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COMPARISON OF MISSISSIPPI AND LOWER RIO GRANDE VALLEY LABORATORY REARED STRAINS OF BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOHEMAN: COTTON LEAF BIOASSAY OF AERIALLY APPLIED MALATHION

R. Nelson Foster and Chris Reuter
USDA-APHIS-PPQ-CPHST-DS&PMSL
Phoenix, AZ
Allan T. Showler and Thomas W. Sappington
USDA-ARS-SARC
Weslaco, TX
Leeda Wood
USDA-APHIS-PPQ-CPHST-PDD&ML
Edinburg, TX
Timothy J. Roland, Daryl E. Hill and Bruce D. Radsick
USDA-APHIS-PPQ-AEO
Mission, TX
Osama A. El-lissy
USDA-APHIS-PPQ-BWEP
Riverdale, MD
Robert G. Jones
USDA-APHIS-PPQ
Starkville, MS

Abstract

The laboratory colony of boll weevils historically located in Starkville, Mississippi was relocated to Edinburg, Texas in 2000. A new strain based on Lower Rio Grande Valley, Texas, field-collected weevils has also recently been established in Edinburg, Texas. Differences in untreated and malathion induced mortality between the historic Mississippi strain and the new Texas strain both reared with modified Texas rearing procedures are presented. Additionally, differences between the Mississippi and Texas colonies of the historic strain produced with Mississippi and Texas procedures respectively are discussed.

The Texas reared Mississippi strain exhibited higher mortality in untreated control populations and appeared to be more susceptible to malathion exposure compared to the recently established Texas reared Lower Rio Grande Valley strain. Differences between Mississippi and Texas colonies of the historic Mississippi strain appear evident in size and survival rates.

The differences between the two strains appear substantial, as do the differences between colonies of the Mississippi strain reared in Mississippi and Texas. The differences between strains are likely genetic. However, the differences between colonies are apparently a result of rearing procedures. These differences should be carefully considered when comparisons of data based on weevils from the two strains or from the two colonies with different rearing procedures are conducted.

Introduction

The boll weevil has been reared on artificial diet since 1957 and a colony has been maintained for research purposes for more than 30 years at the Agricultural Research Service, Robert T. Gast and/or Biological Control and Mass Rearing Research Unit at Mississippi State University, Starkville, Mississippi (Sikorowski et al. 1984). The expansion of the boll weevil eradication program (BWEP) into Mississippi in Aug. 1997 made it necessary to relocate the boll weevil rearing facility outside of the eradication zone. On Jan 1, 2000 the Mississippi rearing facility was officially closed and the USDA-APHIS, Pest Detection, Diagnostic and Management Laboratory (PDD&ML) at Moore Air Base near Edinburg, Texas officially assumed the responsibility for rearing the Mississippi strain of boll weevil. While the genetic stock and basic rearing diet formula used in Mississippi were transferred to Texas, the new rearing procedures developed and implemented in Texas are not identical to those used in Mississippi. Additionally, since the transfer of the Mississippi colony of boll weevils to Texas occurred, a second strain based on Lower Rio Grande Valley, Texas field-collected material has also been colonized at the Edinburg, Texas facility. This strain is termed the Texas Lower Rio Grande Valley strain but in this paper will be referred to as the Texas (TX) strain. The Mississippi (MS) strain is the major colony in the Texas facility and is the strain used for all research use requests. Use of this strain provides some continuity with previously conducted research where mass laboratory reared boll weevils from Mississippi were used. However, no comparative studies were conducted between the two procedures used for rearing the MS strain, and some differences have been recently observed between the MS strain reared in Mississippi and Texas. To help identify differences that may exist between the Texas reared MS strain (a very old strain)

and the Texas reared TX strain (a very new strain, 12–13 generations), comparisons were made in survival responses to field applied malathion and untreated adult longevity in laboratory bioassay cages between the two strains with the newly modified Texas rearing procedures. These comparisons may also provide information on potential response differences that may have evolved over the years in the Mississippi mass-reared laboratory colony. In addition, limited literature and unpublished data comparing the Mississippi colony and the Texas colony of the MS strain were reviewed. Hereafter, to alleviate confusion, these strains will be referred to by abbreviations (MS and TX) and rearing locations of colonies will be referred to with unabbreviated state designations.

Materials and Methods

Study Site

The study was located in Hidalgo County of the Lower Rio Grande Valley of Texas. The center of the study area was approximately 2 miles WSW of Hargill. The entire study utilized 6 separate cotton fields provided by William Durbin. The general location and specific fields were selected because this study could be conducted simultaneously with one designed to evaluate reduced rates of malathion combined with cotton seed oil, Showler et al. 2002.

Two plots, 0.34 – 0.52 acres (75 feet x 200-300 feet) were established in each of 6 fields for the study. One was designated as an untreated check plot and the other as a treated plot. This size accommodated one aerially applied swath. All plots were separated by a minimum of 200 yards.

Treatments

One plot in each field was treated with Fyfanon ULV at the BWEP rate of 12 fluid oz/acre (0.93 lb AI/ acre). All treatments were applied with a Cessna Ag-Truck aircraft owned by the USDA, Animal and Plant Health Inspection Service (APHIS) and equipped with winglets (DBA-AG Tips: Clack Oberholzer, Alberta, Canada). Winglets were added to the spray aircraft to reduce the production of fine droplets and to improve handling characteristics. A USDA-APHIS pilot operated the aircraft.

The aircraft was equipped with a standard commercial spraying system and was operated at ca. 10 feet (boom height) above plant canopy during applications. The aircraft and spraying system were calibrated for a 75 feet wide swath. Prior to application, the aircraft spray system was calibrated to operate under parameters that resulted in delivery of spray within one percent of the desired rate per acre. Calibration was accomplished by collecting and measuring the amount of material sprayed through each of the end nozzles for a predetermined amount of time, and making adjustments in pressure until the desired output was achieved. The Fyfanon ULV treatments were applied at 125 mph through 10 (8002) Flat Fan Tee Jet stainless steel nozzle tips oriented straight down. Winds during application ranged from 0.5 mph to 1.3 mph.

Bioassay

A bioassay utilizing cotton leaves untreated and treated with 12 fluid oz/acre (0.93 lb AI/acre) of Fyfanon ULV was used to assess each of the boll weevil strains. Insects of both strains were exposed to both treated and untreated cotton leaves from each of the 6 fields at 6 selected post treatment intervals of exposure. Each of the 6 fields was considered a replicate. Each replicate consisted of 10 weevils exposed per assigned experimental treatment. Therefore, 360 weevils were used for each of the experimental treatments examined.

Cotton leaves from canopy level within one row and 10 yards of the center of each plot were collected at 0, 1, 2, 3, 4, and 5 days after application. Two leaves were collected from each plot at each post treatment interval. Leaves collected in each plot were placed in zip lock plastic bags and transported to the laboratory (Figure 1). Twelve cages (100 mm x 15 mm plastic petri dishes modified with a screen-covered 45 mm diameter opening on top for ventilation), 2 cages per plot, were established for each treatment on each post treatment interval. Each dish was stocked with 1 leaf and 5 active adult laboratory reared boll weevils (Figure 2). Weevils were furnished by the USDA, APHIS, PPQ, PDD&ML, Boll Weevil Rearing Facility near Edinburg, Texas. Both strains were reared identically except for the addition of Calco red dye to the diet in the Mississippi strain. It is important to note that the rearing procedure in the Texas facility is substantially different than that used for years in the Mississippi facility. Disposable gloves and freshly washed scissors were used during collection of cotton leaves to prevent contamination. Dishes were maintained under florescent lights, 14:10 daylight:darkness at 81^{o} - 83^{o} F. in an isolated laboratory at the PDD&ML near Edinburg, Texas (Figure 3). Mortality was recorded daily for 5 days after weevils were exposed to leaves in petri dishes. Weevils were categorized as dead when no movement of any kind could be detected. Weevils were also categorized as seriously affected when there was detectable movement but when death was obviously imminent. These weevils were judged incapable of causing damage by feeding, mating or oviposition. Such weevils were considered functionally dead and were combined with physically dead individuals for analysis.

Analysis

Bioassay data were expressed as percent mortality based on the pretreatment population. A one-way analysis of variance was conducted with the Tukey multiple comparison test (Systat 6.0, 1996) used to separate means. Results from the analysis with the observed data are presented. Further analysis was conducted when percentage mortality values were adjusted with the appropriate untreated check mortality to arrive at percentage control data (Connin and Kuitert, 1952). The resulting means were then converted to ranks and compared as before.

Results

The mean mortality of boll weevils exposed to 0-5 day-old residual treatments of malathion on cotton leaves and untreated leaves for each strain of laboratory reared weevils is shown in Table 1. Mortality is shown for the progressive exposure to treated and untreated leaves for 0 through 5 days.

The strong activity of malathion masked any differences in susceptibility between the two strains on the day of treatment or with residuals one day-old. Significant differences in susceptibility, in terms of mortality, of the two strains to malathion was not evident until residuals were at least two days old. A significant difference was seen after weevils were exposed to 2 day-old residuals for one day with the TX strain demonstrating lower susceptibility. However, this difference disappeared when weevils were held for 2–5 days of exposure to 2 day-old residuals. When weevils were exposed to 3 day-old residuals the TX strain demonstrated significantly lower susceptibility than the MS strain for weevils held for 1–5 days of exposure. Significant differences were evident with 4 and 5 day-old residuals when weevils were exposed for 4 or 5 days. Differences at 1 to 3 days of exposure with the older residuals were likely not evident due to loss of activity of the malathion. Therefore, longer exposure to older residuals was required to elicit differences in susceptibility. Results from another study conducted simultaneously also showed that significant malathion activity in field cages and laboratory bioassays was not detected after malathion residuals exceeded 3 days of age (Showler et al. 2002).

When untreated populations of the two strains were compared no significant differences in mortality were detected until weevils had been held for at least 4 days. At 4 days, TX strain weevils showed significantly higher survival in 3 of the 6 residual comparisons. At 5 days, this result had become consistent for all of the comparisons. Additionally, when the untreated populations for all of the residual comparisons were combined for each strain and compared, the TX strain demonstrated significantly higher survival than the MS strain (Table 2). At 1-5 days the TX strain demonstrated 26%, 45%, 43%, 30%, and 37% as much natural mortality respectively as the MS strain.

Discussion

Comparison of Strains

During this study it was noted that the older Texas reared MS strain boll weevils were more physically active than Texas reared TX strain weevils. This increased activity may result in greater exposure to droplets of malathion on cotton leaves and thus explain higher mortality/susceptibility in the older Texas reared MS strain. Greater activity could also reflect increased metabolic activity and thus decrease natural survival rates, which may help explain increased mortality in untreated populations of the MS strain. These results are also consistent with increasing homogeneity within a laboratory population, which may lead to less resistance progeny. Additionally, unlike field populations laboratory populations are not continuously challenged by insecticide treatments. Thus laboratory populations may develop and demonstrate less resistance to insecticides than field populations and in extreme cases, younger strains may exhibit more resistance than older strains.

Comparison of Colonies

While this study dealt with differences between two strains reared in Texas, it is important to discuss some of the known differences between weevils of the same strain produced in the two different locations. Again, it is important to reemphasize that the rearing procedure in the Texas facility is substantially different than that used for years in the Mississippi facility. These rearing differences may contribute to physical and behavioral differences seen between the two colonies of the same strain. Texas reared weevils are substantially larger in size than Mississippi reared weevils (Joseph E. Mulrooney, unpublished data, personal communication 2001). That assessment agrees with visual estimates of weevil size in studies over the last three years conducted by several of these authors. Intuitively, this size difference would suggest that larger weevils may require more toxicant to achieve similar results. If that is the case, lower and/or slower mortality of untreated populations would result with the larger Texas reared weevils compared to Mississippi reared weevils. However, the opposite appears to be occurring in three very similar studies where three of these authors have been involved in over the last three years (Foster et al. 2000; Foster et al. 2001 and Foster et al. 2002).

Because differences in this strain comparison study were evident at 4 days of exposure we focused on possible differences at 4 days between untreated populations of the Texas and Mississippi reared MS strain (colony comparison). There are both

published and unpublished documented differences in the natural mortality between the Texas reared MS strain and the Mississippi reared MS strain when weevils have been used in laboratory and/or field studies (Table 3). Other than size, these differences are most evident as varying levels of survival among untreated control populations in the different studies. The longer holding periods for untreated controls in those studies simply magnify the differences.

Petri dish bioassay data on untreated control populations of boll weevil are most commonly found for periods of 24 - 48 hrs exposure. Little 72 hr exposure data is recorded in the literature and almost no 96-hour or longer exposure data is readily available. A limited examination of the literature and exploration of unpublished data has identified 6 sources for such data including this study. In those studies at 4 days of exposure (Table 3), Mississippi reared MS strain untreated control populations of weevils demonstrated 10.3% mortality (unpublished data from Jones, 1997) and 11.7 % mortality (Foster et al. 1999). The Jones, 1997 study was conducted in Abilene, Texas with shipped weevils and the Foster et al. 1999 study was conducted in Carlsbad, NM with shipped weevils. Numerous other studies with MS strain Mississippi reared weevils have resulted in little or no mortality at 4 days (Joseph E. Mulrooney, personal communication, 2001). High levels of mortality ranging from 27.8% to 44.4% occurred in untreated populations of Texas reared MS strain in 4 recent studies (Table 3). Two of those studies relied on unshipped weevils, thus indicating that increased mortalities may not necessarily be attributable to shipping procedures. This preliminary evidence seems to indicate that different rearing procedures apparently impact survival rates and does not necessarily implicate shipping as the reason for differences in survival.

Conclusions

When reared in Texas the long-established MS strain demonstrated higher mortality in both treated and untreated populations compared to the recently established TX strain. The MS strain appeared to die sooner and is apparently more susceptible to malathion than the TX strain or is at least less healthy and therefore more likely to succumb to insecticide treatments than the new TX strain.

While these differences exist between the two strains there is a separate issue of differences between the Mississippi and Texas colonies of the historic MS strain. Some data indicate that mortality rates of untreated populations of the same strain may show substantial differences depending on the rearing location/procedures.

Comparisons of data based on the Mississippi colony and recent data based on the Texas colony should be undertaken with caution. Generally, in studies where untreated control mortality is available, the untreated test populations of the Mississippi colony survived longer than the untreated test populations of the Texas colony. These differences may be attributed to rearing procedures or as yet other unidentified factors.

A definite explanation of these differences may be difficult because boll weevils are no longer reared in Mississippi. Additional studies (including aspects related to both rearing and shipping procedures) should be conducted to help clarify why these differences have occurred.

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References

Foster, R. N., K. C. Reuter, J. B. Pierce, P. E. Yates, L. A. Wood and J. Ellington. 2001. Encapsulated malathion for boll weevil: Field comparisons of selected doses of three aerially applied formulations of malathion in New Mexico. 2001 Proceedings Beltwide Cotton Conferences Anaheim, California. 2:1070-1077.

Foster, R. N., K. C. Reuter, Joshua D. Siepel, Jane B. Pierce, Patricia E. Yates, Rex Kirksey, Leeda A. Wood, and Joe Ellington. 2002. Evaluation of selected doses of three formulations of aerially applied malathion for boll weevil. 2002 Proceedings Beltwide Cotton Conferences Atlanta, Georgia. (in press).

Foster, R. N., K. C. Reuter, S. Liesner, J. B. Pierce, T. Carrillo and J. Ellington. 2000. Field evaluation and leaf bio-assay of selected formulations and doses of aerially applied malathion against boll weevil in New Mexico. 2000 Proceedings Beltwide Cotton Conferences. San Antonio, Texas. 2:1188-1194.

Showler, A. T., T. W. Sappington, R. N. Foster, K. C. Reuter, T. J. Roland and O. A. El-Lissy. 2002. Comparisons of aerially applied standard rate malathion against reduced rates of malathion and cotton seed oil for boll weevil control. Southwestern Entomologist (in press).

Sikorowsky, P. P., J. G. Griffin, J. Roberson, and O. H. Lindig. Introduction by T. B. Davich. 1984. Boll Weevil Mass Rearing technology. University Press of Mississippi. Jackson. 172 pp.

Jones, R. G. 1997. Tests with diatomaceous earth for boll weevil control in organic cotton in Texas. In: Accomplishments Report January – December 1997. Phoenix Plant Protection Center, Brawley Plant Methods Station and Boll Weevil Plant Methods Station. USDA-APHIS, Phoenix, AZ. 70-73.

Table 1. Mean number of dead boll weevils (10 per replicate) after exposure to treated or untreated cotton leaves in petri dishes.

	Days After Exposure*				
Treatment	**1**	**2**	**3**	**4**	**5**
0 day residual					
Malathion 12oz MS	9.2 a	9.8 a	10.0 a	10.0 a	10.0 a
Malathion 12oz TX	8.8 a	9.3 a	9.7 a	9.8 a	10.0 a
Untreated – MS	0 b	0 b	0.3 b	1.1 b	5.3 b
Untreated – TX	0 b	0.1 b	0.3 b	0.6 b	2.1 c
1 day residual					
Malathion 12oz MS	8.7 a	9.3 a	9.5 a	9.8 a	9.8 a
Malathion 12oz TX	8.2 a	9.3 a	9.5 a	9.7 a	9.7 a
Untreated – MS	0 b	0.6 b	1.3 b	3.4 b	5.9 b
Untreated – TX	0 b	0.1 b	0.1 c	0.9 c	2.1 c
2 day residual					
Malathion 12oz MS	8.2 a	8.3 a	8.8 a	9.2 a	9.8 a
Malathion 12oz TX	5.3 b	7.6 a	8.1 a	8.8 a	9.0 a
Untreated – MS	0.4 c	0.9 b	0.9 b	1.7 b	6.0 b
Untreated – TX	0.1 c	0.3 b	0.7 b	1.4 b	2.6 c
3 day residual					
Malathion 12oz MS	6.2 a	7.3 a	7.8 a	9.0 a	9.8 a
Malathion 12oz TX	2.3 b	3.7 b	4.5 b	5.3 b	6.8 b
Untreated – MS	0.1 b	0.1 c	0.4 c	2.1 c	6.0 b
Untreated – TX	0 b	0.1 c	0.1 c	0.4 c	1.7 c
4 day residual					
Malathion 12oz MS	1.7 a	2.0 a	2.3 a	3.5 a	6.7 a
Malathion 12oz TX	0.8 a	0.8 a	1.2 a	1.5 ab	2.7 b
Untreated – MS	0.6 a	0.6 a	1.0 a	3.7 a	7.9 a
Untreated – TX	0.1 a	0.3 a	0.4 a	0.6 b	2.7 b
5 day residual					
Malathion 12oz MS	0.2 ab	0.2 a	0.8 a	3.8 a	8.0 a
Malathion 12oz TX	0.5 a	0.5 a	0.5 a	1.2 b	2.7 b
Untreated – MS	0 b	0.1 a	0.9 a	5.9 a	9.4 a
Untreated – TX	0 b	0 a	0.3 a	1.6 b	4.1 b

* Means in a column at each residual day followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an analysis of variance with a Tukey multiple comparison procedure.

Table 2. Mean percentage mortality of two strains of adult boll weevils at indicated day of study.

	Mean % Mortality at Indicated Day of Test				
Treatment	**1**	**2**	**3**	**4**	**5**
Untreated – MS	1.9 a	3.8 a	7.6 a	30.5 a	68.8 a
Untreated – TX	0.5 a	1.7 a	3.3 b	9.0 b	25.7 b
P =	0.069	0.072	0.010	< 0.0001	< 0.0001

* Replications from residual days 0 – 5 combined.

Table 3. Adult boll weevil mortality after 96 hours in untreated control populations of selected petri dish/cotton leaf bioassay based studies.

Study Source	Study Location	% Mortality	No. Weevils	Handling
	Mississippi Strain reared in Mississippi			
Jones, 1997	Abilene, TX	10.3	500	shipped
Mulrooney, 1997 *	Starkville, MS	8.3	?	not shipped
Foster et al. 1999	Carlsbad, NM	11.7	600	shipped
	Mississippi Strain reared in Texas			
Foster et al. 2000	Carlsbad, NM	44.4	1700	shipped
Foster et al. 2001	Tucumcari, NM	33.5	1700	shipped
Showler et al. 2002	Mission, TX	27.8	420	not shipped
This study	Mission, TX	30.5	360	not shipped

* Unpublished data.

Figure 1. Boll weevils confined in ventilated petri dish cages with a cotton leaf were monitored daily for mortality.

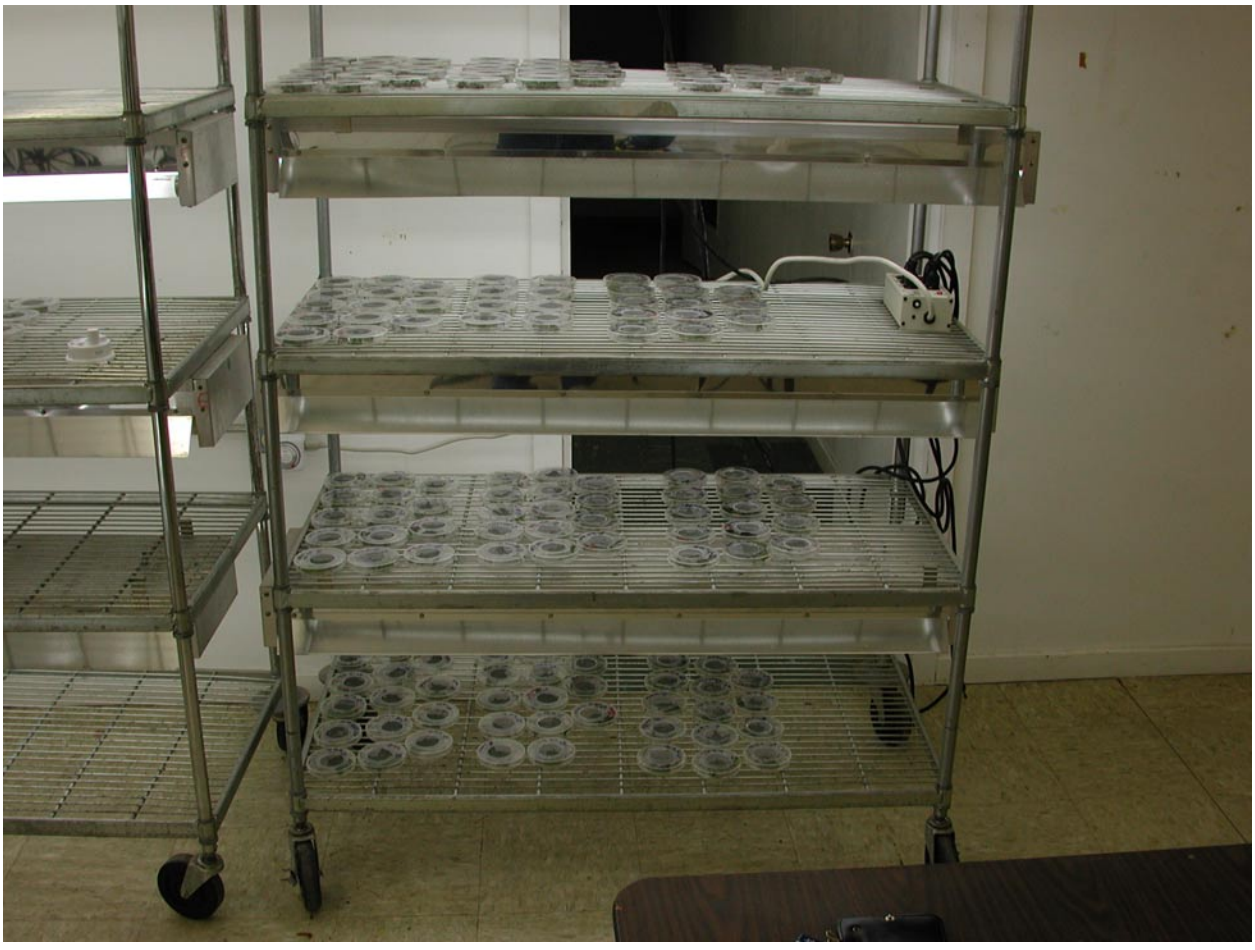

Figure 2. Test insects were held in a laboratory at 81° – 83° F with a 14:10 hr light:dark cycle.