LEPTON™ HTK - A HELIOTHINE DIAGNOSTIC TEST KIT: AN UPDATE

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Abstract

LepTon™ HTK is a new, patented Heliothine diagnostic test kit co-developed by CSIRO, Australia; Cotton Incorporated, USA; and Abbott Laboratories Inc., USA. It differentiates between the tobacco budworm and cotton bollworm at egg and larval stages. LepTon™ HTK utilizes species-specific monoclonal antibodies to distinguish between the two species. It is easy to use, accurate and results can be obtained in 30 minutes. The test kit can be used in both conventional cotton and Bt cotton.

LepTon™ HTK will be commercially available during the 1998 field season. It will be a bollworm positive test kit (Light purple color = bollworm; No color change = Tobacco budworm). If used correctly, LepTon™ HTK has the potential to reduce significantly the overall control cost of bollworm and budworm; therefore, maximizing profitability for cotton growers.

Introduction

Correct identification of insect species is one of the most important factors in a successful pest management program. In cotton, management of tobacco budworm (*Heliothis virescens*) is especially challenging because of the co-occurrence of cotton bollworm (*Helicoverpa zea*) populations throughout the entire growing season. The tobacco budworm and the cotton bollworm are known to have different susceptibilities to insecticides, as well as to Bt cotton. Hence, in order to effectively manage their field populations, it is important to be able to distinguish these two close relatives as early as possible; preferably at the egg stage and during early larval instars. Although subtle morphological differences between eggs of the two species have been described (Bernhardt & Phillips 1985), they have not proven practical for wide-scale use in identification (Greenstone 1995).

Here we report the development of the LepTon™ HTK diagnostic test kit. This a new, patented technology co-developed by CSIRO, Australia; Cotton Incorporated, USA; and Abbott Laboratories Inc., USA. It differentiates between the tobacco budworm and cotton bollworm at egg and larval stages. It is easy to use, accurate and the results can be obtained in 30 minutes.

1997 Field Program

During the 1997 field season, a LepTon HTK prototype test kit was introduced to gain experience in using the test kit under actual field conditions. However, the prototype test kit had several limitations. The limitations were:

1) The prototype test kit potentially had about 20-25% errors.
2) The bollworm positive antibody (AB), ABz44, had to be used at low strength to minimize these errors, and this made the positive signals weak and difficult to read.
3) ABz44 was good for differentiating at the egg stage but not the larval stages.

Despite these apparently severe limitations, the overall responses to the LepTon HTK prototype test kit from the cooperators were very positive. Many of the cooperators commented that the LepTon HTK will be a very valuable tool to help making Heliothine management decisions. Against our advice, a few cotton consultants even used the prototype test kit to help make spray decisions and experienced positive outcomes. If used correctly, LepTon HTK has the potential to reduce significantly the overall control cost of bollworm and budworm in both conventional and Bt cotton. Therefore, it can help to maximize profitability for cotton growers.

Like any new technology with positive benefits, the LepTon HTK has its own challenges and limitations. At times, some of the cooperators experienced difficulties reading and interpreting the light purple positive signals in the prototype test kit. Another challenge was the time commitment expectation to use the test kit, especially when it came to squashing eggs onto the membrane. The third challenge was to collect a sample size of 100 eggs from a management unit or farm to fill 100 spots on the membrane.

The difficulties in reading the positive signals were expected for the prototype antibody and will be significantly improved with the introduction of better antibodies. In terms of the time required to squash eggs onto a membrane, many cooperators commented that it will be easier and require less time as they gain experience.

Regarding the sample size of 100 insects per membrane, this is an area which will require judgment and depend on the field knowledge and experience of the consultant/grower. The reason a sample size of 100 is recommended is that statistical theory predicts the margin of error based on 95% confidence limits (i.e. the margin of error will be valid 19 times out of 20). For example, if the LepTon HTK indicated that 50% of the sample from a given cotton management unit or farm were bollworm, then statistically:

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1) Bases on a sample size of 100, the true percent of bollworm population in that farm can fall between 40% to 60%.
2) However, with a sample size of 30, the true percent of bollworm population can fall between 32% and 68%.
3) And with a sample size of 10, the true bollworm population may fall between 15% and 84%.

Hence, as the sample size decreases, the margin of error increases. It is important to note that the above example assumes that a representative random sample of insects was collected from the cotton management unit or farm in question.

In reality, the insect sample size required to perform the LepTon HTK test for a given cotton management unit or farm will depend on:

1) The acceptable risk (knowing the margin of error) which a consultant/grower can tolerate, and
2) A consultant/grower's experience and knowledge of the historical distribution of bollworm and budworm population in the cotton field which is being managed.

Obviously, this is a challenging issue. Over time and with experience of using the LepTon HTK under field conditions, the optimal sample size may vary among cotton management units or farm and from one cotton consultant/grower to another.

Current Project Status

A series of new bollworm-specific antibodies have been identified over the past 12 months. Among the newly identified bollworm-positive antibodies, ABz9 and ABz15 were found to be the most promising for the commercial LepTon HTK. Both these antibodies are very different from the old ABz44 which was used in the 1997 prototype. ABz9 and ABz15 are highly specific and have an accuracy of greater than 95% for eggs. They are good for both egg and larval stages, and the positive signals are significantly stronger and clearer than the old ABz44, making visual interpretation a simpler process.

Despite the improvements, both of the new antibodies have individual inherent strengths and weaknesses which made it challenging to decide the best one for commercial release. ABz9 and ABz15 both react positively to cotton bollworm and do not react to tobacco budworm. The major differences between the two antibodies are:

1) ABz9 also reacts positively to armyworm and looper whereas ABz15 gives no reaction with either armyworm or looper.
2) ABz9 provides $\geq$ 95% accuracy in differentiating both eggs and larvae between bollworm and tobacco budworm. The accuracy of ABz15 in differentiating between bollworm and tobacco budworm was $\geq$ 95% for eggs but only about 90% for larvae.
3) ABz9 provides excellent positive signals with eggs and good positive signals with larvae. ABz15 only provides excellent positive signals with eggs. The positive signals for larvae were fair to poor at times.
4) ABz9 required a higher concentration of conjugate than ABz15.
5) ABz9 is more difficult and costly to mass produce than ABz15.

After careful evaluation, it was determined that ABz9 was the best antibody for the 1998 commercial test kit. The selection of ABz9 was mainly based on the technical requirements for a good commercial test kit. This choice was made despite ABz9 requiring a higher concentration of conjugate to work well and the greater difficulty and cost of mass production.

To minimize the problems and costs associated with large scale production of ABz9, it was decided that the only practical option currently available is re-use of ABz9 conjugates; same as the Australian LepTon test kit. Hence, in the 1998 commercial test kit, a single bottle of ABz9 conjugate (Reagent 2) will be provided to develop five membranes. The conjugate will be saved after use (i.e. poured back into the same Reagent 2 bottle) and re-used four times. Research will continue in order to improve the antibody and its production for future commercial test kit.

1998 Commercial LepTon™ HTK

The 1998 test kit will be a bollworm-positive test kit. Following development of the membrane with a series of reagents, spots on the membrane squashed with eggs and larvae of bollworm develop into a light purple color, whereas no color change is observed on spots squashed with eggs and larvae of tobacco budworm (Light purple color = bollworm; No color change = tobacco budworm).

Each LepTon HTK can be used to perform 5 separate tests. A test kit will be packaged in a styrofoam container with dimensions of 10" high X 6.5" wide X 9.5" long. It will weigh about 1 pound. The packaging is designed to fit into a refrigerator (NOT FREEZER) since it requires cold storage. The contents of the commercial LepTon HTK are:

a) 5 Nitrocellulose membranes
b) 5 bottles of Reagent 1 (Blocking Reagent); each bottle for developing a membrane
c) 1 bottle of Reagent 2 (Antibody Conjugate); to be used 5 times. Reagent 2 is very expensive and it is very important to **SAVE** Reagent 2

after each use. **ONLY** discard after Reagent 2 has been used to develop all 5 membranes

d) 5 bottles of Reagent 3 (Wash Concentrate); each bottle for developing a membrane
e) 5 bottles of Reagent 4 (Substrate); each bottle for developing a membrane
f) 1 Dropper bottle Reagent 5 (Color Developer); enough for 5 uses
g) An instruction booklet.

Some of the reagents are heat-sensitive and require cold storage (4ºC or 40ºF) but they must not be frozen. Hence, the test kit must be kept in a refrigerator when it is not use. The nitrocellulose membrane is sensitive to moisture and should be stored under dry conditions.

Critical Factors In Using The Test Kit

Use the test kit when the Heliothine pressure is at or above the economic threshold level and when a control decision is needed. It is recommended that eggs and larvae for use in the test be collected during routine scouting. It is important to collect a representative random sample of insects from the field. If there is more than one egg on a given leaf or one larva in a given leaf terminal, use only one of the eggs or larvae. This is because the eggs or larvae may come from the same moth.

To obtain an accurate estimate of the percent bollworm and tobacco budworm on a given farm, only eggs and larvae of bollworm and tobacco budworm should be used to perform the test. Using eggs and larvae from other lepidopteran species will reduce the accuracy of the test.

When writing information (such as location and date) onto the nitrocellulose membrane prior to processing with various reagents, use ONLY pencil. Ink from a pen may bleed onto the membrane and interfere with the readability of the light purple positive signals.

Before using the reagents to develop a membrane, it is very important to allow all of them to warm up to room temperature. If needed, a tepid water bath may be used to speed up this process but if the bath is warmer than tepid, the test kit may be permanently inactively.

When developing a membrane, it is important to set aside 30 minutes of uninterrupted time. It is recommended the test be performed in a well-lit, comfortable room such as an office or a kitchen.

After processing with the reagents, the membrane must be read immediately. This is because the light purple positive signals will fade over time. Light purple spots indicate bollworm whereas not color change will be observed on spots squashed with tobacco budworm. The intensity of the purple spot can vary, hence any light purple color (however faint) should be counted as positive. A pencil or a pen can be used to check the positive spots as the membrane is read. Record the number of positive spots at the bottom right-hand corner of the membrane.

The membrane may be kept for future reference but do not attempt to re-read the membrane. This is because the light purple positive signals will fade over time and the most accurate reading is obtained immediately after completing the test.

It is important to return the "SAVE" Reagent 2 (Antibody Conjugate) and unused reagents back into a refrigerator after completion of the test.

Benefits Of Using The Lepton™ HTK

The LepTon HTK determine if an egg or larvae is a bollworm or tobacco budworm. Most importantly, it gives an accurate estimate of the percent bollworm and tobacco budworm present in a given cotton management unit or farm. This information will help to determine appropriate control measures, if needed, throughout the season.

The LepTon HTK can be used to monitor the Heliothine complex in both conventional and Bt cotton. There are several benefits of using the test kit. It will help to minimize the chances of control failure associated with the mis-identification of Heliothine species. It will help the consultant and/or grower to select appropriate sprays targeted specifically to bollworm or tobacco budworm, and avoid incorrect sprays. re-sprays and crop damage.

The following hypothetical example illustrates the potential financial benefits of the LepTon HTK test kit. Assume that a grower uses a LepTon HTK to monitor the Heliothine complex in a 200 acre cotton farm (either Bt or non-Bt cotton) for the entire growing season. If the LepTon HTK helps the grower to avoid making the wrong spray decision only once during a season, it can save the grower as much as $4000.00 (assuming a total spraying cost of $10.00/acre). Hence, when used correctly, LepTon HTK can potentially help the cotton grower to save significantly in total Heliothine control costs and maximize profitability.

Conclusion

The LepTon HTK will be a valuable new tool for management of Heliothine species in cotton. It will reduce overall Heliothine control costs and assist growers to maximize their profitability. However, as with many other technological tools, users of the test kit will have a significant influence over its reliability and accuracy. Just like a computer, LepTon HTK will only perform at a high level of accuracy with a quality input. Therefore, when using the test kit, it will be vital to obtain a representative random sample of insects from each field and to follow the test kit user guidelines closely.

Acknowledgments

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References

Bernhardt, J. L., and J. R. Phillips. 1985. Identification of eggs of the bollworm, *Heliothis zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F). Southwestern Entomol. 10, 236-238.

Greenstone, M. H. 1995. Bollworm or budworm? Squashblot immunoassay distinguishes eggs of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae). J. Econ. Entomol. 88, 213-218.