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Title: Sustainable management of subterranean insect pests in peanut

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Introduction

The loss of soil insecticides labeled for southern corn rootworm has reached the point where rescue applications to control outbreaks are no longer possible. Only Lorsban retains a label and this is for prophylaxis, useful only in situations where rootworm infestations are a consistent problem. No proven means of control are available and no resistant variety of peanut is adapted to Texas growing conditions for rootworm management. A sustainable method whereby this insect could be consistently managed across the state would reduce not only the damage to peanut, but stress on growers who must worry whether their field will be infested.

The lesser cornstalk borer is a statewide problem for peanut growers. A key pest of peanut, its subterranean habits make it difficult to find until an outbreak occurs. The sporadic nature of the infestation in many fields make the need for scouting important, but this is not always practical for growers if an IPM program or dedicated consultants do not operate in their area. Although more pesticides are labeled for remedial action with this pest than with the rootworm, these may be expensive and must be applied before damage occurs. This implies good scouting practices and timely information on infestations.

Recent research in plant biology has found a broad class of plant-produced compounds collectively known as cysteine proteinase inhibitors. Cysteine proteinases are a group of enzymes produced by insects that help them to digest proteins from their food supply. One way in which plants fight back is by producing inhibitors to these proteins that cause the insect to starve. A second group of compounds known as serine proteinase inhibitors also known to inhibit digestion in insects and may in fact work better on moth larvae, such as the lesser cornstalk borer.

Many species of plants produce these inhibitors, including legumes. An insect-active cysteine proteinase inhibitor gene from soybean has been identified and isolated and will produce the compound in cell culture. This gene and cell culture are available to us for experimentation. Serine proteinase inhibitors have also been isolated from legumes and are available commercially from chemical supply companies. Although not yet identified from *Arachis*, these inhibitors almost certainly occur in them. In natural settings, the inhibitors may be constitutive (present in the cells as a matter of course) and used for storage. They may also be inducible, produced when the cells are damaged, and then used as a feeding deterrent or even as an insecticide. The inducibility factor is important in that it means the plants do not produce the inhibitors until they need them. No extra energy is expended and the plant roots do not shed large quantities of toxins into the soil environment, as occurs in plants engineered to express *Bacillus thuringiensis* toxins, such as Bt corn.

Published reports indicate that some plant pests including southern corn rootworm

larvae, the damaging stage in peanut, are susceptible to proteinase inhibitors. Larvae of the western corn rootworm are also susceptible to these inhibitors, as are caterpillars of some moth species. Mites and nematodes have also been documented as susceptible to inhibition by these compounds.

Methods and Materials

Insects: Southern corn rootworm larvae were obtained commercially as eggs and reared on diet obtained from BioServ®. Lesser cornstalk borer larvae were collected from field-infested peanut, reared to adult and larvae obtained as the F_1 or F_2 generation from this stock. Larvae were reared on a commercial diet formulation obtained from BioServ®.

Rootworm Bioassay: Bioassays of rootworm larvae were conducted against the CPI obtained from soybean. A control and three dose levels – 0.25 mg/ml, 0.75 mg/ml and 1.5 mg/ml of CPI per diet were used. CPI was added to cooled, but still liquid diet and blended before feeding to the larvae. Four larvae per dose and four replicates were tested to determine the effect over a period of 7 days. Weights and mortality relative to the control were then compared.

LCB Bioassay: Commercial diet was prepared and cooled to just above the melting point. From this, 50ul were transferred to each of 10 wells in an ELISA plate for the control group. Serine protease inhibitor (Type II-L: Lima Bean Trypsin Inhibitor, Sigma) was added to the remaining diet to a final concentration of 1mg/ml. Again, 50ul plugs of the diet were then transferred to the ELISA plate in 2 groups of 10 for the experimental group. The diet was allowed to cool to room temperature. First instar LCB caterpillars were then transferred to the diet plugs and cotton was used to plug the wells. The experimental unit was placed in a humidity chamber (to prevent desiccation of the diet) inside a 31°C incubator.

Native inhibitors in peanut: Peanut varieties Coan, Florunner and Tamrun 96 were germinated and grown under greenhouse conditions. Seedlings were frozen and used in the study. DNA and cDNA from the seedlings was sequenced and the results compared to known cysteine proteinase inhibitors to identify the presence of the gene before attempting to isolate and clone it.

Results

Insect bioassays: The southern corn rootworm appear to be susceptible to the cysteine proteinase inhibitor from soybean. Figure 1 shows increased mortality with an increasing amount of inhibitor present in the diet. The assay for lesser cornstalk borer was not usable due to problems with the insect diet and must be repeated. Since this insect is not commercially available and was not sustainable on the diet, and new formulation is needed and new insects from the next field season used to complete the study.

Native inhibitors: A piece of DNA recovered from Tamrun-96 has been found to be a very close match for known sequences of cysteine proteinase inhibitors. The current

sequence is incomplete, but is enough to demonstrate the presence of such genes occurring in the peanut genome.

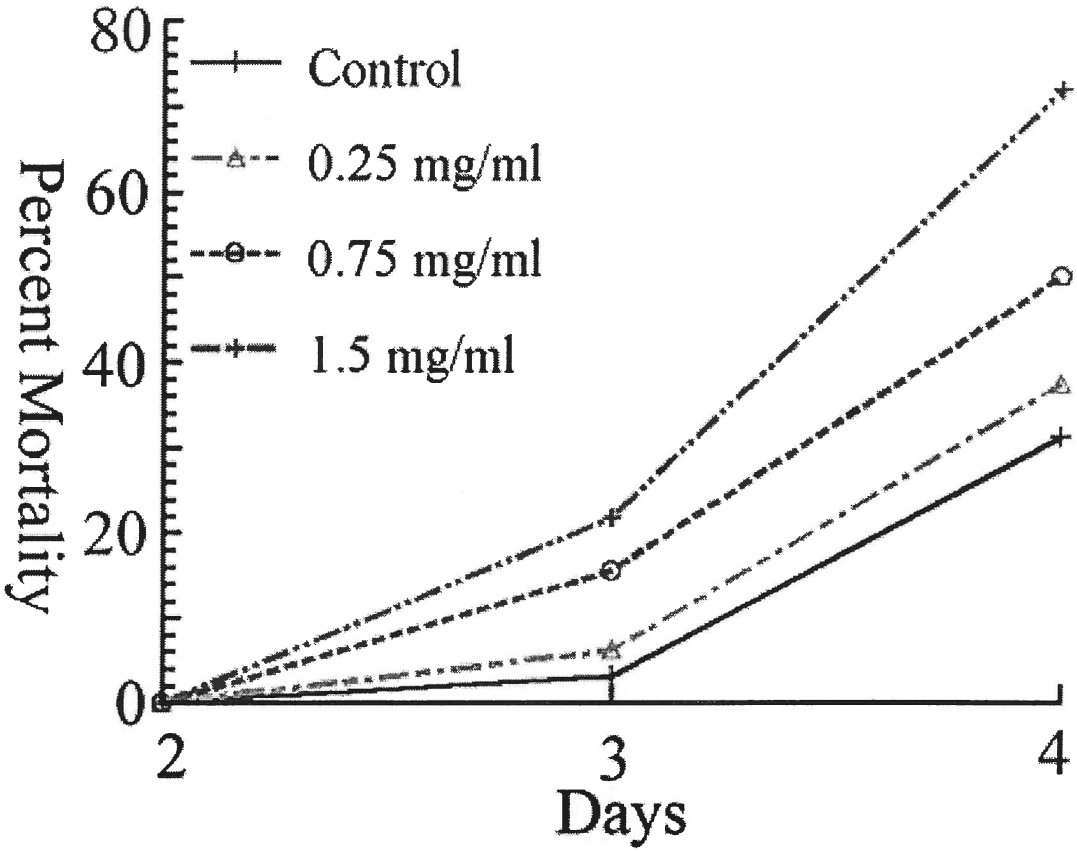

Figure 1. Percent mortality of southern corn rootworm plotted over days 2-4 of the bioassay.

Discussion

Bioassay systems have now been developed for both the southern corn rootworm and the lesser cornstalk borer for testing the effects of proteinase inhibitors. Further data, as yet unanalyzed, have been collected for southern corn rootworm and support the information in figure 1. Continued testing on the LCB must wait until the 2002 field season for the appearance of fresh moths. In the interim, the difficulties with the artificial diet have now been solved and a large light trap has been installed near a peanut field so that adult moths can be captured during the field season and used to provide eggs for the assays, even should the insect diet not be able to sustain a colony for longer terms. Sequence data from the peanut genome indicate the presence of at least one endogenous proteinase. Although not yet isolated, work is continuing and the known portion of the

sequence will assist in excising and cloning of the gene. Expressed proteinase from the cloned gene will then be usable in the bioassay systems and may be then be tested for insecticidal activity. Research on all portions of this study is continuing and additional information will be available by the end of the current field season.