids). Although most antifeedants likely act by stimulating a deterrent receptor, that in turn sends a signal ("do not feed") to the feeding center in the insect's central nervous system, some antifeedants are thought to block or otherwise interfere with the perception of feeding stimulants, whilst others may cause erratic bursts of electrical impulses in the nervous system preventing the insect from acquiring appropriate taste information on which it may choose an appropriate feeding behavior.

As a cautionary note, it is possible that many if not most antifeedants have some physiological or toxic action in insects, depending on the dose. In evaluating 14 antifeedants (based on investigations against stored product pests) against caterpillars, we found that only three strongly deterred feeding, but these three compounds were also significant growth inhibitors via topical administration (Nawrot *et al*., 1991). Other investigations have found a lack of correlation between feeding deterrence and toxicity in plant-feeding insects (Cottee *et al*., 1988).

Azadirachtin (neem) as a paradigm

With the isolation of azadirachtin by David Morgan of Keele University and the subsequent demonstration of its outstanding antifeedant effect on the desert locust (*Schistocerca gregaria*) in the late 1960s, neem seed extracts and formulations have long been praised as an outstanding example of a commercially successful antifeedant (Isman 1997). But is azadirachtin truly an example of an antifeedant that has demonstrated efficacy in the field?

Any evaluation of the efficacy of azadirachtin against insects in the field is undoubtedly confounded by the potent insect growth regulatory actions of this substance against insects. While the antifeedant effects are highly variable among pest species, the IGR effects are more consistent among insects, leading most investigators to conclude that it is the physiological effects, rather than the behavioral ones that carry the day as far as neem or azadirachtin are concerned. Even for species where suppression of feeding appears as a major contributor to crop protection, the response may arise from a post-ingestive toxic effect rather than a direct behavioral one. Although there may well be pest species for which the antifeedant effect is the major one responsible for crop protection in the field, investigations where this has been unambiguously demonstrated are few and far between.

Problems with antifeedants

As crop protectants, antifeedants must meet the same criteria as insecticides, *viz*. they must show selectivity towards the target pest (and thus non-toxic to mammals and other non-target organisms such as natural enemies and pollinators), and they must have sufficient residual action to protect the crop through its window of vulnerability to the key pest(s).

But much more so than insecticides, antifeedants suffer from greater interspecific differences in bioactivity. For example, while azadirachtin is a remarkably potent antifeedant to the desert locust (deterring feeding by 50% at a concentration of 0.05 ppm), the migratory grasshopper, a pest of cereal crops and rangeland grasses in North America is completely insensitive (feeding undeterred at 1000 ppm) (Champagne *et al*. 1989). Using a standard binary leaf disc choice test, the antifeedant potency of azadirachtin was determined for six species of noctuid caterpillars. EC_{50} values (effective concentration deterring feeding by 50%) varied more than 30-fold between species, with the tobacco cutworm (*Spodoptera litura*) the most sensitive and the black army cutworm (*Actebia fennica*) the least (Isman, 1993). A recent investigation of a series of silphinene sesquiterpenes as antifeedants found profound differences in activity of individual compounds when tested against the

Table 2. Potency of some plant-derived antifeedants against noctuid larvae.

Compound	Concentration (mg cm−2) deterring feeding by 50%		
	Trichoplusia ni	*Peridroma saucia*	*Spodoptera litura*
Azadirachtin	0.016	0.013	0.001
Toosendanin	24.8	8.0	0.59
Cedrelone		27.2	
Rocaglamide		3.5	
Xanthotoxin	0.50		0.33
Digitoxin	50.0		
Thymol	40.0		85.6

cotton leafworm (*Spodoptera littoralis*), the Colorado potato beetle (*Leptinotarsa decemlineata*) and five species of aphids (Gonzalez-Coloma *et al.*, 2002).

Another operational problem specific to antifeedants is the potential for insects to rapidly desensitize (habituate) to a feeding deterrent. Several investigations have demonstrated that individual (naïve) insects initially deterred by an antifeedant, become increasingly tolerant upon repeated exposures or through continuous exposure. Under no-choice conditions, feeding by tobacco cutworm larvae on cabbage discs treated with azadirachtin was initially deterred by 90%, but with continuous exposure, the response had waned by more than one-half within 5 hours (Bomford and Isman, 1996) (Figure 5). In the case of the antifeedant toosendanin, feeding deterrence was completely abolished at 4.5 hours. The salient point is that a crop treated with an antifeedant might only enjoy protection from a pest for a few hours before the insect becomes habituated and can then feed with impunity.

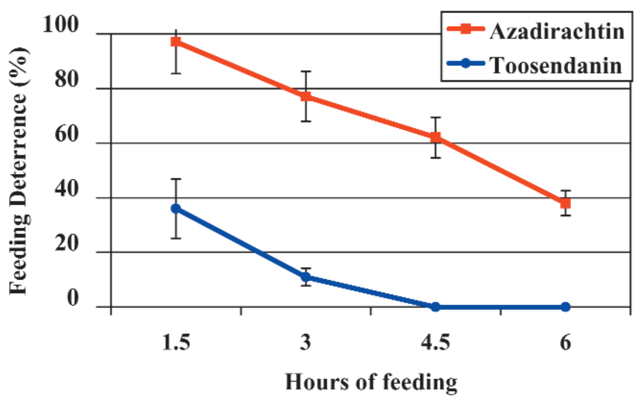

Figure 5. **Desensitization of *Spodoptera litura* larvae to the antifeedants azadirachtin and toosendanin under no-choice conditions and continuous exposure.**

In an ongoing study of this phenomenon in plant-feeding insects, we have demonstrated that caterpillars can become habituated to a variety of plant secondary metabolites, and importantly, they can become cross-habituated. In simple terms, exposure of caterpillars to one antifeedant can render them less responsive to other, unrelated antifeedants days later. Fortunately, habituation to antifeedants can be mitigated in insects, by presenting mixtures of antifeedants. Armyworm larvae (*Pseudaletia unipuncta*) can habituate to either xanthotoxin or thymol, but do not become habituated to either when exposed to a mixture of the two compounds. We had previously shown that *S. litura* larvae could habituate to pure azadirachtin, but less so to a neem extract containing the same absolute amount of azadirachtin (Bomford and Isman, 1996) (Figure 6). Similarly, larvae became rapidly habituated to toosendanin (95%), but less so to a mixture of limonoids of which toosendanin constituted 60% (Gelok and Isman, unpublished data).

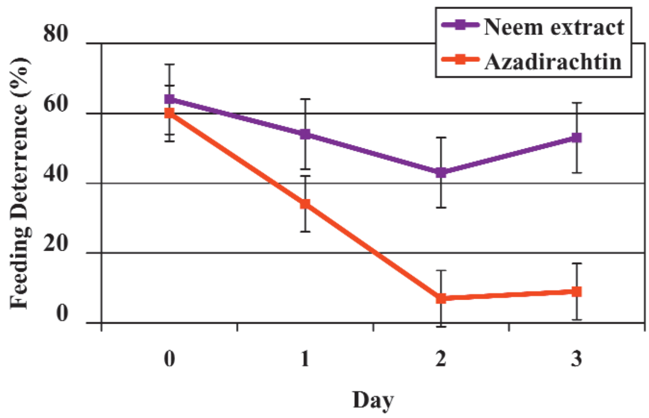

Figure 6. **Desensitization of *Spodoptera litura* larvae to azadirachtin, but not to a neem extract containing the same amount of azadirachtin, under choice conditions with successive daily exposures.**

Potential uses of antifeedants

The simplest method of using an antifeedant as a crop protectant is to apply it as a water or oil-based spray in the same manner used to apply an insecticide. However, apart from neem products, there are few actual demonstrations of antifeedant efficacy in the field. John Pickett and collaborators at the IARC-Rothamsted have shown that application of polygodial or methyl salicylate resulted in reduced aphid populations with concomitant increases in yields of winter wheat, in one case comparable to that achieved with the pyrethroid insecticide cypermethrin (Pickett *et al.*, 1997).

But given that many antifeedants do not kill pests outright, and even their behavioural effects may be ephemeral under field conditions, their utility may ultimately depend on deploying them with more creative strategies. For example, Griffiths *et al.* (1991) investigated the joint effects of an antifeedant, leaf extract of *Ajuga* spp., and the insect growth regulator teflubenzuron, on the mustard beetle *Phaedon cochleariae* and larvae of the diamondback moth *Plutella xylostella* feeding on mustard plants. The antifeedant suppressed beetle and caterpillar feeding for several days, but with minimal mortality after two weeks, whereas the IGR did not prevent feeding in the first 48 hr after application, but did kill all beetles and larvae after two weeks. In applying the two protectants in combination, foliar consumption was reduced by at least 50% and pest mortality was greater than 75%.

As the tender, upper leaves are more valuable than the older, lower ones, leaf damage can be better tolerated on the lower leaves. With that in mind, the investigators utilized the two protectants in an even more intriguing manner. They sprayed the upper parts of mustard plants with the antifeedant, and the lower parts with the IGR. Under this treatment regime, beetles were quickly driven to the lower leaves where they came in contact with the IGR. The result was virtually no damage to the upper parts of the plants, and modest damage to the lower portions but with complete mortality of beetles using a reduced amount of the insecticide.

Another strategy that can include antifeedants is the stimulo-deterrent diversionary strategy (SDDS), sometimes called the "push-pull" strategy (Miller and Cowles, 1990). In this case the "push" can come from an antifeedant applied to the crop needing protection, while the "pull" can come from an attractant applied to an adjacent trap crop or trap rows of the main crop. However, this form of insect behaviour manipulation requires a highly mobile pest, likely to abandon an otherwise suitable host plant and move to another potential host plant some distance away. The best use of this strategy may be to dissuade gravid female insects from depositing their eggs on the target crop, provided there are suitable substances that deter oviposition and more attractive alternative hosts or hostplant chemicals available to provide the "pull."

Prospects for commercial use

Given the aforementioned limitations to the use of insect antifeedants, *viz.* differences in response between pest species, potential desensitization of pests, and rapid environmental degradation, it is most unlikely that an antifeedant will emerge with sufficient field efficacy to act as a stand-alone crop protectant. Assuming though, that there are insect antifeedants (1) with minimal bioactivity in mammals and other non-target organisms, and (2) available on a commercial scale, there are likely specific crop-pest combinations where an antifeedant can play a significant role as part of an integrated pest management system. Whether the market(s) for such a specific protectant can justify the costs of development remains to be seen. Ongoing research into insect sensory systems, neuropharmacology, and organic chemistry may ultimately mitigate the limitations to antifeedants observed at present and lead to a suite of new crop protectants based on deterrence of insect feeding and oviposition.

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