DEVELOPMENT OF HELIOTHIS ANTIBODIES FOR THE LEPTON™ HTK INSECT DIAGNOSTIC TEST KIT

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Abstract

Species specific monoclonal antibodies for cotton bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*) were identified. Specific antibodies that identified the eggs of cotton bollworm were used to develop a prototype diagnostic test kit for heliothis: the LepTon™ HTK. The LepTon™ HTK test kit will be used to differentiate between eggs of cotton bollworm and tobacco budworm, which are the two major pests in U.S. cotton. We presented data on the accuracy of the current LepTon™ HTK prototype and we also discuss plans for commercial development of the test kit.

Introduction

Correct identification of the insect species prior to application of control measures is important for the successful control of the insect pest. Inaccurate identification of species may result in control failure, wasting time, money and effort. In many cases however, it is very difficult to identify the species accurately when they are present as eggs and early larval instars. This is particularly a problem with closely related insect species, whose eggs and early instar larvae may appear visually identical, even under the microscope.

Between 1990 and 1992, Trowell et al. developed a patented diagnostic test kit; The LepTon™ test kit, for differentiating the eggs and larvae of *Heliothis armigera* and *Heliothis punctigera* (Trowell et al., 1993). These two species are the major pests in Australian cotton production. The LepTon™ test kit uses species-specific monoclonal antibodies to differentiate the immature stages of the two insects in 10 minutes. Rapid identification of the species composition of eggs and/or larvae in a cotton field gave the farmer or cotton consultant the information needed to make an optimal management decision, almost instantly.

In 1993, Abbott laboratories commercialized the LepTon™ test Kit for the Australian cotton market (Trowell et al., 1994). Since then, the LepTon™ Test Kit has played an important role in the Australian Insecticide Resistance Management Strategy and has significantly increased the cost-effectiveness of controlling *Heliothis armigera* and *Heliothis punctigera* in the Australian cotton region.

In the U.S., the tobacco budworm (*Heliothis virescens*) and the cotton bollworm (*Helicoverpa zea*) are the two major pests in the cotton belt. The eggs and early larval instar of tobacco budworm and cotton bollworm look very similar, so it is very difficult to differentiate between these two species at these early life stages. However, differential insecticide resistance and susceptibility to transgenic cotton make it important to be able to distinguish them.

In light of the success of the LepTon™ Test Kit in Australia, Cotton Incorporated (USA), Abbott Laboratories (USA) and CSIRO (Australia) are collaborating in a project to develop a LepTon™ Test Kit for the U.S. Similar to the Australian version, it will be known as LepTon™ HTK and will be used for differentiating both eggs and larvae between tobacco budworm and cotton bollworm.

The main goal was to develop a commercial test kit for U.S. cotton in time for the 1998 growing season, with commercial release one year earlier if at all possible. The latter was an extremely aggressive time line since we contemplated compressing a three year project into 18 months. Here, we report on the development status of the LepTon™ HTK and some of the results and challenges from our research and developmental program.

Results and Discussion

Since the initiation of the project in September 1995, a number of potential tobacco budworm- and cotton bollworm-specific monoclonal antibodies have been identified. Microplate and squash immunoassays using eggs and haemolymph of tobacco budworm and cotton bollworm were used to select for species-specific antibodies.

Two cotton bollworm-specific monoclonal antibodies; ZAb28 and ZAb29 were initially selected as most promising. Unfortunately, this pair of antibodies was found to be unsuitable for use in the current LepTon assay format. A third antibody, ZAb44, performed better in the test kit format and was selected for further development. ZAb44 was found to react specifically with cotton bollworm eggs. However it is not suitable for discriminating between the larvae of cotton bollworm and tobacco budworm using the same procedure as for eggs. Nonetheless, because of the urgency of the time constraints and the need for the test kit in the field, we decided to pursue the development of ZAb44 as a potential antibody for the test kit; specifically for the 1997 season. We proceeded to mass produce ZAb44 for manufacture of a prototype test kit. At the same time we continued to evaluate and to characterize ZAb44. In early December 1996, we were able to produce enough of ZAb44 for a program of test kit validation studies.

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We conducted a range of studies to determine the accuracy of the test kit using ZAb44 as the antibody. Here, we describe two of the validation studies.

In the first study, we squashed 50 eggs of cotton bollworm on half of the LepTon test membrane while the other half was squashed with 50 eggs of tobacco budworm. We then processed the membrane with the standard kit reagents, including the ZAb44 antibody conjugate. Each membrane was scored immediately after processing. Four replicates were performed. The results obtained from the test kit are compared with the known, actual, ratio in Table 1. The results confirm that ZAb44 is a cotton bollworm-egg-specific antibody. However, it potentially has about 6% false positives using this particular evaluation format.

The second study was a "blind" test to further evaluate the accuracy of the prototype test kit. In this study, six different ratios of cotton bollworm:tobacco budworm eggs were used: 1) 0:100, 2) 20:80, 3) 40:60, 4) 60:40, 5) 80:20, 6) 100:0. For all cotton bollworm and tobacco budworm ratios tested, the eggs were randomly squashed on the membranes. The membranes were processed using the test kit. Again, each membrane was read immediately after processing. The person who scored the results had no prior knowledge of the cotton bollworm:tobacco budworm egg ratio, nor of the pattern of the eggs placement.

The results of this study were presented in Table 2. The test kit gave a 10% false positives when 100% tobacco budworm eggs were used in the test. At a cotton bollworm:tobacco budworm ratio of 60:40, the test kit indicated there were 74% of cotton bollworm eggs. This was 14% higher than the actual value. When 100% cotton bollworm were used, the test indicated that 99% of the eggs were cotton bollworm. Since, with this antibody, the test is capable of delivering up to 14% false positives, it was decided that ZAb44 is not specific enough for commercial use.

Based on the results, we concluded that ZAb44 is not suitable for a commercial LepTon™ HTK and we therefore decided that we will not market the test kit during the 1997 field season. However, we feel that ZAb44 can potentially be used in a prototype test kit for some research and development purposes. It will allow us to estimate, approximately, the species-composition of eggs lay under a variety of geographical and other conditions. It will also allow us to iron out any other potential teething problems with the test kit format and may allow a number of end-users to gain hands on experience with the test procedure.

Future Plan

For the 1997 field season, therefore, our plan is to provide a limited number of prototype test kits (probably using ZAb44) to researchers and consultants who are interested in working with us, and learning how to use the test kit. We feel that it is important for the end-users to gain some experience in using the test kit prior to the commercial introduction of the LepTon™ HTK.

At the same time we are continuing to improve the performance of currently available antibodies as well as seeking to isolate new improved antibodies. We are confident that we will be able to have the test kit commercially available during the 1998 growing season.

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References

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Table 1. Percent of cotton bollworm and tobacco budworm eggs as determined using LepTon™ HTK prototype with ZAb44. Actual ratio of cotton bollworm and tobacco budworm eggs was 50:50 in all cases.

Membrane Number	Percent Cotton Bollworm Eggs		Percent False Positive Observed Using Test Kit
	Actual	Test Kit	
1	50	54	6
2	50	52	4
3	50	51	4
4	50	51	6

Table 2. Validation results with the LepTon™ HTK Test Kit using different ratios of eggs of cotton bollworm and tobacco budworm.

Egg Ratio Cotton Bollworm Vs. Tobacco Budworm	Percent Cotton Bollworm Eggs		Percent Errors Observed Using Test Kit
	Actual	Test Kit	
0:100	0	10	10
20:80	20	27	7
40:60	40	48	8
60:40	60	74	14
80:20	80	83	3
100:0	100	99	1