DEFENSE OF PLANTS THROUGH REGULATION OF INSECT FEEDING BEHAVIOR

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ABSTRACT

Feeding deterrents, or antifeedants, are chemicals that can protect plants from insect herbivory through regulation of insect feeding. The sensitivity of a given species of an insect herbivore is dependent upon the quantity and chemical structure of the feeding deterrent.

Feeding deterrency can be caused by an effect of the chemical on chemoreception and/or on the centers that regulate feeding and metabolism. Feeding deterrents primarily affect the feeding behavior of insects, but often they are also toxic if fed upon.

Examples of feeding deterrents that play an important role in the multichemical defense of plants from insects are azadirachtin isolated from *Azadirachta indica* (Meliaceae) and rhodojaponin III isolated from *Rhododendron molle* (Ericaceae). These two terpenoidal plant products, so effective in nature, are currently being tested for use in commercial insect control.

RESUMEN

Hay químicos que disuaden o que impiden que coman, y que pueden protejer a las plantas de insectos herbívoros a través de la regulación del acto de comer. La sensibilidad de una especie en particular de insecto herbívoro, depende de la cantidad y de la estructura química de lo que lo disuade que coma.

El que no coman puede ser causado por el efecto del químico en el receptor químico, y o por el centro que regula el acto de comer y del matabolismo. Los disuadores de que coman, principalmente afectan el compartamiento de cóno comen los insectos, poro a menudo también son tóxicos si se comen.

Ejemplos que disuaden el comer y que juegan un importante papel en la defensa multi-química de plantas hacia insectos, son azadiractín aislados de *Azadiracta indica* (Meliaceae) y aislados de rodojaponín III de *Rhododendrom molle* (Ericaceae). Estos dos productos terpenoidales de plantas, tan efectivos en la naturaleza, se están actualmente probando para su uso en el control comercial de insectos.

Feeding deterrents, also known as antifeedants, are substances that result, either temporarily or permanently depending on potency and structure, in the cessation of feeding (Kubo & Nakanishi 1977). The importance of these compounds in plant-insect relationships has been well-documented and it is probable that in nature, all non-host plants contain feeding deterrents (Schoonhoven 1982).

Although insect feeding deterrents can be found in many classes of plant compounds, some of the most potent and widespread are terpenes (Harborne 1989). In this paper, we will discuss two plant terpenes, the tetranortriterpene azadirachtin isolated from

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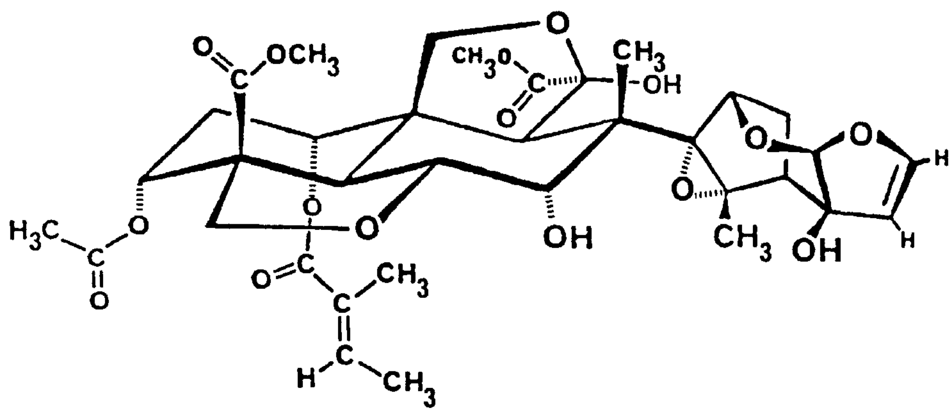
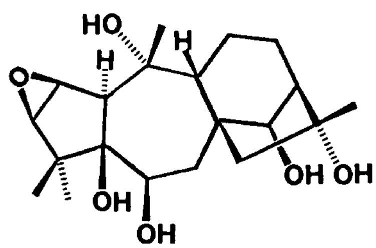


AZADIRACHTIN



RHODOJAPONIN III

Fig. 1. The chemical structures of two naturally occurring insect feeding deterrents.

Azadirachta indica A. Juss. (Meliaceae) and the diterpene rhodojaponin III isolated from *Rhododendron molle* (Bl.) G. Don (Ericaceae) (Fig. 1), which protect the plants in which they are found through regulation of insect feeding behavior.

Both *A. indica* and *R. molle* have long been used as insecticides in the areas where they grow (Perry 1980, Grainge & Ahmed 1988). For example, the effects of the leaves of *A. indica* on insect feeding behavior were reported over fifty years ago in India, where the tree is indigenous (Volkonsky 1937) and the flowers of *R. molle* indigenous to China, have been sold for many years as a commercial insecticidal preparation in Chinese drug stores (McIndoo 1945).

In more recent times, due to the rapid developments in the isolation, identification, and bioassay of plant products, a number of biologically active compounds from *A. indica* (Jones et al. 1989) and from *R. molle* (Klocke et al 1990a) have been reported. These compounds, representing various structural types eliciting various biological activities against insects, are components of the multichemical plant defense against herbivory so often observed in nature (Kubo et al. 1983). Although feeding deterrency forms only a part of the multichemical defense of these plants, its importance in nature and its implications for commercial insect control warrant its study.

Materials and Methods

The potency of the antifeedant effect of azadirachtin and rhodojaponin III against several species of insects was determined using host plant leaf disk bioassays. The compounds, following their isolation and purification from plants (Yamasaki et al. 1986, Klocke et al. 1990a), were dissolved in acetone and applied (25 μl) to the surface of disks (50 mm^2) punched from suitable host plants (i.e., potato for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) and cotton for the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and the tobacco budworm, *Heliothis virescens* (Fabr.) (Klocke et al. 1990b). Following solvent evaporation, the leaf disks were arranged with their upper (treated) surfaces exposed in a circle between two moistened absorbant pads. In this way, the untreated lower surface remained in contact with moisture to prevent drying out, while only the treated surface was exposed to the insects. In the "no choice" leaf disk bioassays, the insects (third instar larvae) were presented only with treated disks. In the "choice" leaf disk bioassays, the insects were presented with both treated and untreated disks simultaneously. The PC_{95} value, the minimal protective concentration of compound (μg/disk) at which >95% of the control, while <5% of the treated, leaf disks were eaten was determined for each compound from 8-10 replicates/concentration (Klocke & Kubo 1982, Yamasaki & Klocke 1989).

The plant compounds were bioassayed further using suitable whole plants (i.e., potato for *L. decemlineata*, cotton for *S. frugiperda*, and tomato for the tobacco hornworm, *Manduca sexta* (L.)). The compounds were either solubilized in 0.05% tween-80 and sprayed (20 ml) onto the foliage or solubilized in 0.5% Triton-X-100 and poured (100 ml) onto the soil of potted plants (Hu et al., submitted; Klocke et al. 1990b). After 24 hours, 15-20 third and fourth instar larvae were transferred to each of the treated plants. After the control plants were almost completely consumed, plant damage and larval survivorship were assessed. Three replicates were used for each treatment.

The plant compounds were tested also in an artificial diet bioassay (Chan et al. 1978, Kubo & Klocke 1983). Neonate larvae of *H. virescens* and *S. frugiperda* were transferred to treated diet in a controlled environment (relative humidity, temperature, and photoperiod) and observations were made on their nutrition, growth, development, and survivorship. After 10 days, LC_{50} and EC_{50} values, the lethal concentration for 50% mortality and the effective concentration for 50% growth inhibition, respectively, were determined from log probit analysis (Litchfield & Wilcoxon 1949).

Results and Discussion

In the "choice" leaf disk bioassays with azadirachtin, *S. frugiperda* was the most sensitive (PC_{95} = 0.1 μg/disk) and *L. decemlineata* the least sensitive (PC_{95} = 75 μg/disk) of the species tested. *H. virescens* was of an intermediate sensitivity (PC_{95} = 6 μg/disk). In similar bioassays with rhodojaponin III, *L. decemlineata* was the most sensitive (PC_{95} = 3 μg/disk) and *H. virescens* the least sensitive (PC_{95} = >50 μg/disk). *S. frugiperda* was of an intermediate sensitivity (PC_{95} = 6 μg/disk). As illustrated here, it is difficult to predict the sensitivity of a given species of insect to a given feeding deterrent and therefore sensitivity must be determined empirically.

The acute sensitivity of *S. frugiperda* to azadirachtin observed in the leaf disk bioassays was observed also in whole corn plants sprayed with 600 ppm of azadirachtin, a concentration which, applied as a prophylactic, resulted in complete plant protection from *S. frugiperda* (Klocke & Barnby 1989). This level of activity was the same as that of lannate, a synthetic insecticide. On the other hand, potato plants treated with the same amount of azadirachtin were not protected from feeding by *L. decemlineata*.

The foliage of potted tomato plants was completely protected from feeding by *M.*

sexta by drenching the soil with 10 mg of azadirachtin. Thus, azadirachtin afforded plant protection through systemic translocation from the roots to the foliage of the tomato plants. A similar systemic translocation was observed when the soil of potted potato plants was drenched with 10 mg of azadirachtin; no *L. decemlineata* larvae, which readily fed on the treated plants, were able to pupate (Klocke & Barnby, submitted).

A systemic mode of action in plants is an important attribute of feeding deterrents because insects often prefer to feed on new plant tissue which would be unprotected if not sprayed continuously. Through systemic translocation, even new tissue is protected without additional sprayings (Schoonhoven 1982).

The feeding deterrent effect of azadirachtin was demonstrated using artificial diet bioassays with *H. virescens* and *S. frugiperda*. When fed on treated diet, larvae of *H. virescens* consumed less food, gained less weight, and were less efficient at converting ingested and digested food into biomass (Barnby & Klocke 1987). Consumption and weight gain were reduced at concentrations of azadirachtin (0.1-0.3 ppm) lower than those affecting the utilization efficiencies (0.5 ppm). This reduced consumption may reflect, at least in part, sensory detection and avoidance, as has been demonstrated for this insect (Simmonds & Blaney 1984).

Besides the effects of azadirachtin on deterrent receptors, several reports have attributed a part of its antifeedant effect to a disruptive influence on the centers that regulate feeding and metabolism (Redfern et al. 1982, Sieber & Rembold 1983, Schluter & Schulz 1984). We found that a single dose of azadirachtin injected either orally (1 μg) or directly into the hemocoel (0.3 μg) of mature *H. virescens* larvae resulted in a lower rate of consumption of diet (from 269 mg to 196 mg of diet) (Klocke et al. 1987).

Azadirachtin fed in artificial diet to larvae of *H. virescens* or *S. frugiperda* caused growth inhibition (EC_{50} = 0.07 and 0.4 ppm, respectively) and mortality (LC_{50} = 0.8 and 1.0 ppm, respectively) (Kubo & Klocke 1986). The mortality was due to a disruption in the molting process caused by a reduction in the titers of brain hormone (prothoracicotropic hormone) in the treated insects (Barnby & Klocke 1989). A similar effect on insect development was observed when larvae of *L. decemlineata* fed on potato plants treated systemically with 10 mg of azadirachtin. The larvae, while not deterred from feeding on the treated foliage, were unable to pupate and eventually died (Klocke and Barnby, submitted).

A toxic effect of feeding deterrents is important since starving insects often will attempt to feed on any potential food available to them, even that treated with feeding deterrents. That is, insects can become desensitized to the antifeedant effect (Blaney et al. 1986). In addition, not all species of insects are deterred from feeding on any given antifeedant. Therefore, an effective antifeedant should have feeding deterrency as its primary effect and toxicity as its secondary effect (Klocke & Barnby 1989).

Larvae of *L. decemlineata* were more sensitive to rhodojaponin III than to azadirachtin, both in the leaf disk and in the whole plant bioassays. Potted potato plants sprayed with 75 ppm of rhodojaponin III were completely protected from feeding by this insect. As in the leaf disk bioassays, larvae of *S. frugiperda* were less sensitive to rhodojaponin III since a concentration of at least 5000 ppm was required to deter them completely from feeding on cotton foliage.

Rhodojaponin III was more potent to both *L. decemlineata* and *S. frugiperda* in "no choice" than in "choice" leaf disk bioassays (PC_{95} = 1 and 2 μg/disk in the "no choice" assays, respectively, and PC_{95} = 3 and 6 μg/disk in the "choice" assays, respectively) (Klocke et al. 1990a). A similar result was reported when other plant products were tested against *L. decemlineata*, prompting the authors to hypothesize that at least a part of the antifeedant effect was due to toxicity rather than completely to chemoreception (Alford et al. 1987). In support of this hypothesis, we found rhodojaponin III to be a growth inhibitor (EC_{50} = 3 ppm) and an insecticide (LC_{50} = 9 ppm) when incorporated into artificial diet and fed to neonate *S. frugiperda*.

The effectiveness of feeding deterrents in host plant resistance in nature has led to their study as potential commercial insect control agents (Schoonhoven 1982). For example, azadirachtin is being evaluated in laboratories in both university and industry for its potential in commercial insect control (Klocke 1989). The need for new insecticides with a mode of action specific for target pest insects is widely recognized. Perhaps plant compounds, such as the antifeedants, can provide models for our future needs in commercial insect control.

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