

LEPTON HTK
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Abstract

LepTon HTK is a diagnostic test kit currently being developed for differentiating between the eggs of tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa zea*). The kit is the product of a joint collaboration between the CSIRO (Australia), Cotton Incorporated, and Abbott Laboratories. Development of the specific antibody was initiated in September, 1995, with an anticipated market introduction during the 1998 cotton field season. Procedures for using the test kit have been identified, along with key recommendations for successful application to egg differentiation. The kit will enable the U.S. cotton grower and consultant to accurately identify insect populations and to select the most effective insecticide for control.

Introduction

Tobacco budworm, *H. virescens* and cotton bollworm, *H. zea* are generally regarded as the most important insect pests of cotton in the United States. It is also recognized that acceptable control with conventional insecticides is highly dependent on species composition and accurate timing of insecticide applications to egg or early larval stages. However, until now, it was impossible to distinguish between tobacco budworm and cotton bollworm 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 will improve insecticide selection decisions and benefit resistance management programs throughout the cotton growing areas where both tobacco budworm and cotton bollworm occur on a seasonal basis. The test is the first diagnostic tool to offer improved insect management and fits the current direction of cotton biotechnology.

Current Project Status

During 1996, a large number of antibodies were identified that offer the potential for selective identification of tobacco budworm and cotton bollworm eggs and/or neonate larvae. However, subsequent evaluation of the best candidates on laboratory and field samples of tobacco budworm and cotton bollworm eggs and larvae did not identify an antibody that would provide commercially acceptable species identification. Although the best antibodies can be utilized on a laboratory basis, the LepTon HTK is targeted for uses as a commercial field test kit, and the results can be

affected by excess moisture and high temperatures. As a result, some antibody will be available for further evaluation during the 1997 field season, but full commercial introduction is not planned until the 1998 U.S. cotton season.

LepTon HTK Format

Eggs and/or neonate larvae of tobacco budworm and cotton bollworm are first squashed onto a cellulose membrane. The test kit utilizes a series of reagents, including a blocking agent (protein), a specified monoclonal antibody sensitive to only cotton bollworm, and a color developer that produces a purple color only in response to eggs or larvae of cotton bollworm. The cellulose membrane acts as a substrate or support for the egg protein and also as the background for scoring the purple color response.

Directions for Use

The preference is to develop the membranes in a controlled environment (office or laboratory), and not in the field, to improve overall processing efficiency and to eliminate moisture and high temperature as potential response variables. The first step is to apply a blocking solution to the membrane which binds to all unrelated protein and requires 1 minute to react. The antibody conjugate is applied in the second step for a 5 minute exposure period. The membrane is then thoroughly washed (3 times) during Step 3. The fourth step involves the application of the color developer for a 1-2 minute period. The developed membrane is finally blotted dry in Step 5 and the light purple color response is read immediately. The total elapsed time for membrane development and readings is 12-13 minutes. Maximum efficiency can be achieved by developing multiple membranes in consecutive order. This can be most effectively completed in an office or laboratory environment.

Keys to Success

The LepTon HTK test kit is comprised of reagents and 5 membranes. Of these reagents, only the LepTon antibody pack must be kept stored at cold temperatures, preferably in a refrigerator at 40°F (4°C). The LepTon cellulose membranes should always be stored in dry location. Direct exposure of the developed membrane to sunlight should always be avoided to reduce the degradation of the purple color response.

The developed membranes should be read immediately. A pencil can be utilized to "check off" all the cotton bollworm-positive light purple spots on the membrane. Once the membrane has been "checked", it should never be "re-read" at a later date. The "checked" membranes can be stored for future reference in a dry condition and/or the results can be recorded as a numerical score.

Patent/Trademark Status

A patent has been issued for the LepTon technology in Australia. The U.S. patent application has been filed and is pending approval in the immediate future. LepTon is a registered trademark of Abbott Laboratories.

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References

1. Abbott LepTon Test, Guidelines for Use, Making the Right Decision. Technical Bulletin.
2. Abbott LepTon Test, Instruction Booklet.