THE USE OF ENZYME LINKED IMMUNOSORBENT ASSAYS (ELISA) TO DETERMINE THE ROLE OF *ORIUS INSIDIOSUS* (SAY) TO CONTROL BOLLWORMS IN THE SOUTHERN BLACKLANDS OF TEXAS

C.G. Sansone
Texas Agricultural Extension Service
San Angelo, TX
J.W. Smith, Jr.
Texas A&M University
College Station, TX
P.O. Darnell
Texas A&M University
College Station, TX

Abstract

The bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), were responsible for $48,497,700 in associated crop losses and insecticide costs in Texas cotton in 1995 (Williams 1996). Current tactics for bollworm/budworm control place an emphasis on monitoring pest densities in individual fields and initiation chemical control measures when an economic threshold is reached (Knutson et al. 1994). Several studies have commented on the impact of natural enemies on the survivorship of bollworm/budworm eggs and first instar larvae in cotton, but few have provided quantitative estimates of mortality. Parasitization and impact of pathogens can be quantified by rearing the hosts, but predators are more difficult. A study was initiated to construct partial ecological life tables of bollworm in cotton in the southern Blacklands and develop an immunoassay to determine the impact of the numerically dominant egg predator.

The data in this study indicate that 71% to 84% mortality can be consistently expected in the egg and first instar and thus insecticide decisions should be delayed until all the mortality factors have had an affect. Egg parasitization averaged 3.1% and larval parasitization was 5.3% in 1991 and never exceeded 5% for the remainder of the study, thus implicating predators as an important component of natural control. ELISA techniques were developed to determine the impact of *Orius* spp (insidious flower bug and minute pirate bug) on bollworm eggs. The ELISA could detect the egg protein for a short time after *Orius* spp ingested the egg. However, the time was long enough to measure egg predation from field collected *Orius* spp. The data from this study provide further information in developing a biologically intensive integrated pest management (IPM) program.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1316-1320 (1997)
National Cotton Council, Memphis TN

Introduction

Members of the noctuid genera *Heliothis* and *Helicoverpa* are agricultural pests worldwide, however, only *Helicoverpa armigera* Hubner, *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.) have achieved major pest status (Fitt 1989). The impact of bollworm on cotton production in the Blacklands of central Texas has been well documented (Rummel et al. 1986). The status of the bollworm/budworm complex as a pest is often dependent upon insecticide use patterns. Cotton has a long history of misuse of insecticides to manage pests. However, since the early 1970's, insecticide use has generally declined in cotton. Implementation of IPM programs by the Cooperative Extension Service, private consulting entomologists or individual farmers have contributed to the decline. These programs incorporated some of the aspects of bollworm/budworm natural control into the economic injury levels and economic thresholds that are used to initiate insecticide applications.

Decreasing rates of return on investments in insecticide applications by producers and chemical manufacturers, public dissatisfaction with pesticide use and accelerating rates of pest resistance have aided a renewed interest in the role of natural mortality in pest suppression (Jutsum 1988). Much of the evidence supporting the importance of natural control and predation in suppressing the bollworm in cotton has been attained from studies comparing bollworm damage in insecticide treated and untreated cotton (Quaintance and Brues 1905; Ewing and Ivy 1943; Fletcher and Thomas 1943; Ehler et al. 1973). Despite the importance of cotton as an agricultural crop and bollworm as a pest, few quantitative ecological studies have attempted to identify the causes of mortality, partition age specific mortality or define generation mortality of bollworm in natural or agricultural habitats (Ehler et al. 1973; Sears and Smith 1975; McDaniel et al. 1981; Hogg and Nordheim 1983). A description and analysis of natural mortality are requisite to developing and implementing biologically intensive IPM strategies for bollworm.

The mortality factors identified must be quantified to be of use in the field. Parasitization and the impact of pathogens can be quantified by rearing the age specific host. The impact of predation is harder to quantify for a number of reasons (Stuart and Greenstone 1990). Serological assays, including enzyme linked immunosorbent assays (ELISA), overcome some of the problems associated with determining the impact of predators. The assays rely on the unique response of certain animals to produce an immune reaction to a specific foreign substance. If each positive response in an ELISA is considered to represent one prey consumed, the minimum number of prey eaten can be estimated form predator density (Kiritani and Dempster 1973). The information developed from life tables and ELISA can be used to implement biologically intensive IPM programs.

Materials and Methods

Life table census and population densities of bollworm life stages and predators was conducted in a commercial cotton field at the Stiles Farm Foundation located in Thrall, Williamson County, Texas from 1991-1995. Agronomic practices were standard for the experimental rainfed cotton field except that no insecticides were applied to the field. One hundred plants were sampled three times per week. The entire plant was visually searched for bollworm eggs and all larval stages. All life stages found on the plant were removed for observation. Simultaneous with collection of the bollworm life stage census data, the individual plants were searched for predators.

O. insidiosus was used as the model bollworm predator insect because of the high density found throughout the season in the study field and because of the probable role it plays as a key predator of bollworm in the cotton agroecosystem (Quaintance and Brues 1905, Ewing and Ivy 1943, Fletcher and Thomas 1943, McDaniel and Sterling 1982, Nuessly and Sterling 1994). The ELISA assay employed was a direct antigen coating (DAC) configuration (Mowat 1985). The primary antibody used was a heliothine egg antibody (HVE-C1-B3A, Greenstone and Trowell 1994) that was purchased from Dr. Matt Greenstone, USDA-ARS research entomologist. The amount of time the ELISA specific protein is available for reaction was determined by developing a protein degradation curve. Adult *O. insidiosus* were first observed consuming a frozen *H. virescens* egg and then held without any further animal protein at a constant temperature and relative humidity for 0, 2, 4, 6, 8, 10, 12, 18 and 24 hours prior to quick freezing in liquid nitrogen. Assays were also conducted on adult and nymphal stages of *Orius* spp collected from the same experimental fields used to collect life table census in 1994 and 1995.

Results

The results for the census data are similar so only 1995 results will be presented. The temporal distribution for the bollworm population indicates only one generation per season (Fig. 1). Preliminary studies indicated that two generation are present when cotton is managed under a typical insecticide regime. Table 1 is a partial ecological life table for bollworm in the 1995 season. Age specific and generation mortality was greatest for the egg and first instar larva. Disappearance or unexplained mortality accounted for most of the egg and first instar mortality. Predation is considered a prime candidate for contribution to disappearance of egg and first instar larvae since the disappearance corresponds to the closely with predator densities (Fig. 2).

The ELISA provides an objective method of evaluating predation without interfering with the predator or its habitat. The positive-negative threshold for each assay plate was calculated using the mean plus three standard deviations from the absorbence value of the negative control. Any well in a microplate that gave a value of three standard deviations beyond the mean for the negative control was considered positive. The mean and variance of the negative control can vary from plate to plate and accordingly the positive-negative threshold can change from plate to plate. The proportion of positive *O. insidiosus* from three trials may provide a better indication of how long the bollworm egg protein was detectable (Table 2). From 0 to 10 hours, the average proportion of positive OD readings was stable ranging form 0.958 to .708 (Table 2). After 10 hours, the proportion of positive OD readings declined precipitously. The detection time of 10 hours is advantageous because it ensures that any predation events detected in the field study occurred on the day of collection and that predation is not overestimated.

Table 3 gives the estimates for egg predation by *Orius* spp in 1995. *Orius* spp appear to be extremely efficient at finding eggs early and late in the growing season. Egg predation is detected even when no eggs were detected by plant sampling. Egg predation decreases at peak bloom possibly indicating *Orius* spp shift their prey preference as other food sources increase or the egg protein is not detectable as other sources become available.

Discussion

As in previous studies (Quaintance and Brues 1905; Ewing and Ivy 1943; Fletcher and Thomas 1943; McDaniel and Sterling 1982; Nuessly and Sterling 1994), predators are implicated as an important mortality factor for bollworm in cotton. Predators may act as a stabilizing factor in the agroecosystem and maintain pest densities below economically damaging level early in the season (Riechert 1974; Wiedenmann 1990) when pests are in the latent phase of population growth. Although bollworm densities may eventually reach high levels, the mortality contributed by predators could delay pest population growth into the subsequent epidemic phase. This delay will allow the crop to approach maturity which raises the economic injury level and reduces the reliance on insecticides to produce the crop.

Orius spp are highly polyphagous insects that can survive not only on insects but also pollen and plant juices. This adaptive advantage of being highly polyphagous may increase its importance as a predator of the bollworm/budworm complex in cotton, especially early in the season when prey are scarce. Although *Orius* spp appeared to ship its feeding niche at peak bloom, the early feeding on bollworm eggs may help in the population increase of *Orius* spp and may minimize first generation bollworm/budworm damage.

The management of bollworm in southern Blacklands cotton should now be viewed from an area perspective (macromanaged) as well as a field to field perspective

(micromanaged). The cotton crop must be managed to minimize early season (prior to bloom) insecticide use. Success in controlling bollworm will also depend on the management of surrounding crops. Manipulation of surrounding habitat as well as crop maturity may help in staggering the emigration of pests and predators into cotton and delay the rapid build up of bollworm that often occurs.

Acknowledgments

I would like to thank Dr. Matt Greenstone for the use of the heliothine antibody and his many useful suggestions and comments. Also, thanks to Dr. Forrest Mitchell, with the Texas Agricultural Experiment Station, for his comments and help with developing the ELISA.

References

Ehler, L.E., K.G. Eveleens and R. van den Bosch. 1973. An evaluation of some natural enemies of cabbage looper on cotton in California. Environmental Entomology 6: 1009-1015.

Ewing, K.P. and E.E. Ivy. 1943. Some factors influencing bollworm populations and damage. Journal Economic Entomology 36: 602-606.

Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. Annual Review of Entomology 34: 17-52.

Fletcher, R.K. and F.L. Thomas. 1943. Natural control of eggs and early instar larvae of *Heliothis armigera*. Journal Economic Entomology 36: 357-360.

Greenstone, M.H. & S.C. Trowell. 1994. Arthropod predation: a simplified immunodot format for predator gut analysis. Annals Entomology Society of America 87: 214-217.

Hogg, D.B. and E.V. Nordheim. 1983. Age specific survivorship analysis of Heliothis spp. populations on cotton. Res. Popul. Ecol. 25: 280-297.

Jutsum, A.R. 1988. Commercial application of biological control: status and prospects. Philos. Trans. R. Soc. Lond. Biol. Sci. 318: 357-373.

Kiritani, K. and J.P. Dempster. 1973. Different approaches to the quantitative evaluation of natural enemies. Journal of Applied Ecology 10: 323-330.

Knutson, A., R.D. Parker, R.L. Huffman, C.G. Sansone and J. Swart. 1994. Management of cotton insects in the Southern, Eastern and Blackland areas of Texas. Tex. Agri. Ext. Ser. Bull. B-1204. 12 pp.

McDaniel, S.G. and W. L. Sterling. 1982. Predation of *Heliothis virescens* (F.) eggs on cotton in East Texas. Environmental Entomology 11: 60-66.

McDaniel, S.G., W. L. Sterling and D. A. Dean . 1981. Predators of tobacco budworm larvae in Texas cotton. Southwestern Entomologist 6: 102-108.

Mowat, W.P. 1985. Tulip chlorotic blotch virus, a second potyvirus causing tulip flower break. Ann. Appl. Biol. 106: 65-73.

Nuessly, G.S. and W.L. Sterling. 1994. Mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in cotton as a function of oviposition sites, predator species and desiccation. Environmental Entomology 23: 1191-1202.

Quaintance, A.L. and C.T. Brues. 1905. The cotton bollworm. USDA Bur. Entomol. Bull. 50: 154 pp.

Riechert, S.E. 1974. Thoughts on the ecological significance of spiders. BioScience 24: 352-356.

Rummel, D.R., J.F. Leser, J.E. Slosser, G.J. Puterka, C.W. Neeb, J.K. Walker, J.H. Benedict, M.D. Heilman, L.N. Namken, J.W. Norman and J.H. Young. 1986. Cultural control of *Heliothis* spp. in southwestern U.S. cropping systems, In S.J. Johnson, E.G. King and J.R. Bradley, Jr. (eds.) Theory and Tactics of Heliothis Population Management: I-Cultural and Biological Control. South. Coop. Ser. Bull. 316: 38-53.

Sears, D.E. & J.W. Smith, Jr. 1975. A natural mortality table for the corn earworm on peanuts. Tex. Agri. Exp. Sta. Prog. Rep. 3344C. 4 pp.

Stuart, M.K. & M.H. Greenstone. 1990. Beyond ELISA: a rapid, sensitive, specific immunodot assay for identification of predator stomach contents. Ann. Entomol. Soc. Am. 83: 1101-1107.

Wiedenmann, R. N. 1990. Searching strategy of the generalist predator *Podisus maculiventius* (Say) (Heteroptera: Pentatomidae). Ph.D. Thesis, Purdue University.

Williams, M.R. 1996. Cotton insect losses 1995, In Proc. Beltwide Cotton Production and Research Conference, National Cotton Council, Memphis, TN, pp. 670-689.

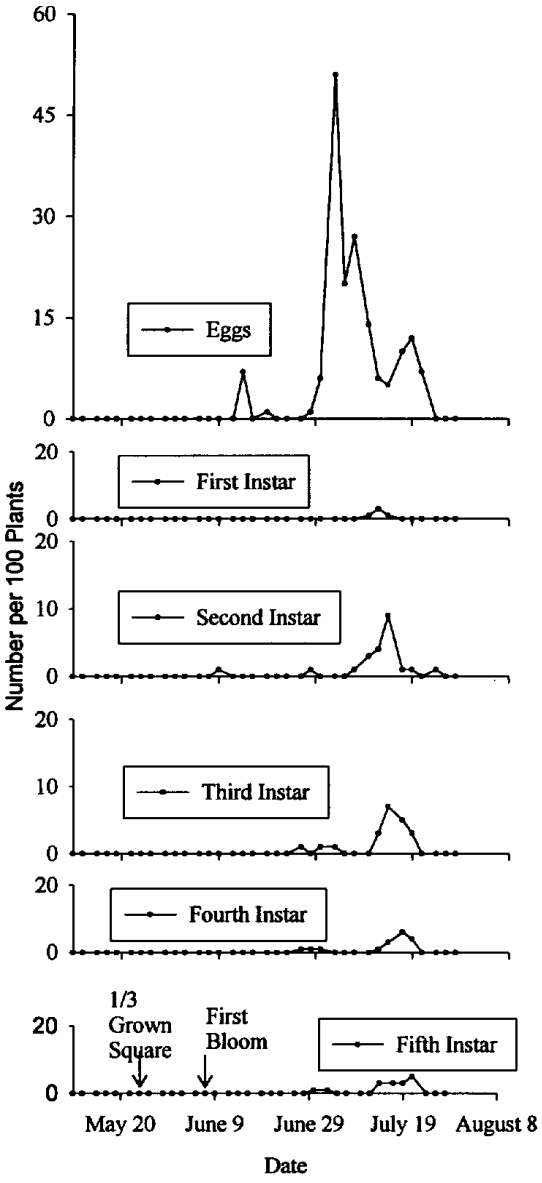

Figure 1. Temporal distribution of the bollworm population. Williamson Co., TX. 1995

Table 1. Partial ecological life table for bollworm. Williamson Co., TX. 1995.

Age interval	No. alive at beg. of x	Factor responsible for dx	No. dying during x	Age specific mort. during x	Gen. mortality during x
x	lx	dxF	dx	qx	rx
Eggs & first instar	32,462	Parasitism	1,855	0.057	0.057
		Unexplained	24,712	0.761	0.761
		Total	26,567	0.818	0.818
Second instar	5,895	Unexplained	464	0.078	0.014
		Total	464	0.078	0.014
Third instar	5,431	Parasitism	217	0.040	0.006
		Unexplained	1,208	0.222	0.037
		Total	1,425	0.262	0.043
Fourth instar	4,006	Unexplained	2,920	0.729	0.089
		Total	2,920	0.729	0.089
Fifth & sixth instar	1,086				
Generation total					0.964

Values are life stages/acre

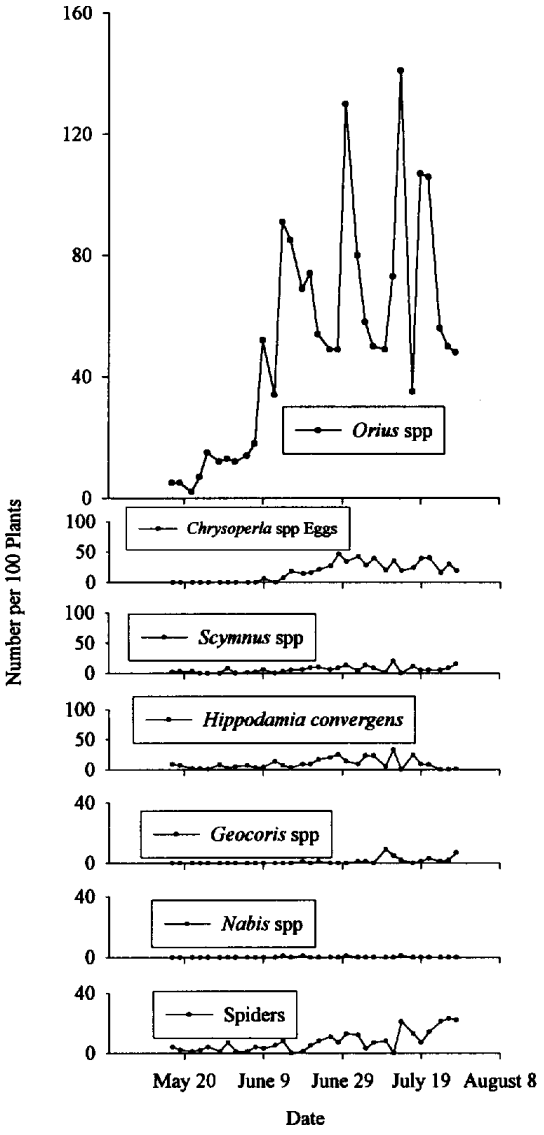

Figure 2. Temporal distribution of common predators in Williamson Co., TX. 1995.

Table 2. Proportion of positive OD readings for O. insidiosus for each feeding trial in the protein degradation study.

Hours post feeding	1	2	3	Mean
0	0.875	0.625	1.000	0.833
2	0.875	0.875	0.875	0.875
4	0.875	1.000	1.000	0.958
6	0.625	0.875	1.000	0.833
8	0.875	0.750	1.000	0.873
10	0.875	0.250	1.000	0.708
12	0.125	0.125	0	0.083
18	0.125	0.125	0	0.083
24	0.125	0.125	0.125	0.125

Table 3. Estimated number of eggs per acre preyed upon by *Orius* spp based on ELISA assays and *Orius* spp density. Williamson Co., TX. 1995.

Date	Plant Stage	No. of *Orius* spp per acre (P)	Pro.Pos. (mi)	Est. No. of eggs detected per acre	Estimated[1] total consumed per acre
June 5	Square	6,337	0.100	0	1,679
7		8,287	0.250	0	5,490
9		23,400	0.067	0	4,154
12		16,575	0	0	0
14		44,363	0.052	3,412	6,113
16		41,438	0.200	0	21,962
19	First	33,638	0.589	487	52,503
21	Bloom	36,075	0.088	0	8,412
23		26,325	0.069	0	4,813
26		23,888	0.036	0	2,278
28		23,888	0.015	487	949
30		63,376	0.030	2,925	5,038
July 3		39,000	0.022	24,375	2,273
5	Peak	28,275	0.067	9,750	5,020
7	Bloom	24,375	0.052	13,162	3,358
10		23,887	0.070	6,825	4,431
12		35,588	0.018	2,925	1,697
14		68,738	0.036	2,437	6,557
17		17,062	0.130	4,875	5,877
19		52,163	0.243	5,850	33,590
21		51,676	0.147	3,412	20,130
24		27,300	0.041	0	2,966
26		24,375	0.093	0	6,007
28		21,937	0.092	0	5,348

[1] (PmT)/t where P= Predators present
m= Proportion *Orius* spp. nymphs and adults reacting positive
T= Period of time prey are available (40.5 hu/15.23 hu/day= 2.65 days)
t= Length of time prey meal remains detectable (1 day)