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Phytochemical diversity of insect defenses in tropical and temperate plant families

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Phytochemical diversity and redundancy

One of the most intriguing features of the chemical ecology of plant–insect interactions is the remarkable number of different phytochemical defenses found in plants. A single plant may contain five or six biosynthetic groups of secondary metabolites and within each group these defenses may include many structurally related analogs and derivatives. Across the different species of higher plants, there is a bewildering array of different substances and modes of actions of substances. During the course of our research on phytochemical defenses in specific plant families, we have become interested in the raison d'être for this diversity, as have many other researchers (Romeo *et al.*, 1996). How much do we know about different types of defenses in plants? How did they arise? Are some of these secondary metabolites "redundant," with no function? How do they interact with one another? In this chapter, we will address some of these issues with observations on the defenses of several plant families against insects, using results from our own research and the published literature.

In general, we do not have a comprehensive picture of the different types of defenses in plants. Over a decade ago, Soejarto and Farnsworth (1989) estimated that of the 250 000 species of flowering plants, only 5000 species had been thoroughly investigated according to the Natural Product Alert (NAPRALERT) database, leaving 98% of species with potential for phytochemical discovery. Taking a more focussed view of the potential for discovering drugs of phytochemical origin in tropical forests, Mendelsohn and Balick (1995) estimated that current prescription drugs represent only 12% of what might be there to discover. Although steady progress in identifying the phytochemical defenses of plants is being made (thousands of compounds per year), the percentage of higher plants studied is

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increasing only slowly. About 70% of angiosperm species are tropical or subtropical and, according to Wilson (2002), we may lose 20–50% of these within the coming decades because of habitat loss and fragmentation. For this reason, there is a great urgency to learn more about their phytochemistry and chemical ecology before they are lost forever.

Progress in evaluating the diversity of defenses in the neotropical Meliaceae

The family Meliaceae (the mahogany family) provides an example of the high level of phytochemical diversity that can be found in tropical trees. The neem tree, *Azadirachta indica*, is a member of this family and its insect antifeedant and growth-reducing effects on insects have generated considerable research interest in the defenses of the family against insects. Intensive study of neem has revealed it to contain more than 50 limonoids and related compounds in its various tissues (Isman *et al.*, 1996). Many of these substances have feeding deterrent effects on herbivorous insects, and the specific mode of action of the most well-known compound, azadirachtin, has been studied at the electrophysiological and biochemical level. The neem tree is one of the most extreme examples of phytochemical redundancy in a single species. Characterization of limonoids from other species has been of considerable interest to the phytochemical research community and a large number of compounds have been identified in Meliaceae and related families of the Rutales, especially from Asian and African forests (Waterman and Grundon, 1983; Champagne *et al.*, 1992, 1996).

Neotropical forests are somewhat less well studied (Table 1.1). Approximately 20% of species have been investigated, although some of the more important timber genera in the family, especially mahogany (*Swietenia*) and tropical cedar (*Cedrela*), have received considerable attention. The insecticidal properties of these trees were known to indigenous peoples, such as the Maya, who valued cedar for its insect resistance and traditionally used pressed oils from its seeds as a treatment for lice (Pennington *et al.*, 1981). Our collaborative group (including R. Mata's laboratory in Mexico, the tropical dendrology group in Costa Rica (L. Poveda and P. Sanchez) and M. Isman's insect chemical ecology group in Vancouver) recently has investigated the insect-deterrent properties and substances in a number of these species. From *Swietenia humilis*, Jimenez *et al.* (1996, 1998) recovered more than 10 limonoids, including six novel humilinolides (Fig. 1.1) that were found in concentrations of 0.021–0.29% in seeds. Several of these were isolated in sufficient quantity for the assessment of insect growth-reducing activity against European corn borer, *Ostrinia nubilalis*, a highly polyphagous insect. These limonoids incorporated into insect diets at 0.005% caused significant reduction in growth, comparable to that seen with the positive control toosendanin (Fig. 1.2), a limonoid that is commercially available

Table 1.1. *Number of species and state of phytochemical investigation in genera of neotropical Meliaceae*

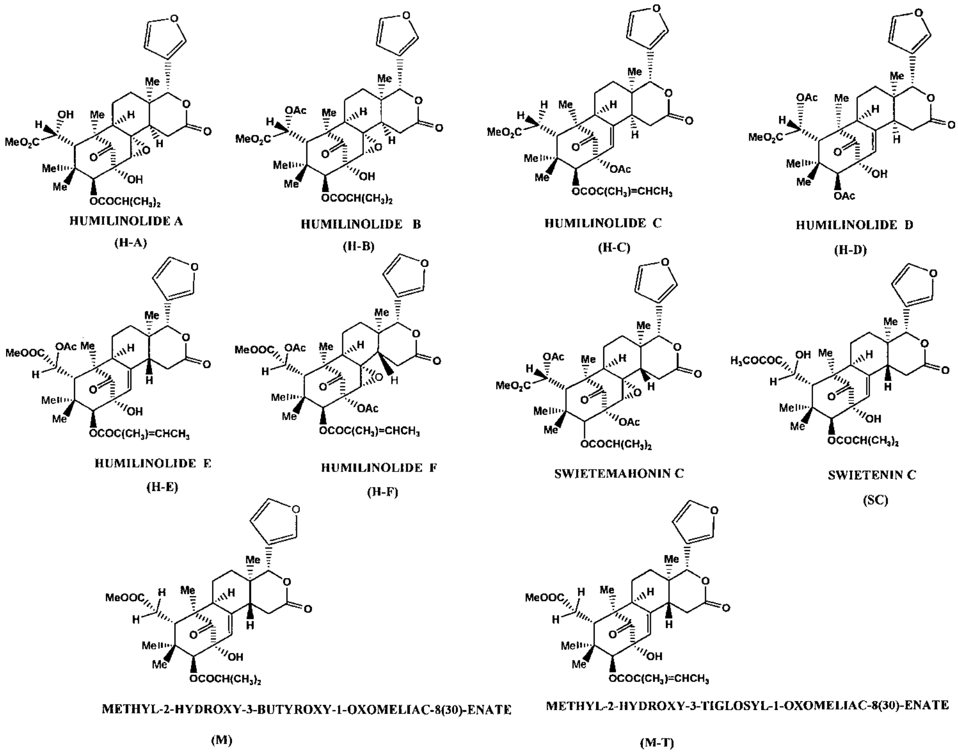
Genus	Number of species	Number of species with phytochemical identifications
Trichilia	70	14
Guarea	35	9
Swietenia	3	3
Ruagea	5	1
Cabralea	1	1
Cedrela	8	4
Carapa	3	3
Schmardia	1	0

Data from Pennington *et al.* (1981) and recent literature.

in China. They also caused delays in time to pupation and adult emergence as well as elevated mortality compared with controls. A recent study (Omar, 2000) with a model phytophagous weevil, *Sitophilus zeamais*, showed that the same compounds caused significant reduction in consumption of treated diets by these insects. Clearly these compounds are present in seeds at 5–50 times the concentration necessary to produce significant effects on generalist phytophagous insects. The presence of more than 10 compounds shows that the multiple defenses found in neem are not unique.

Other recent studies have demonstrated the insect growth-reducing and antifeedant properties of limonoids isolated from seeds. A similar study of *Guarea macrophylla* led to the isolation of six limonoids and protolimonoids, that were active in the *Ostrinia* bioassays (Jimenez *et al.*, 1998). Hirtin, a limonoid isolated from the seeds of *Trichilia hirta*, was active as a growth reducer to dark-sided cutworm, *Peridroma saucia* (Xie *et al.*, 1994). Recently, we isolated two other insect growth-reducing limonoids from the seeds of *Trichilia maritana* (MacKinnon *et al.*, 1997a) (Fig. 1.2). These results and studies by other groups, for example work on the genus *Raugea* (Mootoo *et al.*, 1996), show continuing potential for identification of insecticidal limonoids in seeds.

Plant parts other than seeds have been less well studied and little attention has been placed on insecticidal modes of action of the defenses of the neotropical Meliaceae. Our study of the insect growth-reducing activity of 50 extracts of bark, leaf and wood of Central American Meliaceae showed the potent effect of these extracts against lepidopteran larvae and the potential for isolation of bioactive compounds from a large number of these species. Extracts from the genera *Trichilia* and *Cedrela* (Xie *et al.*, 1994; Ewete *et al.*, 1996a; Wheeler *et al.*, 2001) show exceptional activity. *Trichilia americana* extracts have strong antifeedant activity to *Spodoptera*



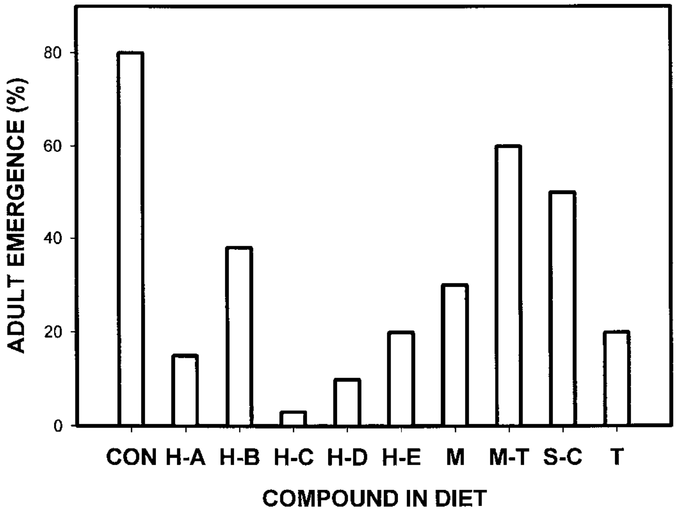
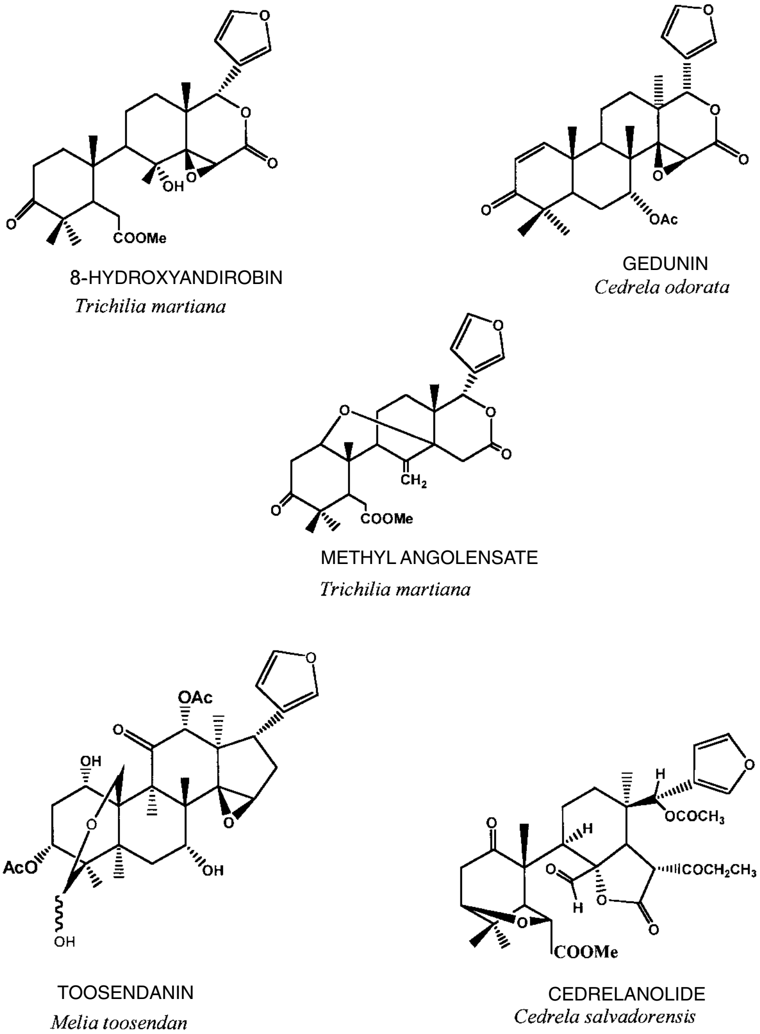


Fig. 1.1. Limonoids isolated from the seeds of *Swietenia humilis* and their effect on adult emergence of European corn borer following dietary administration at 50 ppm. CON, control; T, tenulin (toosendanin; Fig. 1.2); $n = 30$.



8-HYDROXYANDIROBIN
Trichilia martiana

GEDUNIN
Cedrela odorata

METHYL ANGOLENSATE
Trichilia martiana

TOOSENDANIN
Melia toosendan

CEDRELANOLIDE
Cedrela salvadorensis

Fig. 1.2. Limonoids isolated from the Meliaceae used in recent studies with insects.

litura, reducing growth by lowered food intake and lowered efficiency of conversion of ingested and digested food (Wheeler *et al.*, 2001). The common tropical cedar tree of the Americas, *Cedrela odorata*, contains the limonoid gedunin (Fig. 1.2), and closely related derivatives in substantial amounts in the bark and wood. We previously had found gedunin and *C. odorata* extract to have antifeedant properties, and significantly to reduce growth and delay development of lepidopteran larvae (Arnason *et al.*, 1987; Ewete *et al.*, 1996a). Gedunin is also a potent antimalarial compound (MacKinnon *et al.*, 1997b). Little was known about the specific mode of action of this (and other neotropical limonoids), but its relatively easy isolation

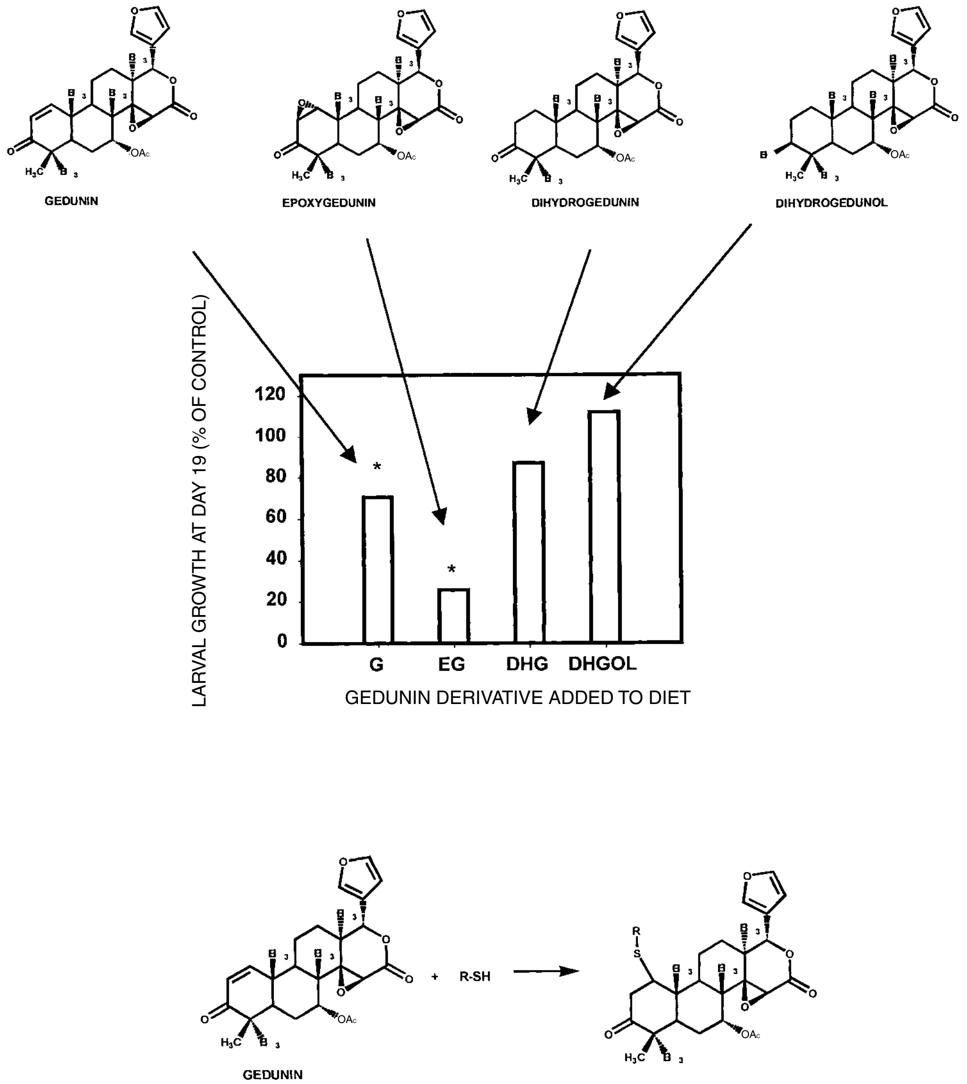


Fig. 1.3. Effect of semi-synthetic derivatives of gedunin on European corn borer growth and proposed mechanism of action. Significant effects.

and purification from wood provided enough starting material for semi-synthesis of derivatives (Fig. 1.3) (MacKinnon *et al.*, 1997b). Modification of the A ring by reduction of the double bond or both the keto group and the double bond eliminated insect growth-reducing activity. In contrast, the epoxy derivative produced an even greater growth-reducing effect than gedunin. These results are consistent with a mechanism of action in which gedunin or its epoxide acts as an alkylating agent, as shown in Fig. 1.3, whereas reduction of the keto group or double bond eliminates the

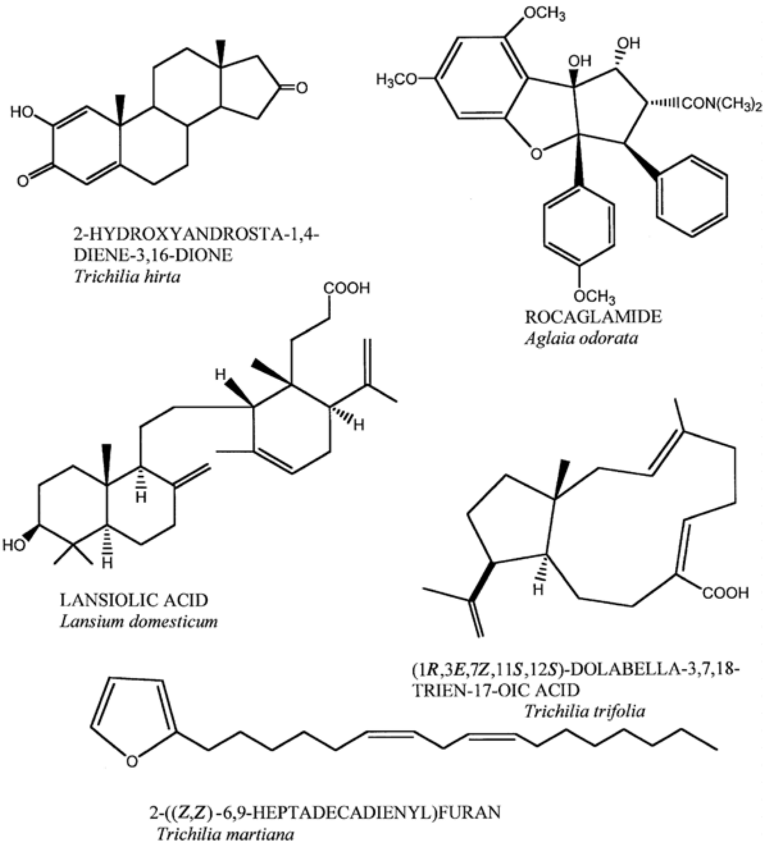


Fig. 1.4. Phytochemical diversity in the Meliaceae, illustrating bioactive compounds other than limonoids.

potential for this reaction. Recent work on a related species of tropical cedar, *Cedrela salvadorensis* yielded the limonoid cedrelanolide (Fig. 1.2), which is comparable in activity to gedunin (Jimenez *et al*., 1996) but which must have a different mode of activity.

Other studies show the presence of a diversity of compounds other than limonoids as defenses in the tissues of Meliaceae. Woody tissues of *Trichilia trifolia* afforded three novel dolabellanes with flexible C_{11}ring structures. These substances were very active antifeedants in the *Sitophilus* bioassay (Ramirez *et al*., 2000). *T. martiana* seeds yielded large amounts of 2-((*Z,Z*)-6,9-heptadecadienyl)furan. *T. hirta* and *T. americana* bark have yielded novel steroids by insect bioassay-guided isolation and application of a nanoprobe nuclear magnetic resonance (NMR) technique for structure elucidation (Chaurest *et al*., 1996). Compounds isolated included hydroxyandrosta-1,4-diene-3,16-dione (Fig. 1.4) and derivatives. However, studies by Wheeler *et al*. (2001) suggest that other unidentified compounds may also be involved in the activity of these species. Asian species have also provided a number of interesting non-limonoids. *Aglaia odorata* contains several benzofuran derivatives including rocaglamide (Fig. 1.4), which is the only compound that we have tested that has insecticide activity comparable to azadirachtin when incorporated in *O. nubilalis* diets (Ewete *et al*., 1996b). We have also isolated nine triterpenes from bark of *Lansium domesticum*, collected in Borneo (Omar, 2000). These compounds, called lansiolides, including lansiolic acid (Fig. 1.4), have insect feeding-deterrent activity as well as antimalarial activity.

Clearly there is still much to discover about the insect chemical ecology of this tropical family. More information is also needed about the chemical ecology of generalist and specialist fauna on these plants, although economically important insect pests, such as the mahogany shoot borer (*Hipsipyla grandella*), have been studied. This pest attacks and damages the shoots of *Cedrela* and *Swietenia* but does not attack the closely related Australian genus *Toona*. Grafts of *C. odorata* shoots onto root stock of *Toona ciliata* are resistant because of the translocation of *Toona* compounds into the graft (da Silva *et al*., 1999).

Overall, the published studies reveal that the defenses of the Meliaceae involve use of multiple defenses in one species and a broad diversity of biosynthetic types of phytochemical defense other than the well-studied limonoids, especially in plant parts other than seeds. Evolution of these diverse defenses was predicted in Ehrlich and Raven's (1964) stepwise co-evolution theory, later described as the "chemical arms race hypothesis" (Berenbaum, 1983), which was developed in part by observation of tropical families, including the Meliaceae, and their associated insect fauna. Although the concept that chance mutation and elaboration of novel defensive phytochemicals has led to the escape of plants from their herbivores continues to provide a plausible explanation for the evolution of phytochemical diversity, many modern chemical ecologists suggest that aspects of the original co-evolution theory requires reworking because of inconsistencies, such as a lack of observed reciprocal effects and ecophysiological constraints on co-evolution (Thompson, 1994).

Analog synergism in the Lepidobotryaceae and Piperaceae

Although plant–herbivore co-evolutionary theory provides a model for the evolution of the phytochemical diversity between species described above, the diversity of closely related compounds or analogs observed in any single species is not readily explained. Jones and Firn (1991), Berenbaum (1985) and Feng and Isman (1995) provided several hypotheses that have looked specifically at the evolution of diversity of phytochemical substances in a single species and the possibility of "analog redundancy" (a duplication of effort or production of compounds with no specific function). Jones and Firn (1991) proposed a "screening hypothesis" in which mechanisms exist to produce and retain a large number of compounds, many of which may have low potency but at least one or a few of which are molecules of high potency that will provide a defensive role. In our research, another example of extreme analog synergism similar to that of *S. humilis* is found in *Ruptiliocarpon caracolito*, a species that produces a remarkable number of unusual triterpene analogs (MacKinnon *et al.*, 1997c). This species was originally thought to belong to the Meliaceae, based on similarities in wood structure, but was more recently identified as the only neotropical member of the family Lepidobotryaceae, which contains two other species in Africa. Extracts of the wood, bark, and leaves were the most active insect growth reducers tested in a survey of *Rutales* species assayed against corn borer and cutworm. From this tree, our group has isolated more than 14 triterpenoids with a unique spiro C–D structure. Published structures are shown in Fig. 1.5. Because of the large number of analogs and derivatives, this is a useful species to test the Jones and Firn (1991) screening hypothesis, which is that these compounds are being produced in large numbers because a few may be highly effective insect deterrents. We isolated and screened 12 of these compounds at 5 and 50 ppm in lepidopteran diets, concentrations well below their naturally occurring levels. Assessed over the larval period, the results (Fig. 1.5) showed that most of the compounds increased larval mortality and significantly reduced the growth of survivors (not shown). They also caused delays in development and mortality at pupal and adult stages. Such a high level of activity suggests screening for a rare effective defense molecule is not occurring.

An alternative hypothesis for this phytochemical redundancy is analog synergism (Berenbaum, 1986). We isolated six of the compounds in sufficient quantity for studies of their activity alone and in mixtures. When tested alone, not all of the compounds reduced growth significantly and the calculated mean effect of the six compounds was to reduce growth of larvae by 50% compared with controls. In the mixture, each of the six individually tested compounds was added at one-sixth of the individual compounds. The growth of insects fed the mix was reduced significantly (80%; $P = 0.01$) suggesting synergism between the analogs (data not shown).

We were able to test the analog synergism in another setting where the analogs could be produced in sufficient quantity by synthesis. The wild pepper, *Piper tuberculatum*, was the most active of 16 pepper plants from Costa Rica assessed in our study (Bernard *et al.*, 1995). Four insecticidal piperamides from *P. tuberculatum* were produced by synthesis (Scott *et al.*, 2002) and the lethal concentrations of the compounds were assessed alone and in binary, tertiary and quaternary mixtures. Although binary mixtures were no more toxic than individual compounds, toxicity increased with three and four compounds in the mixture, while keeping the total

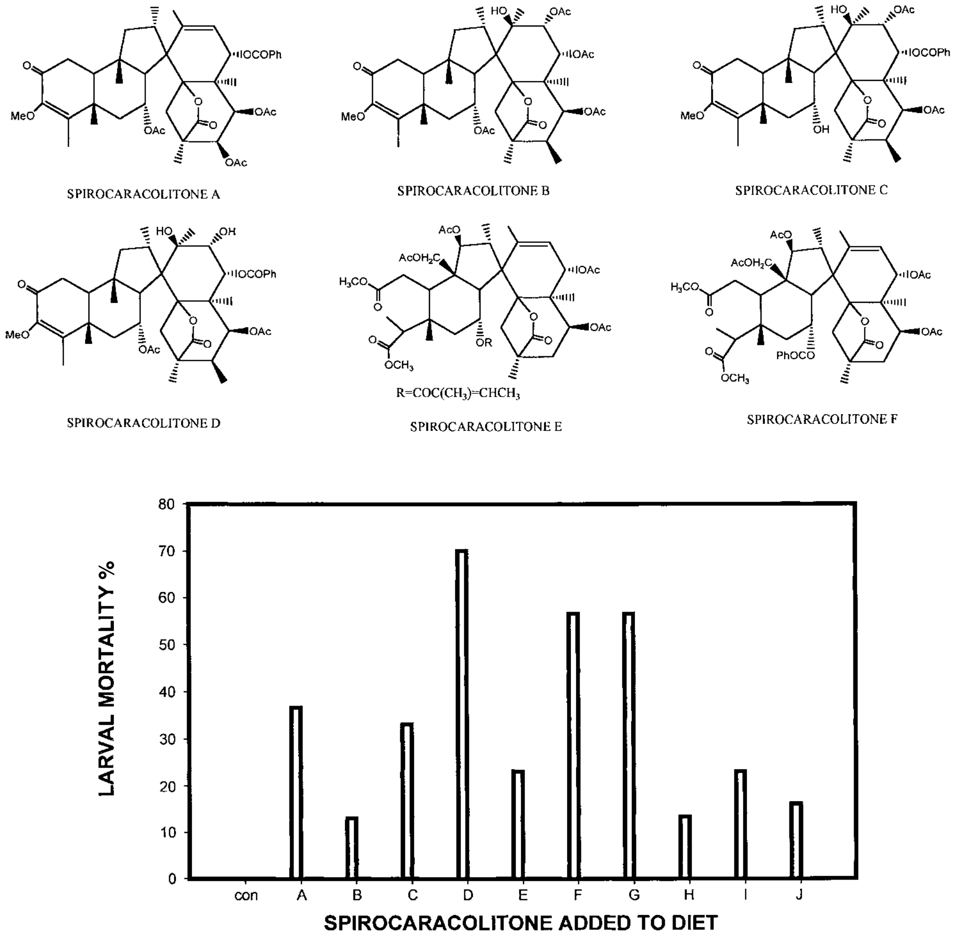


Fig. 1.5. Six of the more than 12 spirocaracolitones isolated from the neotropical tree *Ruptiliocarpon caracolito*. The effect of spirocaracolitones A–J on European corn borer larval mortality is illustrated when added to diets at 50 ppm.

the same. These results provide some support for the analog synergism hypothesis (Fig. 1.6).

Other benefits of large numbers of analogs to plants may include slower evolution of tolerance or lower rates of metabolism of mixtures compared with that of single compounds in herbivorous insects. Feng and Isman (1995) investigated the possibility of adaptation in herbivores by repeated selection of peach aphid colonies with either pure azadirachtin or neem seed extracts containing a large number of limonoids. The colonies treated with azadirachtin soon showed evidence of tolerance of this pure compound, whereas no evidence of tolerance was