LEPTON HTK: A DIAGNOSTIC TEST KIT TO IMPROVE COTTON INSECT CONTROL

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Abstract

Abbott Laboratories, in cooperation with the CSIRO in Australia, has previously developed a Heliothis field test kit (LepTon Test) that is capable of differentiating between two species of Heliothis (*H. armigera* and *H. punctigera)* that occur in Australia and are considered the two primary pests occurring on cotton. *H. armigera* exhibits high levels of resistance to pyre-throid insecticides, while *H. punctigera* does not. Some parts of the North American cotton industry face similar problems to the Australian cotton industry, in that Heliothis/Helicoverpa species show different levels of resistance to pyrethroids and other insecticides.

The application of the Australian LepTon Test technology to United States cotton production will require the development of a similar field test kit (LepTon HTK) that can accurately differentiate between the two key lepidopterous species, *Helicoverpa zea* (*Hz,* cotton bollworm) and *Heliothis virescens* (*Hv*, tobacco budworm).

Abbott Laboratories has entered into an agreement with Cotton Incorporated to develop the LepTon HTK for the U.S. cotton market. Abbott, in coop-eration with the CSIRO, will be responsible for generating monoclonal antibodies capable of recognizing and differentiating between these two key lepidopterous species, incorporating them into a prototype test kit, performing laboratory and field evaluations, validating the test kit for accuracy, producing the kit, and finally marketing the LepTon kit for use in the United States.

The isolation of the lipophorin antigen and the production of antibodies was initiated in September, 1995 with an anticipated market introduction of the LepTon HTK during the 1997 cotton field season.

Introduction

Hz and *Hv* are generally regarded as the most important insect pests of cotton in the United States. The cotton entomologist and industry recognize that *Hz* is susceptible to conventional chemical insecticides, including pyrethroids, while *Hv* has developed significant resistance to these products, and pyre-throids in particular. Therefore, use of these products is restricted to specific times during the production season to avoid excessive selection for resistance.

It is recognized that adequate control with conventional insecticides is highly dependent on species composition and accurate timing of insecticide applications to egg or early larval stages. However, it is currently impossible to distinguish between *Hv* and *Hz* in the egg and early larval stages. The development of the LepTon HTK as a tool to quickly and easily determine species composition based on field samples of eggs and/or neonate larvae would improve insecticide selection decisions and benefit resistance management programs throughout the cotton growing areas where both species occur on a seasonal basis.

Based on discussions with key cotton growers, consultants, and extension entomologists in the U.S., we have concluded that the primary utilization of the LepTon HTK will occur during the transition period when *Hz* populations diminish and are replaced by *Hv* populations. This kit will be utilized as a monitoring or scouting tool by the U.S. cotton growers, consultants, and university cotton research and extension specialists. The LepTon HTK will enable these cotton specialists to almost instantly identify *Hz* from *Hv* populations in the field. This capability will result in more effective utiliza-tion of conventional cotton insecticides utilized for control of *Hz* and *Hv* by selecting insecticides which have not demonstrated resistance development in *Hv* populations.

Product Development Plan

The format and chemistry of the kit has been identified and utilizes an antigen known as lipophorin and specific monoclonal antibodies to discriminate between species. The elements of the test kit are included in Australian, U.S., and Canadian patent applications. The antibody is crucial because its characteristics determine the range of applications, ease-of use, and reliability of the kit. The antibody used will recognize a soluble antigen that is present in all life stages of the insect.

The development of an antibody suitable for differentiation of *Hv* and *Hz* was initiated in September, 1995. The entire project, from initiation to comple-tion, will require approximately 18-24 months. It will take from 6-12 months to obtain a monoclonal antibody with the desired characteristics. Up to 6 months is required to generate optimal immunity in mice and, based on prior experience, perform a number of fusions to achieve a suitable antibody.

Preliminary characterization, scale-up, conjugation and testing will require an additional 6 months. We plan to evaluate a prototype antibody test kit in the 1996 cotton growing season and, if successful, commercialize the LepTon HTK in the 1997 cotton growing season.

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LepTon HTK test membranes have already been prepared for preliminary evaluation of the antibody. Eggs and larvae of both species were collected from both lab and field populations and squashed onto membranes to determine initial antibody and membrane sensitivity and reliability.

Following laboratory testing, the prototype kit will be evaluated in the 1996 field season for sensitivity, precision, and reliability in the differentiation of *Hz* and *Hv* populations. This will be completed with cooperating growers and consultants in key cotton growing states (MS, AR, LA, TX, AL, GA, FL). Field populations will be monitored to determine species composition.

If these field trials are successful, a full scale-up manufacture of the mono-clonal antibody will commence in 1996. Manufacturing of the LepTon HTK will be conducted under Abbott's quality assurance standards and specifica-tions. Abbott will establish laboratory standard operation procedures (GLP's) and manufacturing guidelines (Basic Operating Procedures, BOP's) to insure uniform production of the membranes, reagents, and kit components.

It is anticipated that LepTon HTK will be available to cotton growers during the 1997 cotton growing season.

Results

A number of problems in the development of the LepTon Test in Australia were overcome, including interference from endogenous larval enzymes and demonstrating the accuracy of the kit under field conditions (Trowell et al., 1993). The LepTon Test in Australia has proved as accurate as possible within the limitations of sampling error. Testing of eggs and larvae obtained from the field in Australia indicated a false positive rate of less than 5% and a false negative rate of less than 2% which has proven acceptable from an operational basis.

This new technology has been widely used and has already had a significant impact on pest management in the Australian cotton industry (2). The use and implementation of the kit has been reviewed by Trowell et al., 1992, Forrester, 1993 and Forrester et al., 1993.

Discussion/Conclusions

The synthetic pyrethroid insecticides play a critical role in controlling *Hv* and *Hz*. The cotton entomologist and industry recognize that *Hz* is susceptible to conventional chemical insecticides, including the synthetic pyrethroids, while *Hv* has developed significant resistance to these products. Therefore their use is restricted to key times during the production season to avoid excessive selection for resistance.

Obtaining adequate control with pyrethroids is dependent on species composition and accurate timing of insecticide applications to egg or early larval stages. The development of the LepTon HTK as a tool to quickly and easily determine species composition based on field samples of eggs and/or neonate larvae would improve insecticide selection decisions and benefit resistance management programs.

A similar approach to reduce the development of insecticide resistance in Australian cotton has been effectively employed and utilizes the LepTon Test as a key tool in the insecticide selection process (4). The underlying strategy has been to avoid using insecticides that are susceptible or prone to more rapid resistance development on populations dominated by *Ha*. Some of the economic benefits that have been identified during the test kit introduction in Australia include lower costs and improved efficacy for insect control due to less spray failures, fewer resprays, identification of the least expensive spray options, and use of fewer high cost tank mixtures (3).

As a result of the reliance by the cotton grower on synthetic pyrethroids and organophosphates to control both pests and based on the difference in response of both pests to these pesticides, there appears to be a window of application for the LepTon technology to differentiate populations in the field. This window is directed to the transition period when *Hz* populations diminish and are replaced by *Hv* populations. Historically, *Hz* is the predominant early to mid-season pest on cotton in the Mid-South and is generally replaced by *Hv* during the mid-season cotton growth cycle. This transition usually takes place at the end of June and during July of the growing season. Once the transition is completed, *Hz* generally remains as the dominant pest for the remainder of the cotton growing season.

Based on the discussions with key cotton growers, consultants, and extension entomologists in the U.S., we have concluded that the primary utilization of the LepTon HTK will occur during the transition period. Prior to the transition, cotton insect control relies on biological and conventional chemical pesticides which are effective against *Hz*. After the transition period, insect control continues to rely on conventional chemical pesticides, including pyrethroids, in non-resistant areas and on non-pyrethroid products in SP-resistant areas.

The *Hz* and *Hv* populations can be readily distinguished in the adult phase by the trained observer. These results are currently provided to the grower via printed newsletters and electronic mail services. However, the individual grower must assume that the area wide trap catches are directly correlated to individual field populations in that area. This is not always the case and many SP insecticide application failures are due to the misassumption that *Hz* populations are present based on trap catch reports, while in fact, the

population has shifted to a high proportion of *Hv* in specific fields.

Significant portions of the U.S. planted cotton acreage are not concerned with resistant populations of *Hv*. These areas include the Far West (CA, AZ) and the Southeast (NC, SC, GA, AL) regions where few, if any, complaints related to SP resistance have surfaced or been documented. As a result, the primary utilization of the LepTon HTK will be in the mid-South (MS, LA, AR, coastal TX) where SP resistance has been documented and programs are in place to respond to the development of SP resistance in *Hv* populations.

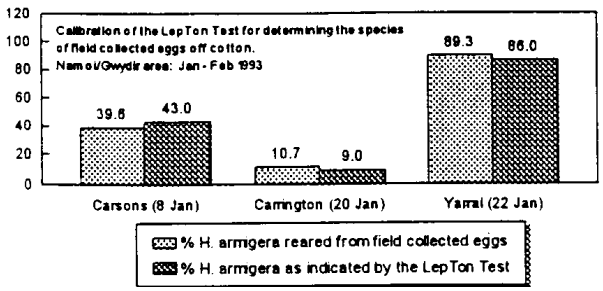

Figure 1.

Acknowledgments

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