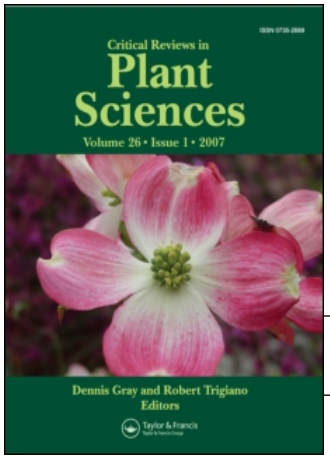

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Phytochemicals and Insect Control: An Antifeedant Approach

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Phytochemicals and Insect Control: An Antifeedant Approach

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Plants based pest control agents have long been touted as alternatives to synthetic chemicals for integrated pest management. Such phytochemicals reputedly pose little threat to the environment or to human health. Bioactivity of plant-based compounds is well documented in literature and is a subject of increasing importance. An antifeedant approach for insect control has been extensively studied, at least at laboratory level, though only a handful of plant-based compounds are currently used in agriculture. The known active plant-based antifeedants belong to groups like chromenes, polyacetylenes, saponins, quassinoids, cucurbitacins, cyclopropanoid acids, phenolics, alkaloids, various types of terpenes and their derivatives etc., and each insect species may process these allomones in a thoroughly idiosyncratic way, so that the same compound may have very different fates and consequences in different species of insects, thus pointing to different mechanisms involved in antifeedant action. It can also be visualized that insect feeding deterrents may be perceived either by stimulation of specialized deterrent receptors or by distortion of the normal function of neurons, which perceive phagostimulating compounds. Some plant antifeedants influence the feeding activity through a combination of these two principal modes of action. Only a few highly active antifeedants have been looked into from a commercial point of view, which makes it impossible to systemize or to predict any molecular motifs in feeding inhibition. Structure activity relationship studies also do not point to any generalization. "Mix and Match" systems may help in developing a cocktail of feeding inhibitors that can be used in developing a customized formulation against a specific category of pests. Application of such

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products will be broad and will not be limited to targeted pests and to plant parts. Decreased deterrence resulting from habituation has been suggested that could pose different implications for pest management than does decreased deterrence resulting from increased tolerance to toxic substances. Genetically modified plants, which could produce the active antifeedant substances in amounts high enough to protect the plants from further herbivorous damage, could be a possibility in the future.

Keywords phytochemicals, allelochemicals, antifeedants, pest control, mechanism of antifeedants, commercialization, chemosensory system, stereoselective perception, habituation

I. ANTIFEEDANTS: A GENERAL VIEW

Plants produce a vast and diverse assortment of compounds and the majority of them do not participate directly in growth and development. These phytochemicals, traditionally referred to as secondary metabolites, often are differentially distributed within the plant kingdom. Although noted for the complexity of their chemical structures and biosynthetic pathways, phytochemicals have been investigated for their chemical properties extensively since the 1850s. Recognition of the biological properties of large number of phytochemicals has fueled the current focus for the search of new drugs, antibiotics, insecticides, herbicides, and behavior-modifying chemicals. Many of these compounds have been shown to have important adaptive significance in protection against herbivory (Croteau *et al*., 2000); in fact, phytochemical diversity of insect defenses in tropical and temperate plant families has been significantly established (Arnason *et al*., 2004) and I think it reasonable to concentrate on the compounds, which interfere with the feeding behavior of insects and accordingly following review is an updated and expanded version of portions of my book *Insect Antifeedants* (Koul, 2005).

In recent past *allomones* have been defined as substance(s) produced or acquired by an organism that, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological response that is adaptively favorable to the emitter but not to the receiver (Nordlund, 1981). As such, allomones are differentiated from the pheromones because they mediate interspecific, rather than intraspecific interactions. Receiving organisms respond to such allomones in a variety of ways. Subtle changes in behavior and physiology of the receiver can result in host-shifts in phytophages or parasites, or extended developmental times due to reductions in nutritional value of foodstuffs. At the other end of the spectrum, violent reactions leading quickly to injury and death are often the result of encounters with highly toxic defensive allomones. This tremendous diversity, coupled with the intensity of allomone-mediated interspecific interactions makes allomonal chemicals potential agents for insect pest control (Koul, 2005). Although allomones mediate a wide variety of complex interactions, allomonal chemicals fall into one of two basic categories. The first of these includes materials produced by the organisms and released into the environment, mostly volatile compounds, which exert their influences at some distance from the emitter. Such volatiles include a wide variety of short chain alcohol and aldehydes, ketones, esters, aromatic phenols, mono- and sesquiterpenes and a host of other secondary metabolites. The second group of allomones includes compounds produced or acquired for defense, which remain in the body of the producer. This group includes toxins sequestered by insects for defense and the vast array of phytochemicals.

Behavioral mechanisms provide a system of avoidance of nonhost chemicals by which insects select their food, though the molecular basis for action of chemical deterrents on both gustatory and olfactory sensory systems in insects is only poorly understood. Among plant antiherbivore chemistry, a strong link does not exist between feeding deterrence and internal toxicity in insects, suggesting that behavioral rejection is not an adaptation to ingested effects but more an outcome of deterrent receptors with wide chemical sensitivity (Mullin *et al*., 1991c, 1994). Many of these substances are bitter and acceptance of host plants by herbivores requires chemoreception of favorable levels of phagostimulants relative to plant antifeedants (Dethier, 1980). This restricts the application of a very liberal definition for an antifeedant, namely, "any substance that reduces consumption by an insect" to a more precise definition "a peripherally-mediated behavior modifying substance (i.e., acting directly on the chemosensilla in general and deterrent receptors in particular) resulting in feeding deterrence" (Isman, 1994). This definition, however, excludes chemicals that suppress feeding by acting on the central nervous system (following ingestion or absorption), or a substance that has sublethal toxicity to the insect (Isman, 2002). Several definitions for the term "antifeedant" exist in the literature (Munakata, 1975; Norris, 1986; Frazier and Chyb, 1995; Glendinning, 1996; Messchendorp, 1998) suggesting that the definition of the term varies widely. A broader approach suggested by Mansson (2005) explains this where volatile and non-volatile preingestive inhibitors have been regarded as antifeedant compounds. The reason not to include postingestive inhibitors in the antifeedant concept is that these inhibitors demand feeding during a longer period than preingestive inhibitors, which may already have caused significant and possibly mortal damage to the plant, when the insect finishes feeding. However, ingestive inhibitors could be borderline cases as chemosensory and accessory cells are involved in both preingestive and ingestive inhibition. Thus, one category could be of repellent and arrestant type of antifeedants where insects avoid feeding without coming in contact with plant material. Similarly, insects are suppressed from biting once contact has been made with plant material leading to antifeedance (suppressants). The most accepted category is that of feeding deterrent phytochemicals, which deter insects from feeding after they have bitten the plant material, i.e., inhibition by gustatory responses.

Feeding deterrents from plants with a wide diversity of structures are not known to directly interfere with insect taste cell

responses to phagostimulants such as sugars (Lam and Frazier, 1991; Schoonhoven *et al*., 1992). Presently the mode-of-action of feeding modifying chemicals in insect gustatory systems is largely unknown (Frazier, 1992; Schoonhoven *et al*., 1992), though some molecular targets have been identified (Koul, 1997; de Bruyne and Warr, 2006). Taste receptor proteins are only now beginning to be biochemically purified and cloned. The determination of the molecular basis for action of feeding deterrents in the insect gustatory system is thus a primary goal among basic and applied entomologists interested in insect-plant interactions or in the control of herbivore pests.

According to the theory of biochemical coevolution it should be possible to develop an evolutionary pattern of antifeedants on the basis of their distribution in different plant families and their biosynthetic pathways. However, the pattern of distribution varies among families. One plant family may concentrate on one type of deterrent molecule like limonoids in the Rutales (Champagne *et al*., 1992) and within a family individual members may have developed further barriers to feeding. For instance, it is clear that flavonoids in plants can modulate the feeding behavior of insects, though mechanisms associated with these behavioral responses are not clearly understood (Simmonds, 2001). Other families may diversify their deterrents; for example, non-protein amino acids (e.g., L-canavanine), alkaloids, cyanogens, and isoflavones are found in the Fabaceae. Plants produce all these and many varied compounds in the first instance as protective devices against insect feeding. Thus a majority of plant families rely on secondary plant metabolites for protection from phytophagous insects. One might surmise that within such a family the more advanced members are better protected than others. Berenbaum (1983) has pointed to good evidence in the Apiaceae where plant defense is based on hydroxycoumarins, linear furanocoumarins, and angular furanocoumarins, which are biosynthetically and toxicologically related.

It is also evident from various studies that as a result of coevolutionary pressures, plants have a startling number of plant chemicals including chromenes, polyacetylenes, saponins, quassinoids, cucurbitacins, cyclopropanoid acids, phenolics, alkaloids, various types of terpenes and their derivatives etc., and each insect species may process these allomones in a thoroughly idiosyncratic way, so that the same compound may have very different fates and consequences in different species of insects (Blum *et al*., 1987; Koul, 1993). These various insect-plant interactions are consistent with the idea of reciprocal evolutionary interactions based on secondary metabolites. In fact, variation in plant secondary metabolites is critical for understanding the evolutionary ecology and biochemical diversity because they act as defense chemicals and markers (Andrew *et al*., 2007). This, however, could be related to the evolution of deterrent receptors in insects too. There is a clear indication that no two insect species are equipped with an identical sensory system. Each species has a unique sensory window, which can discriminate between host and nonhost plants (Schoonhoven, 1982). Even in very closely related species the chemical senses show striking differences (Drongelen, 1979). It can be visualized from such information that the contact chemical senses may in evolutionary terms easily be adapted to changing circumstances as has been well evidenced in two strains of *Mamestra brassicae* in response to sinigrin and naphtyl-β-glucoside (Wieczorek, 1976). Similarly, electrophysiological recordings from the receptor neurons in sensilla chaetica in *Heliothis virescens* during mechanical and chemical stimulation have shown responses of one mechanosensory and of several gustatory receptor neurons. Separate neurons showed excitatory responses to sucrose and sinigrin. While sucrose elicited extension in 100 percent of the individuals in all repetitions, sinigrin elicited extension in fewer individuals, a number that decreased with repeated stimulation (Jørgensen *et al*., 2006).

It can also be visualized that insect feeding deterrents may be perceived either by stimulation of specialized deterrent receptors or by distortion of the normal function of neurons, which perceive phagostimulating compounds. Some sugars are very important components of an insect's sustained feeding; the inhibition of the receptors is an effective antifeedant action. Some antifeedants from plant sources influence the feeding activity through a combination of the two principal modes of action mentioned above.

Initial discoveries of antifeedant chemicals were simply made by chance when organometallic compounds and a few insecticides were found to reduce insect feeding (Ascher and Rones, 1964; Jermy and Metolcsy, 1967). This clearly emphasizes the point that many synthetic compounds could be potential antifeedants for insect pests (Koul, 1993), of course in addition to the allomones or their derivatives from natural sources. As early as 1932 Metzger and Grant tested about 500 plant extracts against *Popilla japonica*, though results were not substantially encouraging. Pradhan *et al*. (1962) evaluated extracts of the Indian neem tree, *Azadirachta indica* that prevented feeding by the desert locusts. Although terrestrial plants produce a diverse array of secondary metabolites, likely more than 100,000 unique compounds (Isman, 2002), today, only about 900 compounds have been identified to possess feeding deterrence against insects (Koul, 2005). In addition to various compounds isolated from plants or synthesized as insect antifeedants, a number of studies demonstrates the antifeedant efficacy in metabolite mixtures of plant essential oils or total extracts against variety of insect species. In recent years studies have revealed the antifeedant potential of plant essential oils against postharvest pests, aphids, thrips, lepidopterans, termites, and mite pests (Hori, 1999; Hou-HuaMin *et al*., 2002a,b; Koschier *et al*., 2002; Maistrello *et al*., 2003). Similarly, during past few years ample emphasis has been in demonstrating the antifeedant efficacy in total plant extracts (Mancebo *et al*., 2000a,b; Wang *et al*., 2000; Jannet *et al*., 2001; Lababidi and Koudseieh, 2001; Schlyter, 2001; Wheeler and Isman, 2001; Mehta *et al*., 2002; Jayasinghe *et al*., 2003; Okabe *et al*., 2004; Zhang *et al*., 2004; Regnault-Roger *et al*., 2005; Debrowski and Seredynska, 2007;

Lehman *et al*., 2007; Owusu *et al*., 2007) as they seem to exhibit the activity as multicomponent systems. However, it is also well known that antifeedants show interspecific variability (Chapman, 1974; Schoonhoven and Jermy, 1977; Isman 1993). Such interspecific differences, as shown for many insect species, encourage the need to search selectively for specific feeding deterrents.

II. MECHANISM OF ANTIFEEDANT ACTION

Food selection among insect herbivores is a highly specialized phenomenon. While olfactory and physical aspects of plants or their organs can be important in insect host finding and acceptance (Miller and Strickler, 1984), the choice of food is primarily based upon contact chemoreception of various allelochemicals (Frazier, 1986; Stadler, 1992). In particular, dietary experience has influenced the ability of insects to taste plant chemicals that may have served as signals of suitability or unsuitability. Certain dietary constituents appeared to suppress the development of taste sensitivity to deterrents in an insect (Renwick, 2001). Avoidance of allelochemicals, when looked at from a behavioral point of view, is the outcome of interactions with chemoreceptors characterized by an often broad sensitivity spectrum of deterrents (Mullin *et al*., 1994).

According to Schoonhoven *et al*. (1992), there are four basic reasons why the chemosensory perception of feeding deterrents by phytophagous insects warrants special attention:

- First, they are apparently more important in host plant recognition than phagostimulants,
- second, a huge number exist with variable molecular structures adding to their diversity,
- third, there are fewer deterrent receptors, and
- fourth, different deterrents may elicit different behavioral reactions, indicating the presence of a differential sensory coding system.

Overall the mode of action of feeding modifying chemicals in insect chemoreceptor systems is largely unknown and no biochemically purified or cloned taste receptor proteins have been identified. However, a number of molecular targets for feeding deterrents have been identified (Koul, 1997; de Bruyne and Warr, 2006) and there is evidence to show the existence of several sensory mechanisms involved. Therefore, to understand the concepts and mechanisms of feeding deterrents in insect gustatory system, a search for candidate neuroreceptors and various behavioral end points is required. To achieve this, the chemosensory equipment involved in the process must be examined.

A. Chemosensory System

The surface of the insect body is richly supplied with sensilla of various shapes and densities. The sensillum is the structural unit from which the majority of insect sensory organs are derived. Ectodermal in origin, a sensillum develops by differentiation from a mother epidermal cell. It consists of cuticular parts, one or more sense cells and two or more sheath cells.

The sense cells vary in number from 1 to 40 or more and have large nuclei located below the epidermis. These bipolar sense cells send their dendrites to the cuticular parts where their form, ultrastructural features, and methods of attachment are characteristic for cells of different modalities. Their axons extend into the sensory nerve parallel with other sensory axons often extending directly to the central nervous system (CNS) before making synaptic connections to second-order neurons. Thus, they are primary sense cells that contain both a sensory receptor area on their dendrites and an impulse-conducting membrane along their axons.

Usually sheath cells vary in number and are of three types, i.e., the basal, the outer, and the inner sheath cells. These cells have a junction among them and form a sort of insulating barrier between the extracellular space surrounding the dendrites and the haemolymph space below the epidermis (Kuppers and Thurm, 1982).

The cuticular projections of insect sensilla are the most visible portions, and their size, shape, and position have been the basis for classifying them. Features of structure and function have been demonstrated with various microscopical examinations and impulse recording techniques. Insect sensilla on the outside of the body consist of the major types based on shape of the cuticular part, the presence or absence of pores and the type of attachment to the cuticle (Frazier, 1985). They have been classified as the following:

- Sensillum in flexible socket with single sense cell containing a tubular body.
- Sensillum without flexible socket containing a sense cell with lamellated dendrite.
- Uniporous sensillum in flexible socket containing one cell with tubular body and one or more cells with dendrites extending to the terminal pore.
- Uniporous sensillum without a flexible socket containing two or more cells with unbranched dendrites.
- Multiporous sensillum with single wall and multiple cells with branched dendrites.
- Multiporous sensillum with double wall and multiple cells with unbranched dendrites.

Out of these six major types, the sensilla, which possess only a single terminal pore (thick-walled) are of gustatory nature and are concentrated on the mouth parts, though taste hairs also occur on tarsae, antennae, and ovipositors. They possess flexible sockets; 2–20 sensory cells, 1 dendrite with tubular unbranched body or inflexible sockets; 2–9 sensory cells and unbranched dendrites. They are usually uniporus. However, uniporous sensilla with inflexible sockets are fewer in number, but dome-shaped

sensilla occur often in the preoral cavity, where they serve to monitor the food being eaten.

Lepidopteran larvae have been observed to carry in each maxilla a palpus and a galea, the latter carrying two sensilla styloconica, non-socketed pegs with an apical papilla. These taste hairs are innervated by four bipolar neurons, the dendrites of which extend through the length of the hollow cuticular peg ending just below the pore at the tip, i.e., within a few milliseconds of diffusion time from the external chemical environment (Schoonhoven, 1987, Descoins, 2001). The tip of the maxillary palp is covered with eight sensilla basiconica. The palp tip sensilla are innervated by 14–19 neurons in total (Schoonhoven and Dethier, 1966). This number, however, varies in different insect species (Devitt and Smith, 1982). As most of these sensilla are gustatory in nature, they are also involved in food recognition (Descoins, 2001). Palpation of the intact leaf surface, prior to biting activity is related to contact chemoreception during which chemicals on the leaf cuticle are perceived (Devitt and Smith, 1985).

An epipharyngeal taste sensillum in *Leptinotarsa decemlineata* larvae was studied using electron microscopy, which showed that the sensillum is innervated by five neurons. Electrophysiological experiments showed that one of these cells responds to water, a second to sucrose and a third to two feeding deterrents that were also effective in a behavioral test. The response of the sucrose-sensitive cell was strongly inhibited by one of the two feeding deterrents and only slightly by the other feeding deterrent. It was concluded that probably both the response of the deterrent cell and peripheral interactions exerted by feeding deterrents on the sucrose-sensitive cell determine the potency of feeding deterrents. These results provide a physiological basis for the hypothesis that the presence or absence of feeding deterrents in potential food plants is a decisive cue in food plant selection by *L. decemlineata* larvae (Messchendorp *et al*., 1998). However, differential neurosecretory response of this insect species has also been recorded, for instance, against glycoalkaloids (Hollister *et al*., 2001)

Thus, one can easily surmise that gustatory chemosensilla must be regulating feeding behavior. It is obvious that many cells furnish information during the feeding sequence. In grasshoppers, for instance, receptor complement is large in number and low in specificity and in caterpillars the number is low and relatively high in specificity (Frazier, 1986). In both extremes there is, however, redundancy among chemosensory cells, both with respect to specificity as well as overlap of sensitivity ranges of individual receptor cells (Blom, 1978). Obviously, it is vital to have extensive and dependable information about plant allelochemicals, which reduce or inhibit feeding. This link between single chemosensory cell input and behavioral output must be known before we are able to correlate the effects of allelochemicals on single cells in electrophysiological studies with their effects on the feeding behavior of the whole insect (Frazier, 1986).

B. Stereoselective Perception

Antifeedant properties of a plant compound may be revealed either by direct observation or by using electrophysiological methods (Gothilf and Hanson, 1994; Marion-Poll and Van der Pers, 1996; Glenginning and Hills, 1997; van Loon and Schoonhoven, 1999) that need thorough understanding of the chemoreceptor system of an insect. The latter procedure provides information on sensory mechanisms underlying the perception of antifeedant chemicals. However, no two insects possess fully identical chemoreceptory systems, but rather show different responses to various stimuli. Consequently, plant compounds may evoke different behavioral reactions even in closely related insect species (Schoonhoven, 1987). According to Schoonhoven (1988), life at a macroscopic scale presents itself usually in symmetrical forms. At the molecular level, however, asymmetry prevails. Nature often produces only one type of a stereospecific molecule and not its stereoisomer(s). Since chemoreception is a process of molecular interactions the phenomenon of stereoisomerism may have consequences for the process of stimulus recognition. Therefore, the question arises: what is the role of stereospecificity of insect chemoreceptors *vis-à-vis* plant antifeedants?

As mentioned above, the sense of taste in insects is localized in specialized receptors on the mouth parts, the preoral cavity, on the tarsi, and on the antennae—often at several of these sites in the same insect. Extensive studies performed mainly on blowfly (Dethier, 1976) and lepidoteran larvae (Schoonhoven, 1987) have shown that receptors are usually not highly specific and responses could be multineural. A correlation of the electrophysiological response with behavioral discrimination in caterpillars has provided evidence supporting the idea that patterns of multireceptor activity constitutes the basic code for recognition and discrimination.

The sensory code may be altered due to the stimulation of specialized receptors or modulation of the activity of receptors tuned to other compounds. In lepidopteran larvae several specialized deterrent receptors have been described that respond to various alkaloids, phenolic compounds and glycosides and that inhibit food intake. The deterrent receptors in different species often overlap in their sensitivity spectra, but show at the same time characteristic interspecific variations (Schoonhoven, 1982).

Feeding deterrents may also change the activity of receptors which signal the presence of feeding stimulants, for instance when suppressing sugar receptors, and thereby act as strong antifeedants (Kennedy and Halpern, 1980). Azadirachtin, a terpenoid isolated from the neem tree, stimulates a deterrent receptor in a number of herbivorous insects (Schoonhoven, 1988), but appears to suppress sugar and inositol receptors in other species (Schoonhoven, 1988).

Overall several specialized deterrent receptors have been described mainly in lepidopteran larvae. For instance, *Bombyx mori* possesses a bitter receptor which is located in one of the two sensilla styloconica on the maxilla and responds to various

alkaloids acting as feeding inhibitors (Ishikawa, 1966) or respond to limonoid inhibitors in the case of *Helicoverpa armigera* and *H. assulta* (Tang *et al*., 2000). *Pieris brassicae* larvae and several other lepidopteran species have one or more deterrent receptors, which overlap in their sensitivity spectra (Schoonhoven, 1982; Chapman, 1982). Colorado potato beetles also have deterrent receptors in their tarsal sensilla, responding to various solanaceous plant alkaloids (Sturckow, 1959). Specific deterrent receptors are also present in the preoral cavity of lepidopteran larvae (Ma, 1972; de Boer *et al*., 1977). Exclusively three pairs of bitter-sensitive taste cells that are located in the medial, lateral, and epipharyngeal sensilla (Glendeinning *et al*., 2006) mediate the gustatory response to bitter taste stimuli in *Manduca sexta*. In *Drosophila*, gustatory receptor neurons (GRNs) occur within hair-like structures called sensilla. Most taste sensilla house four GRNs, which have been named according to their preferred sensitivity to basic stimuli: water (W cell), sugars (S cell), salt at low concentration (L1 cell), and salt at high concentration (L2 cell). Labellar taste sensilla are classified into three types, l-, s-, and i-type, according to their length and location. Of these, l- and s-type labellar sensilla possess these four cells, but most i-type sensilla house only two GRNs. In i-type sensilla, it has been demonstrated that the first GRN responds to sugar and to low concentrations of salt (10–50 m*M* NaCl). The second GRN detects a range of bitter compounds, among which strychnine is the most potent; and to salt at high concentrations (over 400 m*M* NaCl). Neither type of GRN responds to water. The detection of feeding stimulants in i-type sensilla appears to be performed by one GRN with the combined properties of S + L1 cells, whereas the other GRN detects feeding inhibitors in a similar manner to bitter-sensitive L2 cells on the legs. These sensilla thus house two GRNs having an antagonistic effect on behavior, suggesting that the expression of taste receptors is segregated across them accordingly (Hiroi *et al*., 2004)

Electrophysiological studies of Blaney (1980) emphasize the fact that deterrent receptors cannot be of a single and simple category. Therefore, even today the conclusion of Dethier (1980) that in insects with few receptors, multiple receptor sensitivity occurs and that "there is no generalized deterrent receptor" seems to be highly plausible. As it is clear now that deterrent receptors vary from species to species, it won't be an exaggeration to conclude that the contact chemical senses in evolutionary terms easily may be adapted to changing circumstances (Schoonhoven, 1982).

Receptor sensitivity and specificity, however, are genetically determined and changes in them apparently occur by a gradual replacement of certain receptor sites in the dendritic membrane by another type of site, which bind different stimulants. For example, Wieczorek (1976) showed that the deterrent cell in two strains of *Mamestra brassicae* show quantitative differences in their response to some chemicals, which may be explained by a different ratio between two types of receptor sites present in the receptor membrane. This is consistent with the model of Bernays and Chapman (1994), which suggest that differences in taste sensitivity to deterrent compounds could account for the difference in host range. It is also possible that diet breadth has a direct link with sensitivity of the deterrent receptor cells. For instance genetic differences in the sensitivity of the deterrent receptor cells of *Bombyx mori* in relation to diet breadth (Asaoka, 1994) imply that the effect could not be peripheral, however, the same interpretation does not hold true for *Heliothis* species and suggest central nervous system mediated differences (Bernays *et al*., 2000). Reduced feeding on deterrent diets is, in fact, a consequence either of rejection without any ingestion or of rejection following some ingestion. Rejection without ingestion indicates that deterrent compound is detected by chemoreceptors on the mouthparts. Rejection following after some ingestion apparently results from the accumulation of sensory information, since deterrent receptors sometimes adapt relatively slowly (Schoonhoven *et al*., 1998). There could be postingestive feedback that allows limited intake (Bernays *et al*., 2000).

An intriguing question concerns the origin of deterrent receptors. It has been suggested that herbivorous insects, rather than evolving receptors for some specific deterrents, have developed from a "common chemical sense" resulting in a receptor type that is sensitive to a wide variety of compounds, even including chemicals to which a particular species has never been exposed before (Dethier, 1980). It may be concluded from state-of-the-art studies that insect deterrent receptors cannot be considered as a primitive or uniform type of receptor, but rather as compound receptor types with a high degree of plasticity. According to Schoonhoven (1982) this plasticity on the one hand ensures that the insect may quickly adapt to changes in its environment, but maintain the capacity to recognize unpalatable plants, and on the other hand, has led to considerable divergence resulting in no two insects being identical.

In terms of CNS interpretation of the sensory code, feeding activity obviously requires motor output from the CNS, whereas the presence of feeding deterrents signaled via chemosensory input may inhibit feeding motor output, leading to a refusal to eat (Ma, 1972). Presently it is difficult to study the processes underlying the evaluation of sensory input by the CNS that result in either continuation or cessation of feeding activity. However, the sensory inputs can be analyzed and the principles upon which central neural integration is based can be hypothesized. Some basic considerations put forth are in the following statements:

- the gustatory sense has a leading role in feeding activity. The epipharyngeal organs do not add new information to that of the maxillary hairs,
- sensory input from the maxillae is sent to the suboesophageal ganglion, then from the epipharyngeal organ to the tritocerebrum, and
- the message indicating whether or not a plant is acceptable must be hidden in the sensory pattern it evokes (Schoonhoven, 1987).

If the CNS is able to read this message, it is in principle also decipherable to us and accordingly some messages may permit

feeding activity and others may not. Sensory coding of feeding deterrents is based upon neural activity in one or more neurons. The following three basic types of sensory coding are known (Schoonhoven *et al*., 1992):

1. *Labelled lines*: Each neuron conveys a specific message, which can be understood by the CNS without additional information from other neurons.
2. *Across-fiber patterns*: The message is contained in a neural activity pattern, transmitted by two or more receptors, possessing different stimulus spectra.
3. *Temporal patterns*: Stimulus quality affects nerve impulse interval patterns and adaptation rates, which may contain additional information.

These coding principles could be cited in several cases and often occur in combination in insects (Dethier and Crnjar, 1982; Schoonhoven and Blom, 1988). A temporary distortion of such sensory codes can result in the inhibition of feeding. When in *Leptinotarsa decemlineata* the responses were compared between host and nonhost potato saps, the response patterns for the nonhost stimuli appeared to be considerably less consistent than the patterns evoked by the sap from the host plant (Mitchell *et al*., 1990; Schoonhoven *et al*., 1992). This suggests that such variable patterns are interpreted by the CNS as "nonsense" with the result that no feeding or only limited feeding occurs, a pattern which has also been observed in various lepidopteran larvae (Simmonds and Blaney, 1990).

Several chemicals, including some heavy metal ions, may distort the functioning of chemoreceptors in such a way that, even in the presence of an acceptable plant, the neural acceptance profile that the CNS requires for initiating feeding behavior is not evoked (Schoonhoven, 1987; Schoonhoven and Jermy, 1977). From neuroanatomical analysis using *Drosophila* model, it has been demonstrated that *hug*-expressing neurons project axons to the pharyngeal muscles, to the central neuroendocrine organ, and to the higher brain centers, whereas *hug* dendrites are innervated by external gustatory receptor-expressing neurons, as well as by internal pharyngeal chemosensory organs. The use of tetanus toxin to block synaptic transmission of *hug* neurons results in alteration of food intake initiation, which is dependent on previous nutrient condition. The results provide evidence that *hug* neurons function within a neural circuit that modulates taste-mediated feeding behavior (Melcher and Pankratz, 2005), however, if this will apply to other insects remains to be seen.

C. Mechanisms

Secondary plant substances are in principle noxious because they interfere with normal structure and function of insect cells and thus disturb their integrity. Thus, insects, like other animals, have developed various mechanisms to reduce or prevent harmful effects of secondary plant substances when contacting them or after ingesting them (Brattsten and Ahmad, 1986). As we have seen above, chemoreceptors in insects are primary sense cells and thus true neurons are generally protected from the deleterious effects of secondary plant compounds. This is supported by the fact that insects have sensory neurons that respond to sugars, amino acids, or salts and function normally despite the presence of these host-specific noxious compounds, as was demonstrated in the case of polyhydroxy alkaloids against *Spodoptera* and *Helicoverpa* species (Simmonds *et al*., 1990).

If some receptor cells have retained their primordial sensitivity to different kinds of secondary plant compounds, they would be ideally suited to signal the presence of chemicals to be avoided. Thus, the primitive, unmodified taste cell may be considered as the primordial deterrent receptor, which still possesses sensitivity to odd plant substances originally shown by all primitive neurons. That does not mean that the present-day deterrent receptors are unchanged and wholly identical to their ancestral neural cell type. The modern deterrent receptors, while retaining sensitivity to various secondary plant compounds, have developed a physiological mechanism, which protects them against the harmful effects of their adequate stimuli. Not only has the basic sensitivity to secondary plant substances been preserved in these receptors, it also became connected to the action potential generating system, resulting in a change of impulse frequency upon stimulation (Schoonhoven, 1991). Thus, in contrast to sugar and salt receptors, deterrent receptors have preserved their general sensitivity, which has been linked to a neural response mechanism. In fact, all lepidopteran larvae possess a pair of maxillary palps that "drum" the surface of foods during feeding. These chemosensory organs contain over 65 percent of a larva's taste receptor cells, but their functional significance remains largely unknown. Their role in rejection of plant allelochemicals was examined, using the tobacco hornworm, *Manduca sexta*, as a model insect and an extract from the plant, *Grindelia glutinosa*, as a model stimulus. This system was selected because hornworms reject foods containing *Grindelia* extract, and because preliminary studies indicated that their maxillary palps respond to this extract. It was hypothesized that *Grindelia* extract elicits rejection through stimulating the following: (i) olfactory receptor cells, (ii) taste receptor cells, (iii) oral mechanoreceptors, and or (iv) a postingestive response mechanism. The results were consistent only with hypothesis (ii); larvae approached *Grindelia*-treated diets without apparent hesitation, but rejected it within 6 seconds of initiating biting. *Grindelia*-treated solutions stimulated taste receptor cells in the maxillary palp, but not the other gustatory chemosensilla; and ablating the maxillary palps eliminated rejection of *Grindelia*-treated diets. The results demonstrate that taste receptor cells in the maxillary palps mediate rejection of *Grindelia* extract, and provide the first direct evidence for a role of maxillary palps in rejection of plant allelochemicals (Glendinning *et al*., 1998).

The possibility exists that insects use some other codes for taste quality, such as assessment of the temporal sequence of firing, which gives a continuous evaluation of the activity of individual neurons. It is also likely that simultaneous evaluation of inputs from different neurons allows contradictory signals,

indicating the presence of phagostimulants and/or antifeedants, to be assessed concurrently (Schoonhoven, 1987).

In addition to these neural mechanisms it should be mentioned that some other targets are also vulnerable to antifeedants, like GABA antagonistic mechanisms, biogenic amine inhibition, etc.

1. γ-Aminobutyric Acid (GABA) Antagonisim

GABA and related aminobutyric acids are known to stimulate feeding and evoke taste cell responses among herbivorous insects of various taxa, like Orthoptera, Homoptera, Coleoptera, and Lepidoptera (Mullin *et al*., 1994). However, it has also been established that allelochemicals antagonize GABA phagostimulants, like the isoquinoline alkaloid papavarine does in the Colorado potato beetle, thereby inducing feeding deterrence (Mitchell, 1987). GABA-gated chloride channels respond to many classes of chemicals in insects (Sattelle, 1990; Anthony *et al*., 1993). The antagonism of GABA binding allows increased depolarization within an excitable cell and affects function at both the neuromuscular junction and central synapses within the nervous system of insects. The present view is that inhibitory $GABA_A$ (Cl^- conducting) receptors belong to a gene superfamily of ligand-gated ion channels that include excitatory nicotinic acetylcholine (Na^+, K^+) and inhibitory glycine (Cl^-) receptors (Anthony *et al*., 1993). In turn, the α-carboxylated and precursor form of GABA, glutamic acid, gates a more distantly related family of both excitatory K^+/Na^+ and inhibitory Cl^- channels (Darlison, 1992; Sattelle, 1992). On the whole it has been shown that the $GABA_A$ and glycine receptor complexes must incorporate two or three different four-transmembrane-domain subunits (Mullin *et al*., 1994).

Association of GABA/glycine receptors with sensory systems has been demonstrated. For instance, bicuculline insensitivity at $GABA_A$ sites in insects has been found in CNS interneurons of the cockroach (Walker *et al*., 1971) and *Manduca sexta* (Waldrop *et al*., 1987). In *M. sexta* GABA was found to mediate olfactory behavior via inhibitory interneurons in the antennal lobe of the deutocerebrum. However, only β-like subunits of GABA receptors from the CNS of *Drosophila* spp. (Henderson *et al*., 1993) and yellow fever mosquito, *Aedes aegypti* (Thompson *et al*., 1993) have been cloned from insect species.

An interesting study of Mullin *et al*. (1991a; b) shows the association of an antifeedant with a GABA/glycine-receptor. Epoxy sesquiterpene lactone antifeedants from sunflower exhibit picrotoxinin-like GABA-gated chloride channel neurotoxicities in adult western corn rootworm. In fact, terpenoid epoxides and isoquinoline and related alkaloids, such as azadirachtin (a strong antifeedant from neem) (Koul, 1996), bicuculline, etc., are interesting antifeedants of this category.

Mullin and coworkers (1994) have used three-dimensional structure-function relationships in *Diabrotica* to demonstrate antifeedant potency of compounds proposed to interact at a common binding site. Compounds were co-fitted through use of Alchemy III molecular modeling software (Tripos Associates, Missouri). Common binding features for high antifeedant activity among the polycyclic terpenoid epoxides like azadirachtin, agrophylin, picrotoxinin, caryophyllene oxide, etc., include an epoxide and π bonding sites separated by 0.5–0.6 nm, one or more electronegative oxygen centers, and a trisubstituted oxirane. Polyoxygenation may maintain sufficient polarity to allow diffusion to and interaction with the taste receptor. The 3D structural similarity between argophyllin (Mullin *et al*., 1991b) and picrotoxinin and dieldrin (Matsumura *et al*., 1987) suggest action through a shared picrotoxinin receptor site.

The above studies also indicate that optimal polarity for molecular interactions at an exterior chemosensory receptor is different from internal interaction requirements with excitable cells since membrane penetration and transport by binding proteins are not necessary (Mullin *et al*., 1994). A hydrophobic nature of compounds makes them noninhibitory to feeding as has been determined by using partition coefficient techniques. Many deterrents tested against *Diabrotica* have been shown to cause firing of a single taste neuron and this chemosensory response correlates well with their feeding deterrency. In fact, GABA antagonism at the taste cell level may after neural processing result into net inhibition or excitation, respectively, of the dominant adductor with a converse effect on the adductor. Clearly higher CNS inputs into mandibular opening and closing are also required. The actual inhibitory and excitatory inputs at each synaptic level, their means of integration and the responsible neurotransmitters, receptors and ion movements for insect gustation mostly remain to be clarified (Frazier, 1992).

2. Biogenic Amine Inhibition

Biogenic amines are widely distributed within the insect CNS and thought to act as neurohormones, neuromodulators, and/or neurotransmitters (Evans, 1980). To get information about the mechanism of insect feeding, the insect response at biogenic amine levels against the feeding deterrents has been investigated (Ikemoto *et al*., 1995). For example, chlordimeform and aristolochic acid are well-known insect antifeedants and have been used as probes of antifeedant activity. Five typical biogenic amines (5-hydroxytryptamine, dopamine, epinephrine, norepinephrine, and octopamine) using HPLC with an electrochemical detector have been investigated in the CNS of last instar *Spodoptera litura* larvae. It has been demonstrated that chlordimeform causes an increase in N-acetyldopamine levels in cerebral and suboesophageal ganglia and a decrease in 5-hydroxytryptamine (5HT) and n-acetyloctopamine levels in the cerebral, suboesophageal, and thoracic ganglia. On the other hand, aristolochic acid I, an antifeedant from *Aristolochia* species, did not cause any significant change in any amine levels except for dopamine and 5-hydroxytryptamine in suboesophageal ganglia and tyranine in thoracic ganglia (Ikemoto *et al*., 1995). Decrease in 5HT has also been reported in the cockroach cerebral ganglia (Omar *et al*., 1982). Inhibitory activity of chlordimeform against N-acetyltransferase has been shown in several insect species (Wierenga and Hollingworth,

1990), which suggests that some plant-based antifeedants have a mechanism of action through bioamine system in insects. The relationship between such alterations and the antifeedant treatment is not clear yet, but this could be one of the directions to study the mechanism of antifeedants and to understand the biochemical and physiological meaning of such alterations occurring due to feeding deterrents.

3. Mechanisms Related to Specific Antifeedants

Koul (2005) illustrates about 900 compounds that inhibit feeding of a variety of insect species using various bioefficacy procedures. However, the question remains how do these chemicals affect the insect chemosensory cells, though a generalistic concept has been discussed above, and do these compounds indicate the multiplicity of actions that can reduce feeding?

Substantial data have been obtained in this regard for a diverse group of allelochemicals, the alkaloids. They inhibit impulse generation in sugar sensitive cells in lepidopterans (Frazier, 1986; Simmonds *et al*., 1990) and competitively block sucrose responses in flesh flies (Morita *et al*., 1977). They also reduce the firing of the sugar sensitive cells. Alkaloids as inhibitors of pyranose and furanose receptor sites have been established for flies (Wieczorek *et al*., 1988)

The steroidal glycoalkaloids elicit irregular firing from several cells in the galeal and tarsal sensilla of adult and the larval α-sensilla of the Colorado potato beetle (Mitchell and Harrison, 1985). On the contrary, deterrent effects of various alkaloids, when tested against black blow flies, *Phormia regina*, in order to determine tarsal threshold for mixtures of sucrose and alkaloids, using kinetic analysis of electrophysiological data, ruled out competitive, no competitive and uncompetitive inhibition at receptor sites; although no correlation of thresholds with available data on lipid solubility or octanol/water partition coefficients was observed. This suggests that there is no uniform limiting mechanism for this multiform array of compounds (Dethier and Bowdan, 1989).

Terpenes of various classes also inhibit insect feeding. Azadirachtin, one of the most potent deterrents known, has been shown to induce the firing of one cell in the labial palps and one in the A_3 sensillum of the clypeo-labrum of *Schistocerca gregaria* (Haskell and Schoonhoven, 1969). Azadirachtin also induces the firing of cells in the medial sensilla styloconica of *Pieris brassicae* and *Lymantria dispar* larvae (Schoonhoven and Jermy, 1977; Schoonhoven, 1982). Azadirachtin effects in other caterpillar species are characterized by the firing of large spikes in the lateral and medial sensilla styloconica. This cell appears to fire independently of the sugar-sensitive cell (Simmonds and Blaney, 1984). This confirms a general observation that the effects of azadirachtin (and many other compounds as well) are different in different species, i.e., affecting more than one chemosensory cell type in more than one way. Luo *et al*. (1995) describes a significant correlation between behavior and response of the medial deterrent cell for three triterpenoids, azadirachtin, salannin and toosendanin. They showed a relationship between sensory input and feeding inhibition, supporting the hypothesis that the response of the medial deterrent cell directly causes inhibition of feeding in *Pieris brassicae* (Messchendorp *et al*., 1996). However, interference with the lateral glucosinolate- and sugar sensitive receptor cells measured for toosendanin (Schoonhoven and Luo, 1994) did not contribute to a closer relationship between sensory response and inhibition of feeding on cabbage leaf discs in *P. brassicae* mentioned above. Toosendanin has been shown to modulate the sensory code underlying feeding behavior via several different peripheral sensory mechanisms, i.e., stimulation of the deterrent receptor cell located in the medial maxillary sensillum styloconicum and inhibition of responses of both the sugar and glycosinolate receptor cells (Schoonhoven and Luo, 1994).

Other limonoids have also been shown to deter feeding in a variety of insect species (Champagne *et al*., 1992), but there is no electrophysiological data available to compare the effects on taste receptor cells. This information gap is mainly due to the fact that limonoids are insoluble in water and this makes it difficult to apply the tip recording technique in an electrophysiological bioassay of limonoids. Some workers have solved the problem by using mixtures of 50% tetrahydrofuran and 50% aqueous sodium chloride as a solvent system (Waladde *et al*., 1989). In these studies, compounds like deoxylimonin, obacunone and pedonin were used to show inhibition of the sugar receptor cells of *Eldana saccharina* maxillary styloconic sensilla.

The sesquiterpene warburganal produces irregular firing of more than one cell and then blocks the responsiveness of the sucrose and inositol-sensitive styloconic cell of *Spodoptera exempta* (Ma, 1977). In this case the deterrent acts via interaction with protein sulfhydral groups located at the receptor membrane. Some studies also suggest that warburganal reversibly blocks chemoreceptors, but the observation that feeding behavior of larvae of *Spodoptera eridania, Schistocerca gregaria* and *Manduca sexta* is little affected may indicate that sensory input to the brain in these species does not inhibit food intake (Schoonhoven and Yan, 1989). It is well evident that such dialdehydic sesquiterpenoids (including polygodial, muzigadiol, etc.) not only affect the phagostimulant receptors, but also the deterrent cells located in the medial hair of insects (Schoonhoven and Yan, 1989). This suggests a mechanism of interference common to all taste receptors. Therefore, it remains unexplained why different receptors show different degrees of inhibition and different recovery periods. However, what is certain is that these sesquiterpenoids induce antifeedant effects in various insect species by the following: i) stimulation of a deterrent receptor and ii) decreased sensitivity of most or all other receptors. This variability is obvious from identified insect odor and taste receptors from *Drosophila melanogaster*, *Anopheles gambiae*, *Bombyx mori*, and *Heliothis virescens*. The chemical specificities of many of the *D. melanogaster* receptors, as well as a few of the *A. gambiae* and *B. mori* receptors, have now been determined either by analysis of deletion mutants or by ectopic expression in in vivo

or heterologous expression systems, and have been comprehensively discussed (Hallem *et al*., 2006) in order to understand the molecular and cellular basis of odor and taste coding in insects.

Clerodin, an antifeedant diterpene induces greater feeding deterrency when applied to the maxillary palps as compared to the sensilla styloconica (Antonious *et al*., 1984), which is in contrast to what has been observed in formamidine compounds. Ginkgolides from *Ginkgo biloba* when tested electrophysiologically for neural responses in the maxillary taste sensilla, show a strong stimulation of the deterrent receptors of two types in *Pieris brassicae* and *P. rapae*. However, in *P. brassicae* the medial sensillum is more strongly stimulated than the lateral sensillum, whereas in *P. rapae* the reverse is true (Yan *et al*., 1990). This illustrates the marked difference between the chemoreceptory systems of the two species.

Drimanes with a lactone group on the B-ring appear to be the most potent antifeedants at a concentration of 5 mM (Messchendorp *et al*., 1996). The positive correlation between feeding inhibition and response of the deterrent cell suggests that these compounds exert a direct inhibitory effect on the feeding centres in the CNS. At the same time few compounds, though highly deterrent, do not evoke strong responses from the deterrent cells. This suggests that other mechanisms, either sensory or postingestive, are also involved in feeding inhibition. One of the drimanes tested in the above studies depressed the neurons sensitive to feeding stimulants. Whether or not this interference contributes to feeding inhibition remains to be elucidated. What could be concluded from this study is that highly effective drimane antifeedants can be selected electrophysiologically on the basis of response intensity of the medial deterrent cells, but further details of the mechanisms underlying feeding inhibition await to be revealed. There is also the evidence that mechanisms for antifeedants isolated from plants may vary for analogous drimanes for a species. In another study, for instance, 11 analogous synthetic drimane antifeedant compounds were evaluated for their feeding inhibiting effects on larvae of the large white butterfly *Pieris brassicae* in no-choice tests on the host plant *Brassica oleracea*. The results show that the five analogous antifeedants differentially influence feeding behavior and locomotion activity. Some are most likely sensory mediated antifeedants. Habituation to these compounds occurs soon after the onset of the tests (i.e., within 0.5–1.5 hours). Others, like confertifolin, probably are not direct sensory-mediated antifeedants and rather induce postingestive anorexia. In conclusion, the behavioral observations performed in this research indicate that analogous drimanes inhibit feeding by *P. brassicae* larvae through multiple mechanisms of action (Messchendorp *et al*., 2000).

The antifeedant activity of chalcones, flavones and flavanones is due to the predominant stimulation of the deterrent neurons in the medial sensillum stylonicum and more than one receptor may be involved (Simmonds *et al*., 1990). These studies suggest that there are at least two different receptor types involved, each having a different structure-function type of response.

From the above discussion it is clear that the molecular structure of compounds *vis-à-vis* the neural responses associated with feeding deterrence mechanisms should throw some light on various molecular parameters such as chirality, functional groups, molecular size, lipophilicity of the compounds, etc. However, it appears difficult, if not impossible, to ascertain any common molecular conformation to all active molecules and their induction of a specific type of neural/receptor response towards a specific deterrent.

III. SOURCES AND CHEMISTRY

Since less than 1 percent of all secondary plant substances, estimated to number 400,000 or more, have been tested against a limited number of insect species only, several effective compounds may remain to be discovered. Researchers, when testing candidate compounds, use only a few or even only one species for evaluation. Effective feeding deterrents to a particular insect will easily escape attention. For example, a well-known antifeedant azadirachtin tested against seven orthopterans, the interspecific differences span six orders of magnitude. Compounds known as insect antifeedants usually have a more oxidized or unsaturated structure. However, molecular size and shape as well as functional group stereochemistry also affect the antifeedant activity of a molecule. Antifeedants can be found amongst all the major classes of secondary metabolites such as limonoids, quassinoids, diterpenes, sesquiterpenes, monoterpenes, coumarins, isoflavonoids, alkaloids, maytansinoids, ellagitannins, etc. However, the most potent antifeedants belong to the terpenoid group, which has the greatest number and diversity of known antifeedants. Amongst terpenoids, limonoids are well studied and the most potent example is azadirachtin A (Figure 1, **1**) from *Azadirachta indica* A. Juss of family Meliaceae, chemically synthesized recently (Veitch *et al*., 2007), and which is widely reported insect antifeedant. There are about 12 isomers of azadirachtins in this plant, amongst which azadirachtin B, D, H, and I are also active as antifeedants, but comparatively less than azadirachtin A. This compound is now known to be efficacious against nearly 400 species belonging to the insect orders Blattodea, Caelifera, Coleoptera, Dermaptera, Diptera, Ensifera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Phasmida, Phthiraptera, Siphonoptera, and Thysanoptera (Koul and Wahab, 2004). Variation in the structure influences the activity of this compound (Blaney *et al*., 1990; Rembold, 1989; Ley *et al*., 1993). For instance, hydrogenation of the dihydrofuran ring as in dihydroazadirachtin (Figure 1, **4**) does not effect the activity of the molecule or esters on the A ring do not effect the activity of the compound (Yamasaki and Klocke, 1987), although they could be important in transporting the compounds to the receptor sites. Difference in the level of antifeedance, for instance, among compounds shown in Figure 1 (**1** to **7**) evaluated against four noctuid larvae (Blaney *et al*., 1990) has been attributed to the ability of the respective esters at C-1 or C-3 to transport the molecule to the target site. However, changes in C-1 or C-3 esters in combination with a structural variation at C-11

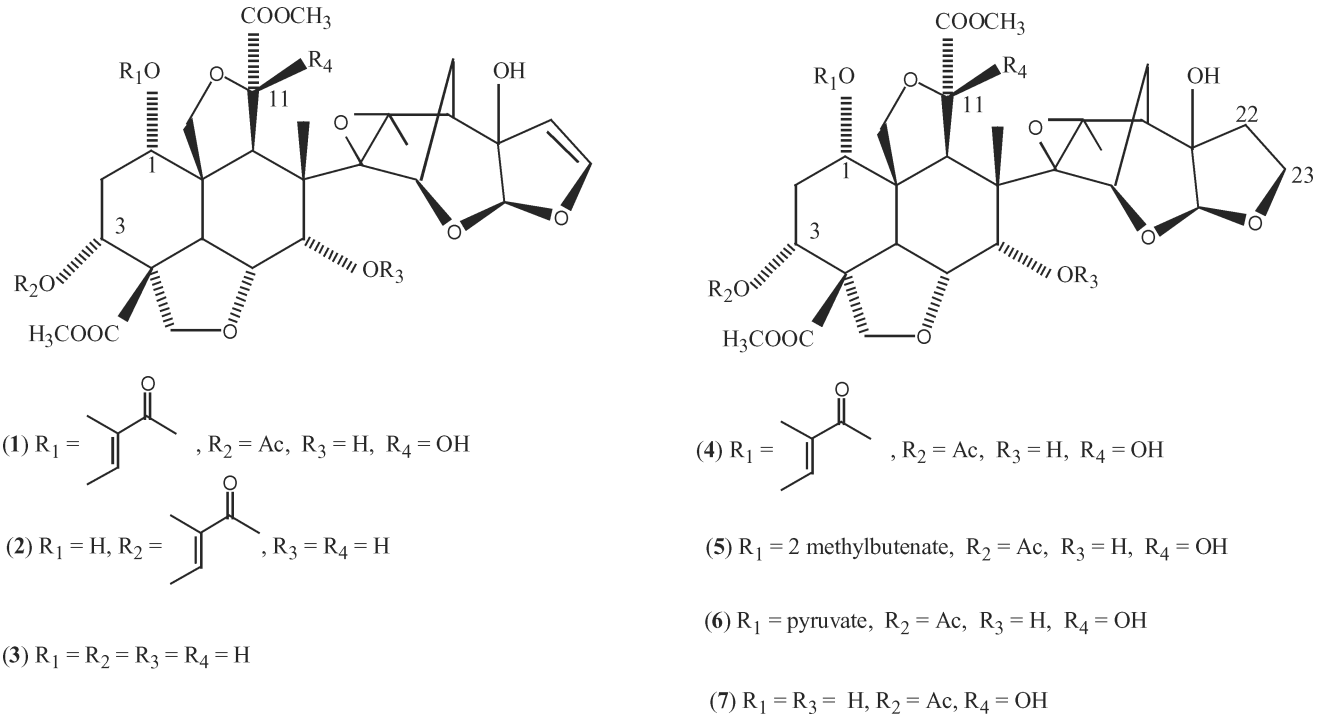
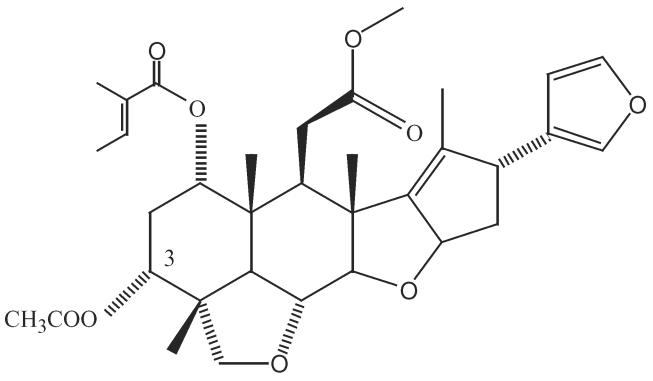


FIG. 1. Structures of azadirachtin and its various derivatives.

resulted in decrease of feeding deterrent activity, especially in *H. armigera*. This suggests that the type of ester present at C-11 is important to deterrent activity. These results also show that hydrogenation of C-22,23 double bond in azadirachtin does not significantly influence antifeedant activity, thus confirming the observations of Yamasaki and Klocke (1987). Hein *et al*. (1999) also report the hydroxy group at C-11 in azadirachtin A is important for high mortality rates and a single bond between C-22 and C-23 increases the degree of efficiency. An exchange of the large ester group ligands at C-1 and C-3 with hydroxy groups in combination with a single bond between C-22 and C-23 and a hydroxy group at C-11 leads to high feeding activity and a degree of efficiency of about 100%. Ley and his co-workers (1993) have synthesized a large number of compounds to establish structure-activity relationships. For instance, 31 compounds were screened related to azadirachtin against *Spodoptera littoralis* that point to hydroxyfuranacetal moeity in the high level of potency of this compound. Stereochemistry at C-7 is crucial and the bridging oxygen substituent at C-6 may play some role. The precise spatial and electrostatic requirements of all the various oxygen substituents, according to Ley, need more detailed studies. These studies also reveal reduction in activity by increasing bulk at C-23. However, similar things do not hold true for other evaluated species like *S. frugiperda* or *H. armigera*. In fact the bulky isopropoxy substitute results in a compound with very potent antifeedant activity against *S. frugiperda* (Blaney *et al*., 1990) and less bulky ethoxy substitution quite active against *H. armigera*.

Another interesting example of a limonoid from neem showing potential antifeedant activity is salannin (Figure 2), which deters feeding in about 10 insect species (Koul, 2005). In addition to neem this compound also occurs in *Melia azedarach* L., *Melia dubia* Cav. and *Melia volkensii* Guerke. Fourteen derivatives of salannin when bioassayed against Colorado potato beetle, *Leptinotarsa decemlineata*, larvae have revealed four target points, which after modification change the activity pattern of salannin. These targets are : (i) hydrogenation of the furan ring, (ii) replacement of the acetoxy group, (iii) modification of the

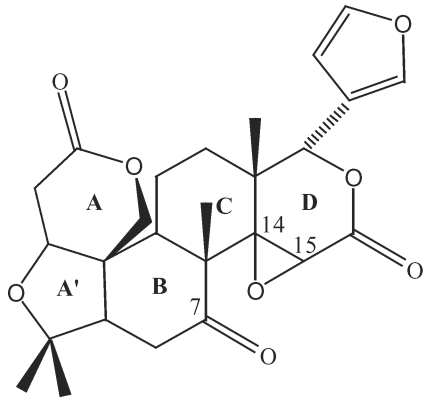


Salannin

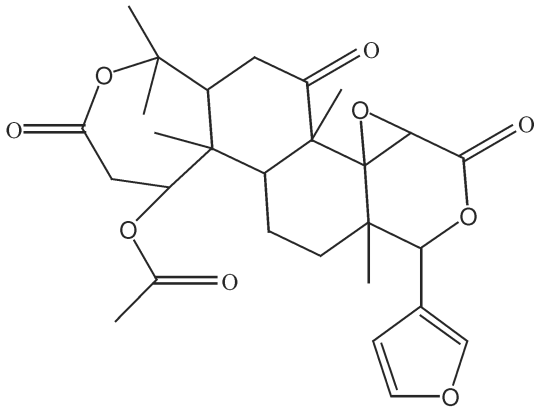
FIG. 2. Structure of Salannin.

tigloyl group, and (iv) saponification of the methyl ester. The hydrogenation of the furan ring to the tetrahydrofuran ring increases the antifeedant activity. The replacement of the acetoxy group at position 3 (Figure 2) by a methoxy group increases the activity, and a similar increase occurs when the acetoxy group at position 3 is replaced by hydrogen. The modification of a tigloyl function, such as hydrogenation increases the activity at least two-fold. On the contrary, deesterification of the tigloyl or the α-methyl butyrene groups result in a reduction of activity. Saponification of the methyl ester at C-11 increases the activity, for instance, salannic acid is at least 8-fold more active than 1,3-diol derivative.

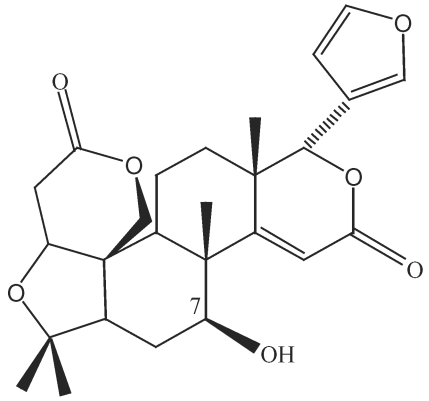
In a number of citrus species the bitterness causative factor is limonin (Figure 3). A few other citrus limonoids, including nomilin, nomilinic acid, ichangin, and obacunoic acid are also bitter. Amongst these, limonin and nomilin (Figure 3) are known to deter feeding in *Spodoptera, Heliothis, Choristoneura, Eldana, Maruca*, and *Leptinotarsa* species with variable efficacies (Champagne *et al*., 1992). It appears that furan and epoxide groups have to play a major role in the activity of these compounds. A possible role of C-7 is implied by the modest activity of the 7-hydroxylated de-epoxy system (Bentley *et al*., 1988). For instance, highly reduced activity of deoxyepilimonol (Figure 3) against limonin demonstrates the above conclusion. In certain cases, the cyclohexenone A ring and the α-hydroxy enone group in the B ring appear to be important for antifeedant activity. Also, the absence of 14–45 epoxide may not drastically reduce antifeedant activity (Govindachari *et al*., 1995). Recently, 23 semisynthetic derivatives of citrus limonoids, with a focus on the changes in C-7 carbonyl and the furan ring, have been evaluated against *Spodoptera frugiperda* larvae. In particular, reduction at C-7 afforded the related alcohols, and from these their acetates, oximes, and methoximes were prepared. Hydrogenation of the furan ring was also performed on limonin and obacunone to establish the significance of furan ring in the antifeedant activity against insects (Ruberto *et al*., 2002).



Limonin

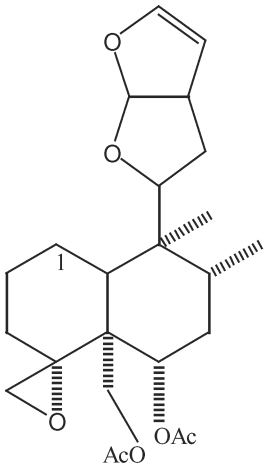


Nomilin

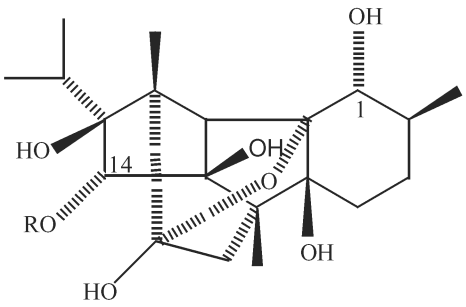


Deoxyepilimonol

FIG. 3. Structure of some citrus limonoids.



Clerodin



Ryanodine type of diterpene

FIG. 4. Structure of basic antifeedant diterpenes.

Quassinoids, which are more like limonoids, rather than degraded triterpenes, also possess anti-insect properties. Compounds such as bruceantin, bruceine-A, bruceine-B, bruceine-C, and bruceine-D from *Brucea antidyseterica* are antifeedant compounds for tobacco budworms, Mexican bean beetles, and southern armyworms (Koul, 2005). These compounds with A-ring enerone function induce potential feeding deterrence to these insects.

Diterpenes, particularly clerodane types of diterpenes have been identified from various plant sources and shown to deter feeding in various insect species (Hosozawa *et al*., 1974; Rose *et al*., 1981; Miyase *et al*., 1981; Koul *et al*., 1982; Wagner *et al*., 1983; Giordano *et al*., 2000). Clerodin (Figure 4) type of compounds from *Clerodendron infortunatum* Gaertn., *C. tricotomum* Thunb., and *Caryopteris divaricata* Maxim. are effective antifeedants against *Spodoptera litura* (Fab.), *S. littoralis* (Boisd.), *Ostrinia nubilalis* (Hubner), and *Euproctis subflava* (Bremer). Considering the feeding deterrent activity of several diterpenes with clerodane skeleton using electronic and conformational behaviors towards *Tenebrio molitor* (Enriz *et al*., 1994, 2000), they seem to mediate at least through two binding sites. The presence of an α,β-unsaturated system, or one spiroepoxide substituent at C-4 in the clerodane structure together with the β-furyl moiety at C-9 is important to evoke antifeedant activity. In addition, the free rotation of the β-furyl group could play a significant role in the biological activity. Recently a new class of insect antifeedants, the ryanodine diterpenes (Figure 4), has been isolated from *Persea indica* (a Lauraceae plant). The structure-activity relationship of these compounds show that C-14 and C-1 substituents play an important role. Aceylation of these centers result in loss of activity, whereas pyrrolecarboxylate at C-14 confers high potency (Gonzalez-Coloma *et al*., 1996). The comparisons of the mammalian toxicity and insect feeding deterrency of these compounds suggest a mechanism of action of these diterpenes in insects different from the Ca^{2+} release channel (Gonzalez-Coloma *et al*., 1996).

Caryophyllene oxide, spathalenol, guaianol, helenalin, eupatoriopicrin, bakkenolide A, bisabolangelone, and various sesquiterpene lactones are active antifeedants against a variety of insect species (Koul, 2005). Similarly antifeedant activity of 53 sesquiterpenes of *Lactarius* origin is known against stored grain pests (Daniewski *et al*., 1995). The sesquiterpenes with lactarane (Figure 5) and marasmane (Figure 5) skeletons are much more active than those with an isolactarane skeleton (Figure 5). The activity of furans is generally higher than their lactonic counterparts. Even some terpenes isolated recently from Rutales have been shown as effective antifeedants for stored grain pests, particularly the spirocaracolitones (Figure 5) being absolute antifeedants (Omar *et al*., 2007).

Sesquiterpene drimane antifeedants like warburganal, polygodial and muzigadial (Figure 6) are also known active compounds (Lam and Frazier, 1987) with a reactive enedial functionality, which interacts with a chemoreceptor site via pyrrole formation. In fact, these compounds, which have been reported to be active against several species of *Spodoptera* and *Heliothis* (Blaney *et al*., 1987), are inactive against aphids. Forty-one sesquiterpenes with a dihydro-β-agarofuran skeleton (Figure 7) have been evaluated against *Spodoptera littoralis* larvae (Gonzalez *et al*., 1997). These studies show activity in 38 compounds, the most active being those with isoalatol (Figure 7) and 4 β-hydroxyalatol (Figure 7) skeletons. Silphenene sesquiterpenes are established chrysomelid antifeedants and have been evaluated against *S. littoralis, L. decemlineata, Myzus persicae, Rhopalosiphum padi, Metopolophium dirhodum, Diuraphis noxia* and *Sitobion avenae* (Gonzalez-Coloma *et al*., 2002) and comprehensively discussed in Koul (2005).

Many monoterpenes from plant sources have been evaluated as feeding deterrents against insects (Koul, 1982). However,

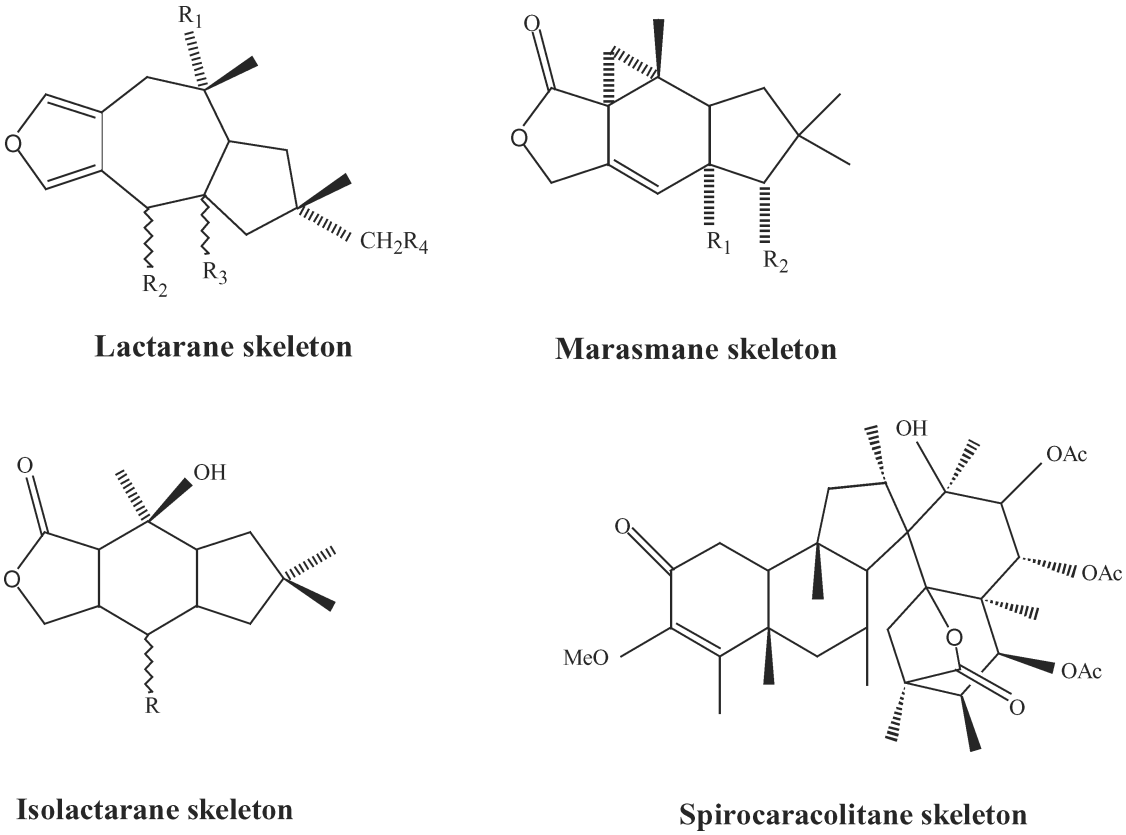


FIG. 5. Structures of various sesquiterpenelactone skeletons from plants evaluated for antifeedant activity.

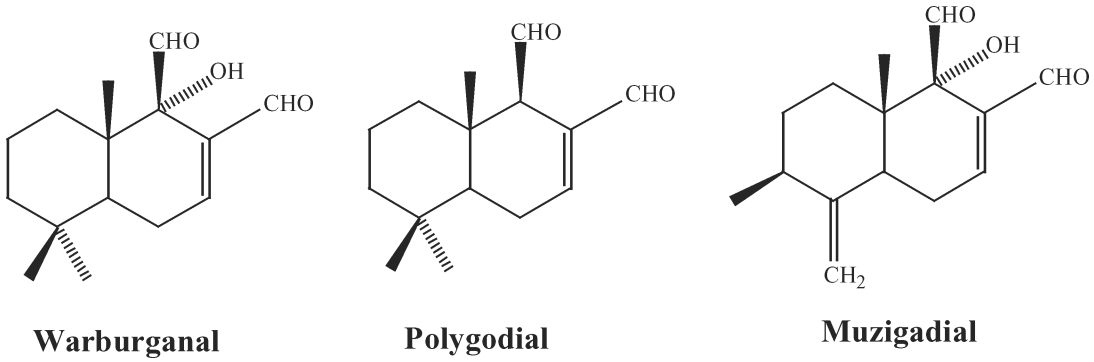
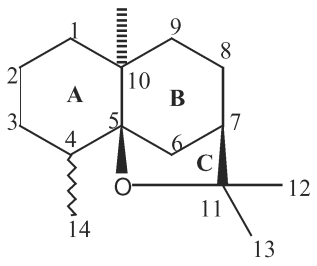


FIG. 6. Sesquiterpene drimanes as antifeedants.



4β - OH, 9α - OBz, 1α,6β,15 - OAc, 8α - OCH_3

Isoalatol

4β - OH, 9α - OBz, 2α,6β,8α,15 – OAc, 1α – OAng

Hydroxyalatol

FIG. 7. Antifeedants of agarafuran skeleton.

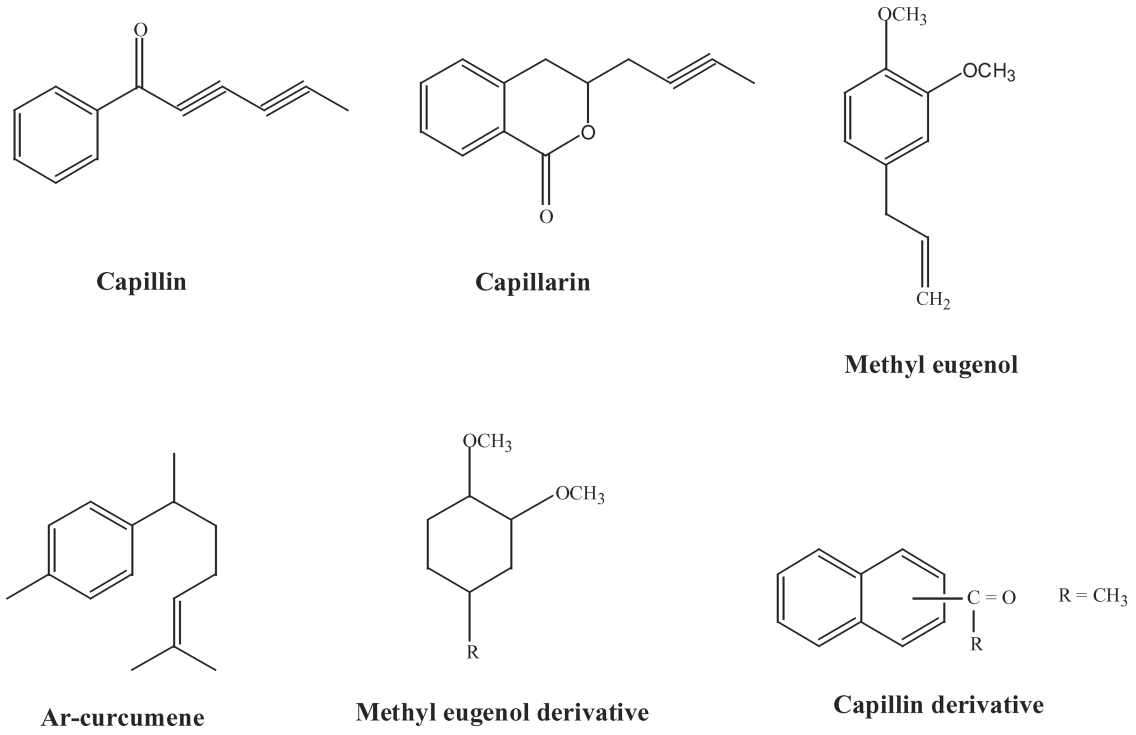


FIG. 8. Structure of monoterpene type of antifeedants from plants.

capillin, capillarin, methyl eugenol and ar-curcumene (Figure 8) isolated from *Artemisia capillaris* have a promise as antifeedant compounds against cabbage butterfly larvae, *Pieris rapae crucivora*. The relative strong antifeedant activity of capillin and capillarin suggest that C=O carbonyl group instead of CH_2 methylene group, a C≡C in a side chain and a lactone ring are some of the many factors that contribute to the biological activity (Yano, 1987). Various derivatives of these base compounds like methyl eugenol reveal that 3,4-dimethyl group and 1-substituent of 3,4-dimethoxy-1-substituted benzenes (Figure 8) contribute to the antifeedant activity (Yano and Kamimura, 1993).

Similarly capillin structure has an aromatic carbonyl group and two C≡C bonds. In order to demonstrate the importance of these two functions for the candidate activity, various derivatives evaluated against *P. rapae crucivora* reveal that arylmethyl ketone with a CH_3 group (Figure 8) instead of an H atom combined with C=O group of aromatic aldehyde is more active than that of aromatic aldehyde alone (Yano and Tanaka, 1995). A relationship between antifeedant activity using phenyl alkynes suggests that C≡C bond in the side chain is associated with antifeedant activity. Terminal groups (R) of side chain of C_6H_5-C≡C-R influence activity considerably and the intensity of activity of various compounds show a reasonable trend in activity with increase in bulk of side chain (Figure 9). This suggests that charge separation of C≡C bond by electron donative effect of alkyl group combination with C≡C bond may be correlated with an increase in antifeedant activity, and that a carbon chain enlargement of the alkyl group results in a decrease of antifeedant activity, probably because of the stereochemical hinderence (Yano, 1986).

Saponins are widely distributed among plants and have a wide range of biological properties. Three alfalfa saponins—zanhic

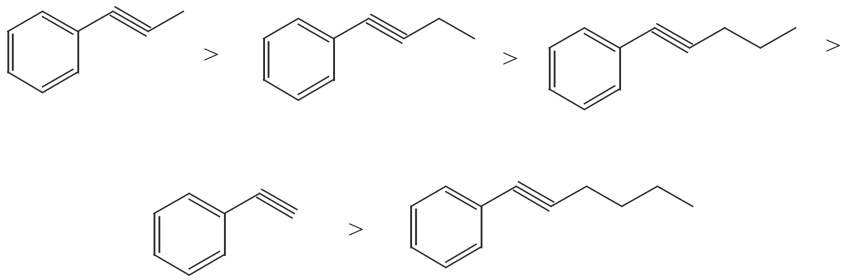


FIG. 9. Intensity of activity among monoterpene compounds, which decreases with the increase in bulk of side chain.

acid tridesmoside, 3GlcA, 28AraRhaXyl medicagenic acid glycoside, and 3GlcA, 28AraRha medicagenic acid glycoside—were tested for their settling inhibition effects on feeding behavior of the aphid, *Acyrthosiphon pisum* using the electrical penetration graph method. Application of saponins to artificial diets affected the probing behavior. In general, saponins incorporated into sucrose-agarose gels significantly reduced the number of aphid probes and extended their duration. Lower saponin concentrations (50 ppm) extended aphid activity and corresponded to phloem sap ingestion. In contrast, higher concentrations (100 ppm) strongly reduced aphid ability to ingest phloem and xylem sap (Golawska, 2007).

In a very recent study the antifeedant effect of six cacalolides and six eremophilanolides were tested against the herbivorous insects *Spodoptera littoralis*, *Leptinotarsa decemlineata*, and *Myzus persicae* (Burgueño-Tapia *et al*., 2007). The test compounds included several natural products isolated from *Senecio madagascariensis* and *S. barba-johannis*. The deterrent activity ranged from moderate to strong action, which was species dependent.

Proteinase inhibitors from plants are also known to induce feeding deterrence in insects. Low-molecular-weight peptidyl proteinase inhibitors (PIs) including leupeptin, calpain inhibitor I, and calpeptin were potent antifeedants for adult western corn rootworm against the phagostimulation of cucurbitacin B or a corn pollen extract. Leupeptin was the strongest (ED_{50} = 0.36 and 0.55 nmol/disk for Cucurbitacin B and corn pollen extract, respectively) among PIs tested with an antifeedant potency much stronger than the steroid progesterone (ED_{50} = 2.29 and 5.05 nmol/disk for Cucurbitacin B and corn pollen extract, respectively), but slightly less than the reference alkaloid, strychnine (ED_{50} = 0.17 and 0.37 nmol/disk for Cuc B and CPE, respectively) (Kim and Mullin, 2003). All active PIs contain a di- or tripeptidyl aldehyde moiety, indicating that PIs exert their antifeedant effects by covalent interaction with putative sulfhydryl (SH) groups on taste receptors as do these PIs with cysteine proteinases. However, opposite inhibition potency against Cucurbitacin B versus corn pollen extracts by two thiol-group reducing agents, DTT and L-cysteine, and the results with other cysteine-modifying reagents obscure the net functional role of SH groups at western corn rootworm taste chemoreceptors. Surprisingly, the model phagostimulant for diabroticites, cucurbitacin B, was more easily counteracted by these feeding deterrents than the stimulants present in corn pollen extracts. Three-dimensional structure–antifeedant relationships for the PIs suggest that a novel taste chemoreception mechanism exists for these peptidyl aldehydes or that they fit partially into a strychnine binding pocket on protein chemoreceptors. Favorable economic benefit may be achieved if PIs are discovered to be useful in adult western corn rootworm control, since both pre- and postingestive sites would be targeted (Kim and Mullin, 2003).

IV. HABITUATION OF ANTIFEEDANTS

There are several possible mechanisms for the decrease in efficacy, including sensory adaptation, motor fatigue, and habituation. Many investigators have used the term habituation liberally without actually proving that the decrement represents habituation and not sensory adaptation or motor fatigue. Habituation, perhaps the simplest form of learning, is defined as the waning of a response as a result of repeated or prolonged presentation of a stimulus, which is not due to sensory adaptation or motor fatigue (Carew and Sahley, 1986). It represents a loss of some particular responses, rather than the acquisition of new ones (Bernays and Weiss, 1996). Habituation differs from sensory adaptation in its ability to be terminated or reversed immediately by a novel or noxious stimulus (Thompson and Spencer, 1966). Different mechanisms may be responsible for the waning of response. Szentesi and Bernays (1984) showed that decreased response to antifeedants following prolonged exposure might result from the effect of mouthpart chemosensory information on the central nervous system, during palpation and feeding, or involving persistent synaptic changes in specific neural pathways (Bernays and Chapman, 1994), or from effects that follow ingestion of the deterrent (e.g., induction of a detoxifying enzyme (Szentesi and Bernays, 1984; Bernays and Chapman, 2000; Bernays and Weiss, 1966). Decreased response to antifeedants following prolonged exposure occurs most readily when a single antifeedant provides a weak inhibitory stimulus (Jermy *et al*., 1982; Szentesi and Bernays, 1984), but not to mixtures of antifeedants (Jermy, 1987). Whether a variety of antifeedants (plant extracts or pure compounds) would produce a decrease in the antifeedant response following prolonged exposure in a generalist herbivore, *Trichoplusia ni* has been recently studied, where *T. ni* was chosen because it is an important polyphagous pest of food, fiber, and ornamental crops throughout the New World (Akhtar *et al*., 2003). The selection of antifeedants was based upon their strong feeding deterrent and growth inhibiting properties on *T. ni* shown in initial screening bioassays (Akhtar and Isman, 2003). Other investigators have also reported similar findings for the chosen antifeedants (*Melia volkensii*, *Origanum vulgare*, digitoxin, cymarin, xanthotoxin, toosendanin, and thymol). The main objectives of the experiments have been the following: (1) to determine under what conditions (different concentrations and larval instars) feeding experience with antifeedants changed subsequent feeding preferences of the cabbage looper, *T. ni*, and (2) to determine if the decreased response to antifeedants following prolonged exposure was the result of habituation in *T. ni*. If so, it should then be possible to demonstrate dishabituation. It is now known that taste receptor cells do discriminate between bitter stimuli (Caicedo and Roper, 2001). In a recent study, discriminating by habituating the caterpillars to salicin and then determining whether the habituation generalized to caffeine or aristolochic acid has enabled to examine discrimination in caterpillars with a modified peripheral taste profile. It was found that the intact and lat-ablated caterpillars both generalized the salicin

habituation to caffeine but not to aristolochic acid. It was also determined whether this pattern of stimulus-generalization could be explained by salicin and aristolochic acid generating distinct ensemble, rate, temporal, or spatiotemporal codes. It was concluded that temporal codes from the periphery can mediate discriminative taste processing (Glendinning *et al*., 2001, 2006)

Knowledge of these factors may have consequences for the use of antifeedants in pest management and might be helpful in understanding host-plant shifts in insects. Although decreases in response to other antifeedants in this study have been observed, it is difficult to ascertain that these are the result of habituation, as it would be necessary to demonstrate dishabituation as well. Further testing of each of the antifeedants using aversive stimuli is necessary before such conclusions can be drawn. A decrease in response to feeding deterrents might enable the insect to feed normally on plant species that belong to the potential host-plant spectrum but are in some degree deterrent to naïve inexperienced individuals (Szentesi and Jermy, 1989) and would permit broadening of diet if the need arises (Bernays and Weiss, 1996). This could be of adaptive value where there is not a strong correlation between deterrence and toxicity of plant phytochemicals (Jermy *et al*., 1982; Jermy, 1987; Bernays and Chapman, 1994). According to Akhatar *et al*. (2003), a decrease in response to antifeedants following prolonged exposure could have many disadvantages from the pest management point of view as their experiments have clearly indicated that continuous contact of a feeding insect with a deterrent-containing food source caused increased acceptance of that food over time, thereby decreasing efficacy of the deterrent, which points to habituation and could offer some solutions for pest management. Decreased deterrence resulting from habituation has different implications for pest management than does decreased deterrence resulting from increased tolerance to toxic substances. Compounds to which insects have become habituated can be made effective deterrents again through the process of dishabituation (Akhtar *et al*. 2003). However, this has substantial implication for integrated pest management and needs extensive experimentation and design of evaluation in order to make such an approach effective.

V. ADVANTAGES AND LIMITATIONS

A. Advantages

The use of antifeedants in pest-management programm has enormous intuitive appeal. They satisfy the need to protect specific crops while avoiding damage to nontarget organisms so that potential value is very great. In fact, insect damage to plants results from feeding or from transmission of pathogens during feeding, therefore, the chemicals that reduce pest injury by rendering plants unattractive or unpalatable can be considered as potential substitutes for conventional insecticides. The host choice of generalists and to some extent specialists may be modified when feeding inhibitors are used. The range of insect species targeted may be chosen by either the chemical structure of the inhibitor or by the composition of a mixture of inhibitors, if different inhibitors are active against different species within the range. Therefore, multicomponent defense strategy of plants themselves could be used, as shown in number of recent studies with non-azadirachtin type of limonoid inhibitors (Koul *et al*., 2003a, b; 2004a, b, 2005) where potentiation among non-azadirachtin limonoids having explicitly two different modes of action, like feeding deterrence and physiological toxicity, play a significant role in the potentiation effect.

Most feeding inhibitors are less stable chemicals than traditional insecticides and act with lower residual activity and environmental impact. Natural predators and parasitoids remain unharmed by feeding deterrents targeting the herbaceous host insects. As the target sites of antifeedants are different, pesticide-resistant insect populations will still be affected by feeding inhibitors. Multicomponent tactics will also slow down the resistance development to these new compounds. In fact, lack of resistance is very useful for practical application of antifeedants as it is unlikely that oligophagous insects could develop general resistance to such deterrents, because this would result in rapid change of their host-plant range, which is determined mainly by the occurrence of such chemicals in the nonhost plants. Different molecular structures of possible antifeedant compounds could be another advantage. The blend of active constituents might diffuse the selection process, mitigating the development of resistance compared to that expected with a single active ingredient. This also supports the earlier mentioned contention that combination mixtures of antifeedants could be more effective than individual compounds.

Systemic action of antifeedants is another useful aspect of their practical application. On one hand it will exert uniform distribution within the plant and on the other it will counterbalance the phagostimulatory effects of plant surface chemicals (Chapman and Bernays, 1989). The systemic action of neem extracts is well documented (Gill and Lewis, 1971; Abdul Kareem *et al*., 1998; Osman and Port, 1990; Koul and Shankar, 1995). Thus, gradual release of neem compounds from neem seed powder incorporated in the soil and their gradual translocation by plant gives neem a considerable persistence as a control agent.

Similarly extracts of *Amora ruhituka* and *A. squamosa* (Islam, 1987) have also been shown to have systemic action. Coumarin is transported in grass leaves and thus unpalatable to *Chorthippus parallelus* and sinigrin is absorbed from water solution and transported to stems and leaves of various plant species. However, if a promising antifeedant is to be established for insect control further investigations into systemic studies are unavoidable.

B. Limitations

From crop protection point of view, antifeedants should meet the same criteria as insecticides. That means they should be selective to the target pests and must have sufficient residual action to protect the crop through its window of vulnerability to the key pests (Isman, 2002). Antifeedants also suffer from greater

interspecific differences in bioactivity. For instance, more than 30-fold variability was seen among noctuid caterpillars using azadirachtin (Isman, 1993). An investigation with silphinene sesquiterpenes as antifeedantas has revealed profound differences in activity when tested against cotton leafworm, the Colorado potato beetle and five species of aphids (Gonzalez-Coloma *et al*., 2002).

Feeding deterrents, if used indiscriminately, may also result in development of resistance. This has been indicated in the studies of selection of resistance to azadirachtin in the green peach aphid, *Myzus persicae* (Feng and Isman, 1995). When two lines of this aphid were treated repeatedly with pure azadirachtin, after 40 generations the AZA selected line developed 9-fold resistance to AZA compared to a nonselected control line. Interestingly this type of resistance did not develop in extracts, treated (with same amount of AZA) insects.

Another operational problem specific to antifeedants is the potential for rapid desensitization to a feeding deterrent. Individual insects initially deterred by a feeding inhibitor, become increasingly tolerant upon repeated or continuous exposure. This has been demonstrated in the case of azadirachtin and toosandanin used against tobacco cutworms (Bomford and Isman, 1996). In fact, insects becoming habituated and cross-habituated is a serious limitation of feeding deterrents however, it can be mitigated by using mixtures of antifeedants in a multicomponent strategy as previously suggested for non-azadirachtin type of compounds (Koul *et al*., 2004a,b) and demonstrated in the combination of xanthotoxin and thymol (Isman, 2002).

VI. PROSPECTS FOR FUTURE AND COMMERCIALIZATION

The present situation for insect antifeedants from plants, from a commercial point of view, indicates that such chemicals have not really made headway towards large-scale use because of the lack of technology to produce them in sufficient quantity and the time consuming and labor-intensive procedures to prepare them. Therefore, in spite of the wide recognition that many allelochemicals possess potential insect control properties, only a handful are in use in some parts of the world, suggesting only use in organic food production, which is estimated to be 8 to 15% in Europe and North America (Isman, 2006).

A. Possible Methods to Proceed

The simplest approach 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, using the protocols of twig tests, extraction, fractionation, and feeding bioassays in laboratory and field have to be the screening procedures for antifeedants. Only these methods could lead to discoveries for both forestry and agriculture. These antifeedant compounds have to be further developed, tested and evaluated before an applied approach can be realized. When these results are obtained, the next step would be to apply them efficiently in the field. Perphaps genetically modified plants could be developed that produce the active substances in amounts high enough to protect the plants from further herbivorous damage. For example, in cotton or crucifers, several insect antifeedants have been identified. To produce plants with higher amounts of these naturally occurring defense compounds would be an attractive method to avoid herbivore attack. Glanded cotton contains more antifeedants than glandless, but the production today focuses more on glandless cotton due to other uses of its seed, particularly cattle food. However, in using genetic strategies one has to take into account possible ecological risks (effects on nontargets, humans, etc.) as well as negative changes in plant energy costs.

As mentioned above, only few highly active antifeedants have been investigated from a commercial point of view, which makes impossible to systemize or to predict any molecular motifs in feeding inhibition. Structure activity relationship studies also do not point to any generalization. Our experience suggests that a "Mix and Match" system may help in developing a cocktail of feeding inhibitor that can be used in developing a customized formulation against a specific category of pests. Application of such products will be broad and will not be limited to targeted pest and to a plant part.

B. Economical Potential

Among 900 insect antifeedant compounds known today (Koul, 2005), only the compounds from neem, *Azadirachta indica* have shown commercial potential and quite a few products are in the market that have met regulatory requirements and have received firm or provisional registrations. The overall picture leads one to surmise that commercial neem products have gained greater significance in the Indian subcontinent where there are commercially marketed products for virtually all types of usage. In all other countries, commercial neem products count for only a modest share of the market. Throughout the world, in those countries where neem trees are grown, the prices for dried seeds are between $0.10 and 2.00 per kg. To effectively control most pests, one hectare of crops must be treated once with between 20–60 g of the main active ingredient, azadirachtin. Thus, given the fact that there are approximately 2 g of azadirachtin per kg of seeds on an average, somewhere between 10 and 30 kg of neem seeds are needed in all. This means that the seed costs alone for the single treatment of one hectare of crops are between $1.00 and $60.00, although in most countries they are somewhere in the narrower range between $5.00 and $20.00 (Status Report on Global Neem Usage, 2000). Because the large multinationals appear to have no serious interest in the development of nonpersistent nature-based biological pesticides (like feeding deterrents), commercialization of such products is mainly taken by small manufacturers. In fact, in India to achieve this goal, provisional registrations have been given to manufacturers and the products are being sold in the market. However, it becomes imperative for producers to fulfill the requirements within the stipulated time-frame, as provided by the regulatory authorities. Western countries should

adopt this policy if botanical biopesticides are to make any impact in the near future in conventional insecticide market. Neem has already provided a modern paradigm for the development of biopesticides and others have to follow the direction.

C. Commercial Prospects

Many feeding inhibitors from plant sources so far have given excellent results in laboratory conditions. In field situations only a few of them are satisfactory alternatives to traditional pest management. The chemical control is usually with broad-spectrum insecticides, and they have to be broad-spectrum by necessity. They have to sell in amounts large enough to accommodate financial development, research, and marketing. The class of antifeedants is tested against one or a small group of insects attacking a specific crop. As a compound, it inhibits the feeding of one species, but for another it may be ineffective or just an attractant. Thus, replacement of a traditional chemical with a specific allelochemical will make pest management more expensive. That is why as of today the only prospect among botanicals is neem. However, apart from neem products, there are few actual demonstrations of antifeedant efficacy in the field. Application of polygodial or methyl salicylate at the IARC-Rothamsted have shown that aphid populations are reduced with concomitant increases in yields of winter wheat, in one case comparable to that achieved with the pyrethroid insecticide cypermethrin (Pickett *et al.,* 1997). Similarly, toosendanin, an antifeedant limonoid from the bark of the trees *Melia toosendan* and *M. azedarach* (Meliaceae) has been subjected to considerable research as a botanical pesticide (Chiu, 1989; Chen *et al*., 1995; Koul *et al*., 2002). Vertebrate selectivity of this compound is very favorable (LD_{50} mice = 10 g/kg) (Isman, 1994). Production of a botanical insecticide based on toosendanin, using a refined bark extract containing approximately 3 percent toosendanin (racemic mixture) as the active ingredient, has recently begun in P.R. China (Zhang *et al.,* 1992). Toosendanin-based insecticides could become a potential commercial product worldwide as formulations based on the technical concentrate are under evaluation in Canada to assess its potential against pests of agriculture and forestry in North America.

D. Future Outlook

The practice of using feeding deterrents from plant sources allows us to develop and exploit naturally occurring plant defense mechanisms, thereby reducing the use of conventional pesticides. However, most of these new strategies need to be developed with four basic facts in mind: organize the natural sources, develop quality control, adopt standardization strategies, and modify regulatory constraints. In fact, all the four areas need substantial effort, if plant-based products are to be successful and competitive. This will definitely give rise to a number of challenges and unexpected problems. For instance, limonene is known to be a bitter antifeedant, but at higher concentrations does cause irritation and allergic reactions when in contact with skin. Therefore, deeper cooperation between industrial and academic research is required that could definitely accelerate the process and give us new environmentally safe methods in future plant protection via plant defense mechanism of secondary metabolites.

Creative strategies need to be deployed. For example, two methods of combining the use of teflubenzuron with insect antifeedant have been studied (Griffiths *et al*., 1991). The strategy of applying the antifeedant and growth inhibitor together relies on stopping the overshoot in feeding that occurs when the insects are poisoned by teflubenzuron. The insect needs to eat $<1\%$ of the leaf disc to acquire a toxic dose but, in the absence of an antifeedant, it eats $>40\%$ even at the highest doses, during the lag phase that occurs between treatment and effect. In laboratory conditions, the combination of antifeedant with teflubenzuron decreased feeding damage by *Plutella xylostella* and *Phaedon cochlearae* without diminishing the toxic effect (Griffiths *et al*., 1991). In the alternative strategy, teflubenzuron and antifeedant were applied separately. Treatment of the growing tips of mustard plants with antifeedant forced insects down the plant to the lower leaves, where they were killed by diflubenzuron. Combination of an antifeedant with a physiological toxin (both may be from the plant source itself) is another choice (Koul *et al*., 2005) to develop a sustainable pest management strategy based on plant products. Manipulation of insect population in this way now forms part of various insect control studies, such as the stimulo-deterrent diversionary cropping (Miller and Cowles, 1990) and the push–pull strategies (Pyke *et al*., 1987; Khan *et al*., 1997).

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