HEL-ID: A TOOL FOR BOLLWORM AND BUDWORM MANAGEMENT

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Abstract

Cotton bollworm and tobacco budworm are among the most important insect pests of cotton. Timely identification of the insects is critical for efficient management of the pests to reduce cotton yield losses. ***Hel*-ID** developed by Agdia and Mississippi State University, can accurately identify both the bollworm and budworm in the egg stage. The ELISA kit based on monoclonal antibodies is simple to use and requires no special laboratory equipment or measuring devices. A large amount of egg samples can be tested in a relatively short time. Two-year results indicate that ***Hel*-ID** is 99-100% accurate in laboratory tests and 95-100% accurate in field trials. Therefore, ***Hel*-ID** can be a convenient tool for cotton producers and consultants allowing them to make bollworm and budworm management decisions precisely within hours.

Introduction

The cotton bollworm, *Helicoverpa zea* (Boddie) and the tobacco budworm, *Heliothis virescens* (F.) are among the most serious insect pests of several economically important row crops in the United States. The two species caused yield reduction of about 2% in cotton, in 1997, resulting in a cost to United States cotton growers of over $180 million (Williams, 1998).

Normally controlled by pyrethroids, the budworm has developed increasing resistance to the insecticides (Luttrell et al. 1991). Bt-transgenic cotton is also being planted as a means to control these species. However the bollworm is more tolerant to Bt-transgenic cotton than the budworm (Luttrell et al. 1998). Maintaining control of these two important species with these accepted methods depends on information about species composition and timing of insecticide applications to eggs and young larvae (Zeng et al. 1998a).

Although identification of bollworm/budworm eggs can be made from visual inspection of the morphological characteristics of eggs (Bernhardt and Phillips 1985), this identification is not always accurate and sometimes requires expensive equipment such as scanning electron microscopy (Zeng et al. 1998b). Other methods of identification include rearing larvae to adults or larger larvae and by estimating percentage of each species as indicated by pheromone and light traps. These techniques are all very time consuming and either delay necessary control measures or precipitate unnecessary insecticidal treatments.

Here we report on the development of a diagnostic test kit designed to address the problem of early species identification of the bollworm and budworm. Our objectives were 1) to design a simple to use test kit which did not require any special laboratory equipment or measuring devices, 2) assure such a test had dependable accuracy, and 3) provide a convenient tool for the cotton producer or consultant that allows them to make bollworm/budworm management decisions within 2-3 hours.

Discussion

***Hel*-ID** is produced at Agdia, Inc., Elkhart, Indiana, using species specific MAbs developed by Zeng and Ramaswamy, Mississippi State University, Mississippi State, Mississippi. The first set of MAbs, produced by hybridoma cell lines Hz46g2E5B and Hz46g2E9B (Zeng et al. 1998b), are specific for *H. zea* antigen only. The second MAbs are produced by cell lines Hv612d6F4d and Hv612d1F4A and are specific only for *H. virescens* antigen (Zeng et al. 1998b). The MAbs produced by these cell lines do not exhibit any cross-reactivity. Hz46g2E5B or Hz46g2E9B was labeled with alkaline phosphatase and Hv612d6F4d or Hv612d1F4A was labeled with horseradish peroxidase.

***Hel*-ID** uses ELISA to identify *H. zea* and *H. virescens* eggs. A kit provides four units which can be used to test 22 eggs each. Eggs are tested by crushing an individual egg on a piece of parafilm with an eggpick and placing it individually into the well of a Nunc Maxisorp ® plate containing 100 µl (2 drops) of phosphate buffered saline (PBS). The wells containing the crushed eggs are incubated for one hour in a humid chamber. After the one hour incubation period, contents are emptied and the wells are washed five times using tap water. After washing, excess water in the wells is removed by tapping on paper towels.

Next 100 µl of the antibody-enzyme conjugates is added to all wells. The wells are again incubated for one hour in a humid chamber. After incubation the wells are washed and excess liquids are tapped out onto paper towels. At this point 100 µl of TBW color indicator solution is added to wells. After an observation period of 5-10 m, wells that turn royal blue are positive for budworm.

After recording the results for budworm, wells are again rinsed five times and excess liquids tapped out, 100 µl of CBW color indicators are added to the wells. After an observation period of 10-20 m, wells that turn a purple-blue are positive for bollworm. Using the ELISA format, ***Hel*-ID** has produced highly accurate results (99%) in all laboratory trials conducted (Table 1).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:77-78 (1999)
National Cotton Council, Memphis TN

***Hel*-ID** was introduced commercially in May 1998. The kit was distributed to 13 different states and the majority of users rated the test kit highly accurate. Results from field data collected in the Mississippi Delta, where ***Hel*-ID** results were compared to known eggs and adults, indicated about 95% accuracy rate (Table 2).

Plans for the 1999 crop season include the introduction of two single species tests. The new product line will include the new tests, ***Hel*-ID: Hz** and ***Hel*-ID: Hv** which can give results for a single species in 1 to 1 ½ hours. ***Hel*-ID: Hz** will provide a one species identification for bollworm and ***Hel*-ID: Hv** will do the same for budworm. Just like Agdia's first product, ***Hel*-ID**, all the products in this line are designed to be convenient and time-saving tools for crop consultants, scouts and growers in making bollworm/budworm management decisions. Agdia is also conducting research on a field test that will have the same quality and reliability of the current ***Hel*-ID** but will only take minutes to perform and obtain results.

Acknowledgments

We thank Sonny Ramaswamy, Fanrong Zeng, Aubrey Harris (Mississippi State University), Dick Hardee, and Larry Adams (USDA, ARS, SIMRU, Stoneville, Mississippi) for their contributions to this study.

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Table 1. Bollworm and budworm eggs tested in laboratory trials using ***Hel*-ID** with three different investigators and multiple egg batches.

	H. zea	+	-	**H. vir.**	+	-
Totals	651	647	4	654	648	6
Percent		99.4	0.6		99.1	0.9

Table 2. Cotton bollworm and tobacco budworm eggs as identified by ***Hel*-ID**. Results are compared to known eggs or eggs reared to adults.

	***Hel*-ID**			**Known Eggs/Adults**		
	# Egg	H. zea	H. vir	#on diet	H. zea	H. vir
Totals	440	242	176	326	179	147
Percent		55	40		55	45