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MODULES, GINS, AND THE THREAT OF BOLL WEEVIL INTRODUCTIONS: WHAT WE KNOW SO FAR

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Abstract

Experiments were conducted to determine the potential threat of boll weevil transport on or in cotton modules constructed in infested areas to gins in weevil-free areas. Surveys in three areas of Texas indicated that live weevils are usually present in defoliated fields just before harvest and one can expect live weevils to be packed into modules. Most weevils dispersed rapidly from untarped module surface when temperatures were warm enough for flight, but a small percentage remained at least to 24 h. Most weevils trapped on the surface under the tarp died from high temperatures. Survival of weevils inside modules was high after 1 and 3 d, but had declined dramatically by 7 d. The greatest threat of reinfestation by weevils dispersing from a module would occur when a module is constructed and transported during cool, cloudy weather, followed by warm weather favorable for flight at the gin yard. Other experiments were conducted to determine boll weevil mortality in various subprocesses in a cotton gin by introducing known numbers of weevils at various points in the system and estimating survival. We found no evidence that weevils can survive in the seed cotton to the gin stand or beyond. The greatest threat for weevil survival and escape from the gin occurs soon after entry, with chances of survival diminishing rapidly the further the weevils progress through the different ginning processes. Small numbers of live weevils can be expected to escape into the rock trap, either as free adults or in infested bolls (Brashears et al. 2003). Thus, it is important that at-risk gins either destroy the trash collected in the rock trap immediately or collect it in a container that will not permit weevil escape. Weevils protected inside unopened bolls can escape alive with the gin trash later in the cleaning process even when passed through a high-speed trash fan. If a mechanical device can be designed and installed to slightly crack open bolls as they move to the fan, this latter problem can be mitigated to a great extent.

Introduction

Several regions in the Cotton Belt where boll weevil eradication zones that have achieved, or are nearing, eradication share a border with a region where weevil populations are still high (El-Lissy and Grefenstette 2002, Kiser et al. 2002, Smith et al. 2002). Some of these still-infested areas have been in an active eradication program for only a short time, while others have yet to organize a program. Responding to a reinfestation is very expensive, so preventing and detecting reintroductions of weevils in eradicated or suppressed zones is a high priority. Boll weevils naturally disperse (Guerra 1986, 1988, Jones et al. 1992, Raulston et al. 1996), and migration from infested zones can slow progress in neighboring eradication zones (e.g., Allen et al. 2001). In the case of movement by flight, little can be done in the suppressed zone other than to remain vigilant until the nearby infested area advances in its eradication efforts. On the other hand, reintroductions can occur through human-mediated transport, and it is in this arena that adoption of preventive measures can have an impact.

We began a study in late 2000 to determine the potential for gins in advanced eradication zones to serve as loci of boll weevil reintroduction through their service to customers harvesting cotton in nearby infested areas. The economic consequences of reinfestation are so great in an advanced or eradicated zone, that the pressure to shut down movement of potentially infested cotton across its borders can become correspondingly great. At the same time, there are negative economic repercussions to the affected gins and growers if such movement is halted, so there is no desire to take unnecessary regulatory actions. Another common situation involves export of gin products out of an infested zone to weevil-free regions in the U.S. or other

countries. Current domestic and foreign quarantine regulations often require fumigation of cottonseed or cotton bales before import, costing the U.S. cotton industry millions of dollars annually. Although no data are available supporting the need for such measures, they are dictated by the economic consequences of a weevil introduction into a weevil-free region, so the reasonable course has been to err on the side of caution. Our research was initiated with the goal of determining the true threat posed by module transport, gin sites, and gin products to weevil-free or nearly eradicated zones, so that minimally intrusive regulatory measures can be designed without endangering weevil-free areas. Our first main objective was to determine whether boll weevil transport on or in cotton modules occurs, the magnitude of such transport if it occurs, and the threat of dispersal from a module before it is ginned. Our second main objective was to estimate weevil survival through different ginning subprocesses, and thus to determine the likelihood of a live weevil occurring in the various products of the gin, including gin trash, cottonseed, motes, and baled lint. This project continues, but preliminary results present an informative picture, and definitive information is available for some aspects. We report here details of module transport experiments, as well as survival experiments for the ginning subprocesses before, at, and through the gin stand. Information on survival through the seed cotton precleaning process and in the bale press is reported elsewhere (Brashears et al. 2002, 2003).

Materials and Methods

Presence of Boll Weevils in Defoliated and Harvested Cotton

Defoliated cotton fields were surveyed immediately before harvest to estimate the magnitude of boll weevil numbers exposed to collection by the cotton picker or stripper. The experiment was conducted in three regions of Texas in 2001 (see Sappington et al. 2002), and in the Lower Rio Grande Valley (LRGV) near Weslaco, TX, and the Northern Blacklands near Waxahachie, TX in 2002. Adult boll weevils present in defoliated cotton were sampled by beat bucket from four fields in each location. Five rows were sampled per field with all plants along a 25-m stretch of each row being shaken vigorously inside a large bucket. Beat-bucketing is a relative sampling method for boll weevils, which likely underestimates the number of weevils present (Raulston et al. 1998). All green or incompletely opened bolls were removed from the same 25 m of row and were dissected to determine percent infestation and the life stage distribution of infesting weevils. When there were more than 100 bolls from a row, a random subsample of 100 was dissected. All samples were taken 0-1 d before harvest. Ten ¼-barrel paper bag samples of seed cotton were collected from active module builders and represented at least two different dumps of the harvesters. Data reported here are from at least five of the bags. Processing continues at the time of this writing. For the 2002 samples, after the bags were returned to the laboratory, 10 weevils marked with paint were killed by freezing and mixed into the seed cotton in each bag to serve as controls for the ability to find natural weevils. The cotton in the bags was sorted by hand to recover marked and unmarked adult weevils. Boll weevils per module were estimated from the number of weevils per pound of seed cotton, assuming a 15,000-Lb (6810-kg) module and that weevils were evenly distributed throughout the module. The number of weevils expected to be packed within 1 cm of the surface was calculated from the estimated number of weevils per module, using module dimensions of 8x9x32 feet (2.4x2.7x9.8 m) (WxHxL).

Boll Weevil Mortality on, and Dispersal from, the Surface of Cotton Modules

Mark-recapture experiments were performed to characterize dispersal behavior of weevils from the surfaces of stationary modules. Twenty-five weevils captured in pheromone traps were marked with fluorescent powder and released onto the surface of modules within a 2-ft diameter release zone marked with module paint in each of five locations per module. Three releases made on the top of each module were immediately covered with the module tarp. One release was made on each of the long sides of the module. Weevils remaining on the modules were recovered after ~10 hr (at night using black lights) to ~24 hr in 2001, and after ~24 hr in 2002. In addition, weevils on the module sides were counted 10 min after release, because many were observed to disperse by flight soon after placement. Controls for baseline mortality consisted of one closed cardboard carton containing 25 marked weevils that was placed on the ground at the base of each module. These control weevils provided a baseline level of mortality. Experiments conducted in Lubbock in 2001 examining weevil dispersal from modules during transport have been described previously (Sappington et al. 2002).

Boll Weevil Mortality over Time inside Cotton Modules

Weevils were placed in cotton pouches, inserted inside a cotton module at different locations, and left for specified times to examine survival. Groups of 20 weevils were placed between two bats of seed cotton, which were sealed inside flat cotton pouches as described by Brashears et al. (2002). On each side of two cotton modules, a set of three pouches was inserted in each of three locations -- approximately 53 (high), 84 (middle), and 142 (low) cm from the top of the module -- by lifting a portion of the module with a fork-lift. From each set of three pouches, one was removed after each of 1, 3, and 7 d. In addition, there was one control pouch for each module side (replication) and duration tested. Control pouches were placed on a laboratory bench and held at room temperature. After removal, each seed cotton bat was searched for weevils which were classified as alive or dead (no movement when the snout was pinched).

Differences due to location within the module for the different days-in-module treatments and for pooled locations (or controls) between days were tested by ANOVA, with location and days as main effects, respectively. Means were separated by the Tukey HSD test ($\alpha = 0.05$). Differences between survival and controls for each number of days were evaluated by a two-sample *t*-test.

Boll Weevil Mortality in the Gin

As indicated by the module studies, live weevils can be expected to enter the gin with the module, either as free adults or in unopened bolls. Thus, studies on the survival of weevils during the ginning process were necessary. Ginning studies were conducted using the full-scale research gin at the USDA Cotton Ginning Research Laboratory in Lubbock, TX. Experiments were variously conducted using free adult weevils obtained from the USDA-APHIS Mission Plant Protection Center, Mission, TX, adult weevils enclosed in desiccated gel capsules (described below), or in naturally infested bolls collected in fields near Lubbock in 2001 and in the LRGV in 2001 and 2002. Free adults and encapsulated adults were marked with fluorescent powder which aided their recovery from samples sorted under a black light.

Many experiments incorporated adult boll weevils encapsulated in simulated pupal cells in addition to free adults. A larva within a boll creates an oblong hollow chamber in which to pupate. This pupal cell consists of tightly packed larval frass which is somewhat hard, but brittle. Field experiments in 2001 revealed that newly eclosed adult weevils can be present in pupal cells in the module. Because the pupal cells are roughly the size and shape of a cotton seed, we deemed it important to test the possibility of weevil survival in pupal cells through the point where seed or motes are removed from the lint. However, we found it impossible to collect pupal cells in the numbers needed for our experiments. As a substitute, we encased adult weevils in empty gelatin capsules (No. 4 size, T.U.B. Enterprises, Almonte, Ontario) which are of a size and shape similar to natural cells. Before use, the capsules were baked at 65°C under a vacuum of 760 mmHg for 24 h, and held thereafter in a sealed plastic bag with desiccant to make them fairly brittle. Although the physical properties of these simulated pupal cells differ from natural pupal cells in many respects, we judged that they would provide a conservative estimate of mortality in the ginning process because they likely offer greater protection than the natural cells. In all experiments, both capsules and weevils were marked with fluorescent powder to aid in recovery.

Before the Gin Stand: Gin Trash. Boll weevils removed by the seed cotton precleaning process (Brashears et al. 2003) can be shunted into the trash fraction at several points preceding the gin stand. Gin trash passes through a fan where it is pulverized before traveling through a cyclone and into a bur hopper, so several fan-tip speeds were tested for their effects on boll weevil mortality. Lots of 300 marked adult boll weevils were each distributed evenly in 20 Lb (9.1 kg) of gin trash, and fed by conveyor belt into an aluminum duct leading to a 42-in (107-cm) diameter fan operating at one of seven fan-tip speeds: 183, 198, 212, 226, 241, 255, and 269 ft/sec. Each fan-tip speed was tested five times, except the 183 ft/sec treatment which was tested 6 times. All debris were collected and sorted by hand to assess boll weevil survival. Live weevils were held in a petri dish with a cotton wick soaked in water until they were checked at 24 hr for survival. In a similar experiment, lots of 50 infested unopened bolls were passed through the fan, with three replications per speed. Aliquots of bolls were dissected prior to each experiment to determine percent infestation and life stage distribution of infesting weevils. Test procedures were the same as above, except the bolls were not embedded in gin trash when fed into the fan.

A follow-up experiment was conducted to determine if slightly cracking weevil-infested bolls before passage through a trash fan increases mortality of the infesting weevils over that observed in uncracked bolls. The distal end of each test boll was slightly cracked along its sutures by pressing with thumb and forefinger, or with pliers. Lots of 50 cracked, infested bolls were fed by conveyor belt at about 2.5 bolls per sec into an aluminum duct and through a fan operating at selected fan-tip speeds. There were two duct treatments. In the first, bolls were carried in an air stream through 134 inches (340 cm) of ductwork to a 90° elbow, followed by 21 inches (53 cm) of ductwork to the fan. The fan was tested at 183, 198, 212, 226, and 241 ft/sec (5 replications per speed). In the second, bolls passed directly down 72 inches (183 cm) of straight ductwork to the fan. The fan was tested at 212, 226, 241, and 255 ft/sec (5 replications per speed). In all cases, a static air velocity was maintained at 1 in (2.54 cm) of water. Weevil survival was determined as described previously.

At and through the Gin Stand. An experiment was conducted to determine survival of marked adult boll weevils, both free and encapsulated, during separation of the precleaned seed cotton into lint, seed, and mote fractions at the gin stand. Groups of free adult boll weevils (300) and encapsulated adults (100) were marked with distinct colors of fluorescent powder. Colors were changed at each replication. This accounted for weevils that may be temporarily caught in the seed roll and thus emerge in the lint, seed, or motes of a later replication. This was not observed. A 300-Lb (136.2-kg) lot of pre-cleaned seed cotton was picked up by the suction telescope and passed again through the cleaning system. As it reached the feeder apron at the gin stand, marked weevils and capsules were sprinkled simultaneously into the seed cotton as evenly as possible. Preliminary tests estimated the time necessary for the 300 Lb of seed cotton to pass over the feeder apron, and one person counted down the time to those introducing the weevils. The resulting lint fraction was collected in a bin and 10 subsamples of ~1000 g each were taken from upper, middle, and lower sections of the lint pile. All resulting seeds and motes, and the subsamples of lint, were searched under blacklight for live and dead weevils, as well as intact or nearly intact capsules. Live free weevils were pooled in a petri dish containing a cotton wick soaked in water, while weevils recovered alive in capsules, were returned to the capsule, and placed together in a petri dish without water. All weevils were examined after 24 hr for survival. Twenty marked free adults and twenty marked weevils in capsules were held in the laboratory at room temperature in a petri dish as controls for mortality. Free adult controls were provided with a cotton wick soaked in water. Mortality among controls was low in all cases, so the data are not presented. The experiment was replicated three times.

Mortality occurs in the separation process itself, but live weevils separated into the seed fraction could also be killed during transit to the seed bin. Thus, we conducted an experiment to determine the potential of free and encapsulated boll weevils surviving separation with the seed to survive the conveyance system from the gin stand to the seed bin. 300 free adult boll weevils and 100 encapsulated adults were marked and uniformly distributed into 50 Lb (22.7 kg) of cottonseed spread evenly along a 10-ft (3-m) conveyor belt. The weevil-seeded cottonseed was dumped into a bin and then fed into the seed conveyance system using a suction telescope. The seed was collected at the outlet through which it is dumped into a receiving truck. All seed thus retrieved was inspected under blacklight for dead and surviving weevils. Continued weevil survival to 24 h was checked as described above. The experiment was replicated three times.

Beyond the Gin Stand: Lint Cleaning. An experiment was conducted to determine survival of marked adult boll weevils, both free and encapsulated, in lint and mote fractions passing through a single saw-type lint cleaner. 300 free adult boll weevils and 100 encapsulated adults were marked with distinct colors of fluorescent powder. Preliminary trials established the amount of time necessary for 300 Lb (136.2 kg) of ginned cotton to pass an opened panel in the ductwork located between the gin stand and the lint cleaner. During the experiment, free and encapsulated weevils were simultaneously sprinkled as evenly as possible into the seed cotton as it passed the open panel while one person counted down the time. The lint was collected at the bale press, where it was lightly formed into a loose 'bale' at a pressure previously determined to cause no mortality (Brashears et al. 2002). The 'bale' was divided into 10 layers. Each layer was parted in the center, and approximately 1000 g (~2.2 Lb) of lint, drawing evenly from top to bottom at the part, was subsampled from each layer. This procedure was intended to reduce any variation caused by uneven sprinkling of the weevils into the seed cotton. The experiment was replicated three times. In addition, a control was included in which weevils were sprinkled into the lint but the lint was not cleaned. It was collected at the bale press and subsamples were taken and processed as described above. The control provided an estimate of efficiency in recovering marked weevils from the lint subsamples.

Results and Discussion

Presence of Boll Weevils in Defoliated and Harvested Cotton

Populations of adult weevils in defoliated cotton just prior to harvest were generally higher in 2002 than in 2001, especially in Waxahachie (Table 1). It is likely that most adults recovered in beat buckets were recently emerged from unopened bolls remaining on the plants. This is supported by a significant correlation ($r = 0.90$, $P = 0.03$) between mean estimated number of teneral adults per acre in unopened bolls and the mean estimated number of free adults per acre. Ongoing analyses of cuticular hydrocarbon profiles, which can be used to estimate weevil age (Sappington et al. 2000), will test the validity of this hypothesis more directly.

A total of 96.3% of marked control weevils were recovered from the bags of seed cotton, suggesting that very few unmarked weevils were overlooked. Unmarked adult weevils were recovered from most samples of seed cotton from the module builders, and their numbers indicated that from 210 to 7215 weevils were packed into individual modules (Table 2). A proportionate number of weevils, 3 to 100, were estimated to have been packed within 1 cm of the module surface, representing potential dispersants before entering the gin.

Boll Weevil Mortality on, and Dispersal from, the Surface of Cotton Modules

Very few boll weevils released on the sides of modules were still present after 24 h, whether dead or alive (Table 3). In most cases, the majority of weevils dispersed from the module sides within 10 min after release. An exception where none of the weevils flew during the first 10 min was a series of releases on the morning of Sep. 24, 2001 in Waxahachie when temperatures were < 18°C. Another exception (Aug 15, 2002, Weslaco) occurred during releases on a morning after a nighttime rain, where only about 20% of the weevils dispersed during the first 10 min. Although it was sunny and air temperature at the time of release on this date was above 27°C, evaporative cooling of the module surface may have influenced weevil behavior. Nevertheless, 90% and 94% of the released weevils, respectively, had dispersed by 24 h. Dispersal was primarily by flight, but a small number of weevils were observed to walk upward from the release circle and into the shade afforded by the tarp by 10 min after release. Weevils generally had little difficulty walking on the sides of the modules, although some occasionally became tangled in the fibers. Among the live and dead weevils still present after 24 h, about half were obviously tangled in the cotton fibers, but an occasional untangled live weevil was observed.

Most weevils recovered on the top surface of the module under the tarp were dead (Table 4), presumably from high temperatures. A few, and sometimes many, released weevils could not be found after 24 h. These weevils most likely burrowed into the loose cotton on the top of the module, and some perhaps escaped lethal temperatures in this way. Direct evidence for burrowing has been reported elsewhere (Sappington et al. 2002). Weevils had great difficulty walking across the top surfaces of modules, and it seems unlikely that any walked from their release sites under the tarp to disperse by flight. We found very few weevils outside the release circles, and most were recovered within a few cm of the center of the circle where they were released, often tangled in cotton fibers.

The behavior of the released weevils suggests that a module represents a relatively hostile environment, particularly in warm, sunny weather, and that most weevils on its surface after module construction will disperse as soon as possible. Heat-induced death is common for those trapped on the surface under the tarp (see also Arnold et al. 2003), although a few may survive by burrowing. Low temperatures can also kill weevils on the surface under the tarp (Arnold et al. 2003). The interval of time between module construction and transport to the gin yard, as well as how long the module rests in the gin yard before being ginned, depends on how busy the gin is at that time. During peak harvest, it is common for modules to remain in the field for 1-2 d, and in the gin yard for up to a week. Our results indicate that the probability of weevils being transported on the surface of a module to disperse later in an eradication zone, either en route or at the gin yard, decreases rapidly with time after module construction. Though few in number, live untangled weevils were occasionally found on the side of a module 24 h after release. The greatest threat would occur when a module is constructed and transported during cool, cloudy weather, which is followed by warm weather favorable for flight at the gin yard. If a module from an infested area must wait a day or more before a gin in an advanced eradication zone is prepared to process it, it would be advantageous if it were allowed to remain in the harvested field during the waiting time rather than in the gin yard.

Boll Weevil Mortality over Time inside Cotton Modules

There were no significant differences in survival of weevils in pouches within the days-in-module treatments among high, middle, or low locations in the cotton modules (F's 0.07-0.91, df = 2, 9, P's 0.44-0.93) (Table 5), so data for locations within days were pooled. Survival was significantly affected by the number of days in the module (F = 79.83, df = 2, 33, $P <$ 0.0001). Survival was relatively high, and not different than in the controls, up to 3 days in the module, but was much less by 7 days (Table 5). Although weevil survival in the control pouches also declined with days (F = 6.77, df = 2, 9, $P < 0.05$), survival in the module to 7 days was significantly less than in the controls (Table 5). The weevils used in this experiment were from the non-diapausing colony at the Mission facility, so it is possible that diapausing weevils would survive longer in the module. However, if the hypothesis is correct that most weevils picked up at harvest are newly emerged adults that have not yet dispersed, then most weevils packed into modules will not be in diapause, and will have low fat reserves with a corresponding low overwintering survival potential (Rummel et al. 1999).

Boll Weevil Mortality in the Gin

Before the Gin Stand: Gin Trash. Our results indicate that few if any weevils survive seed cotton precleaning to pass on to the gin stand in the seed cotton fraction (Brashears et al. 2003). Thus, most if not all weevils that escape seed cotton precleaning alive must do so by exiting with the gin trash. However, all trash, except that exiting into the rock (boll) trap, passes through trash fans prior to final collection. Passage through trash fans provides adequate mortality of pink bollworms if fan-tip speed is high enough (Robertson et al. 1959, Hughs and Staten 1995). In our experiments, a few weevils were found alive shortly after passage through fans operating at tip-speeds of 198 and 226 ft/sec, but all were badly mutilated, and none survived to 24 h (Fig. 1). We found no evidence that a free adult weevil can survive any of the fan-tip speeds tested. The number of dead weevils recovered from the trash that retained at least half a body declined with increasing fan-tip speed, suggesting a corresponding decrease in the likelihood of weevil survival at those speeds.

A small percentage of teneral adults inside unopened green bolls survived even the highest fan-tip speed tested (Table 6). This speed (269 ft/sec) was deemed the maximum at which the fan could be operated safely. A few live larvae were recovered at the two lowest speeds. Thus, it is possible for a small percentage of teneral adults to escape the gin inside unopened green bolls regardless of the fan-tip speed. When infested bolls were slightly cracked before they entered the trash fan, a few adults were still alive when first recovered from the resulting boll debris, but all were severely damaged and none survived 24 hr (Table 7). No larvae or pupae passed through the fans alive. The presence of an elbow in the conduit may enhance the efficacy of the fan by further breaking up the bolls, but the differences between elbowed and straight conduit were not significant for given speeds (t-tests, P's 0.14 - 0.35). We conclude that a device which cracks open green bolls at their sutures would greatly reduce, and perhaps prevent, survival of weevils exiting a gin in the gin trash and passing through a fan operated at any speed. Designs of potential devices for cracking bolls are currently being considered.

At and through the Gin Stand. Lots of 300 Lb (136.2 kg) of cleaned seed cotton yielded 102 - 112 Lb (46.3 - 50.8 kg) of lint, 175 - 183 Lb (79.4 - 83.1 kg) of cottonseed, and 1.8 - 2.0 Lb (0.81 - 0.89 kg) of motes. Only an estimated 11.6 $\pm$ 3.28% of the free adult weevils fed into the gin stand were recovered dead or alive in the lint, seeds, and motes (Tables 8-9), indicating that most weevil bodies were destroyed at the gin stand. Of the weevils recovered, 69.2% were found in the lint, 19.7% were found in the cottonseed, and 11.1% were found in the mote fractions. One recovered weevil was alive at 2 h after passage through the gin stand into the lint fraction, but it was damaged and did not survive to 24 h (Table 8). Hughs et al. (2002) introduced 1000 weevils into cleaned seed cotton at the gin stand in three different runs and recovered no surviving weevils in any of the approximately 70-Lb lots of resulting lint. They did recover a nearly whole, but dead and damaged weevil in one of the lint lots. Thus, it is clear that the probability of survival through the gin stand is close to zero. Only two live weevils were found among the three lots of cottonseed, and only one lived to 24 h (Table 9), indicating a mean survival rate into the seed fraction of 0.1 $\pm$ 0.11% among weevils entering gin stand after precleaning. Five live weevils were found among the

three lots of motes, and four of these lived to 24 h (Table 9), indicating a survival rate into the mote fraction of 0.4 ± 0.11% among weevils entering the gin stand after precleaning.

Of the encapsulated weevils introduced in the three runs, only an estimated 6.6% were recovered dead or alive in the lint, seeds, and motes (Tables 8-9), again indicating that most capsules and the weevils therein were destroyed at the gin stand. Of those recovered, 24.5% were found in the lint, 75.6% were found in the cottonseed, and none were found in the motes. No live weevils were recovered from the lint or motes. Of the nine live encapsulated weevils recovered in the seed fraction, only one survived to 24 h (Table 9).

Mortality of boll weevils segregated into the seed fraction at the gin stand can occur during transport to the seed bin. Any mortality of weevils occurring during passage through the seed conveyance system must be caused by physical trauma associated with striking the walls of the conduits at high speed, especially at elbows. Because there are no fans or other mechanical devices through which the seed passes after separation at the gin stand, it was anticipated that survival would be high. Average immediate survival was indeed high for both free adults (75%) and those in capsules (90%) (Table 10). However, many of these weevils were damaged, as evidenced by the survival to 24 hr, which was only 30% for free adults and 35% for encapsulated adults.

Beyond the Gin Stand: Lint Cleaning. In a control run, boll weevils were introduced into ginned lint which was subsampled after passage to the bale press without the seed cleaning system operating. We expected to recover 61 of the 300 introduced free adult weevils based on the percentage of ginned lint that was subsampled (Table 11). We found 60 weevils, indicating that we could be confident that free adults would not get lost in the long system of ductwork, and that we would not overlook any weevils in sub samples of cleaned lint. We expected to recover 20 of the 100 introduced capsules in the control lint, but only found 10 (Table 11). The reason for the low recovery of capsules is unknown. Because capsules are relatively easy to detect in the lint samples, it is unlikely that they were present but missed. It is possible that the missing capsules became lodged in the system ductwork.

When passed through a single saw-type lint cleaner, no free adults were found alive in the cleaned lint (Table 11). A total of one dead and one live encapsulated weevil was found, but the live weevil was damaged and did not live to 24 h. Thus, we found no evidence that boll weevils can survive passage through a lint cleaner into the lint fraction. Robertson et al. (1963) found that no pink bollworm larvae survived passage through a saw-type lint cleaner. 48.7 ± 6.54% of the introduced free adults and 40.7 ± 9.77% of the encapsulated adults were recovered in the mote fraction (Table 11). The probability of surviving seed cotton precleaning and escaping in the mote fraction was 1.2 ± 0.48% for free adults and 1.0 ± 0.58% for encapsulated adults. Many weevil parts were observed in the mote fraction, suggesting that the bodies of many of the unrecovered weevils were destroyed in the lint cleaning process.

Conclusions

Our results indicate that in boll weevil-infested regions, live boll weevils are usually present in defoliated cotton fields and can be expected to be packed alive into cotton modules. Although mortality of weevils inside a module increases with time, there will likely be live weevils fed into the cotton gin at least up to 7 d after harvest. Dispersal from the surface of the module is rapid under weather conditions which promote flight. We have no evidence that weevils are attracted to modules, and our mark-recapture experiments strongly suggest the module surface is a hostile environment which boll weevils tend to leave quickly. Nevertheless, a small percentage of live weevils were found on module surfaces after 24 h, and these represent potential future dispersants.

We examined weevil survival through various subprocesses in the gin, by introducing large numbers of marked weevils and observing how many were recovered alive in the various outputs of those subprocesses. The cumulative probability of survival to any point in the ginning process can be determined by multiplying the respective probabilities of survival of preceding subprocesses. For example, there has been recent concern in both Arkansas and California regarding the risk of transporting live boll weevils in cottonseed from gins in infested areas to weevil-free areas. The probability of survival into the cottonseed is a product of sequential weevil survival through the seed cotton precleaning process, one or two dryers at a given temperature, the separation process at the gin stand, and transport in the seed conveyance system. We now have data from which to calculate a quantitative estimate of the risk posed by imported cottonseed to weevil-free areas.

Our data provide no evidence that a weevil can survive the seed cotton precleaning process either under a normal (more rigorous) precleaning regime typically used for stripper-harvested cotton or under a minimum (less rigorous) precleaning regime typically used for picker-harvested cotton, and thus we have no evidence that a weevil can survive in the seed cotton to approach the gin stand (Brashears et al. 2003). There is a statistical problem, in that we cannot prove a zero-rate of survival, because one could argue that if the test were sensitive enough (i.e., if the sample size were large enough), we might find a surviving weevil. To set a worst case upper limit on the observed zero-rate of survival, we have chosen to take the most liberal statistical scenario and say that if the sample size were increased by one, the test would have been sensitive enough to

detect one surviving weevil in the minimum seed cotton precleaning process. Under our experimental design, we would have expected to recover 1636 marked weevils (Brashears et al. 2003) from the minimum cleaning process if survival was 100% in the seed cotton. The statistical worst-case parametric mean survival estimate of the observed survival rate of 0% is calculated by increasing the sample by one and assuming recovery of one surviving weevil: 1/1637 = 0.0006 or 0.06%. This worst-case parametric estimate is likely a gross overestimate, because very few weevil parts were recovered in the cleaned seed cotton, and no intact weevils were recovered. In our judgment, actual survival is much closer to the observed 0% than to the calculated worst-case survival rate of 0.06%.

Many gins employ one or two dryers during seed cotton precleaning, and operating temperatures are adjusted depending on the moisture content of the seed cotton. We subjected cohorts of live marked weevils to passage through one or two dryers operating under a range of temperatures (Brashears et al. 2003). Mortality occurred in dryers even when no heat was added, and increased at temperatures above 150°F. Mortality was 100% at 300°F with one dryer, and at 185°F with two dryers. Because gins are sometimes operated with unheated dryers, we do not include this mortality in the worst-case scenario calculations for survival into the cottonseed. Our data from the gin stand experiment indicated a 0.1% rate of survival for any weevils entering the gin stand and segregating into the seed fraction (Table 9). Of those surviving into the seed fraction, 30% can be expected to survive conveyance to the seed bin (Table 10). Thus, using the upper limit for surviving the minimum seed cotton precleaning process with no added heat in the dryers, the probability of a weevil surviving into the seed fraction can be calculated as:

$$0.0006 \times 0.001 \times 0.3 = 0.0000002$$

Assuming a 50% chance of a weevil being female, there is a 0.00001% chance that a female weevil entering the gin will escape alive in the seed under the most liberal statistical assumptions and ginning conditions most favorable for survival.

Estimates of actual numbers of weevils which may survive can be obtained from our survey data on the number of live weevils packed into modules (Table 2). The median estimated number of weevils recovered per module was 1463 (unpublished data) per 15,000-Lb (6810-kg) module. Our experiments have shown that survival of weevils within a module decreases with time (Table 5), but in these calculations we will assume 100% survival into the gin. The number of female weevils surviving into the seed fraction per module under this scenario can be calculated as:

$$1463 \times 0.0000001 = 0.0001463$$ or 1 female in the seed per 6835 modules ginned.

If one assumes 650 Lb (295 kg) of seed produced per bale and 12-bales per module, this translates to about 1 female per 26,652 tons (24,192 mT) of cottonseed. We emphasize that this survival rate is probably an overestimate of actual survival for the reasons explained above, including an expected increase in mortality in any seed cotton dried at temperatures above 150°F. In addition, reinfestation would require that any female weevil surviving into the seed must be mated, and must survive a prolonged period in the packaged seed. This may be possible if the female is in diapause, but a reproductive or prediapausing female can be expected to live only a few days. Furthermore, these calculations are based on weevil numbers in areas not under an eradication program at the time of sampling. Areas where eradication is in progress will have greatly reduced numbers of weevils entering the gin.

In conclusion, although it may be possible for boll weevils to survive into the cottonseed, our experiments provide no evidence that they do. Even under the most liberal statistical extrapolation, the estimated number of weevils that might survive into the seed fraction is still extremely low. When other probable mortality factors are considered, the likelihood of a weevil surviving in cottonseed to later escape into a weevil-free area is infinitesimally small.

The likelihood of a weevil surviving beyond the gin stand and into the bale can be calculated in a like manner. The rate of survival through the gin stand is very low (Table 8; see also Hughs et al. 2002). We observed no evidence of weevil survival through a single saw-type lint cleaner (Table 11), and two lint cleaners are often employed at gins. If a weevil somehow survived to the bale press, it cannot survive the pressures encountered during tie-out in a normal-weight bale (Brashears et al. 2002, Hughs et al. 2002). Thus, the requirements of importing countries for bale fumigation can be lifted with no risk of boll weevil introductions.

The greatest threat for weevil survival and escape from the gin occurs early in the process. Small numbers of live weevils can be expected to escape into the rock trap, either as free adults or in infested bolls (Brashears et al. 2003). Thus, it is important that at-risk gins either expeditiously destroy the trash collected in the rock trap or collect it in a container that will not permit weevil escape. Weevils protected inside unopened bolls can escape alive with the gin trash later in the cleaning process even when passed through a high-speed trash fan (Table 6). If a mechanical device was designed and installed to slightly crack open bolls as they move to the fan, this problem would be greatly mitigated (Table 7).

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Table 1. Mean (± SE) numbers of boll weevils recovered by beat bucket (BW/25m), and in unopened bolls from defoliated cotton (n=20) near Weslaco (Wes), Waxahachie (Wax), and Lubbock (Lub), TX. Mean percentage of bolls infested with live boll weevils, and life stage distribution among those weevils found infesting bolls are indicated: L, larvae; P, pupae; A, adults.

							Stage			*Teneral*
Year	**Loc**	**BW/25m**	***BW/ac*[a]**	**Bolls/25m**	***Bolls/ac*[a]**	**%Infested**	**%L**	**%P**	**%A**	***Adults/ac*[a]**
2001	Wes	6.2 ±1.62	*1004*	148.4 ±37.85	*24,023*	3.9 ±0.64	49.3 ±10.66	20.5 ±9.08	32.5 ±14.66	*304*
	Wax	0.2 ±0.05	*32*	3.5 ±0.65	*567*	0	--	--	--	*0*
	Lub	0.3 ±0.16	*49*	46.4 ±20.06	*7,511*	4.5 ±2.63	26.3 ±14.50	15.0 ±14.01	59.0 ±20.4	*199*
2002	Wes	10.7 ±1.84	*1732*	136.1 ±59.68	*22,032*	27.8 ±12.64	62.0 ±29.24	19.0 ±6.11	19.1 ±9.45	*1170*
	Wax	4.1 ±1.28	*664*	97.3 ±17.64	*15,750*	4.7 ±1.04	8.1 ±5.90	20.1 ±6.35	71.9 ±8.50	*532*

[a]Values were calculated by extrapolation.

Table 2. Boll weevils (mean ± SE) recovered from samples of harvested seed cotton pulled from module builders immediately after harvester dumping (BW/lb) in fields near Weslaco (Wes), Waxahachie (Wax), and Lubbock (Lub), TX.

Year	**Location**	**BW/lb**	***BW/mod*[1]**	***BW near Surface*[2]**
2001	Wes	0.08 ± 0.065	*1200*	*17*
	Wax	0.01 ± 0.014	*210*	*3*
	Lub	0.25 ± 0.154	*3750*	*52*
2002	Wes	0.48 ± 0.314	*7215*	*100*
	Wax	0.15 ± 0.035	*2310*	*32*

[1]Calculated based on 15,000-Lb module.
[2]Weevils calculated to be within 1 cm of the surface, based on module of dimensions 8x9x32 feet (WxHxL).

Table 3. Mean (± SE) fate of marked boll weevils 10 min and 10-24 h after release (25 weevils per release) on side surfaces of cotton modules. Weslaco (Wes) and Waxahachie (Wax), Texas.

					Recovered at 10-24 h		
Year	**Loc**	**Release Date**	**# Releases**	**% Dispersed by 10 min**	**# Alive / Release**	**# Dead / Release**	**% Dispersed by 10-24 h**
2001	Wes	Aug 6-9	16	87.2 ± 2.93	0.3 ± 0.15	0.4 ± 0.15	97.1 ± 0.92
	Wax	Sep 21-24	24	87.0 ± 5.84	0.5 ± 0.34	1.1 ± 0.38	93.7 ± 2.14
2002	Wes	Aug 12-15	24	56.2 ± 5.54	0.6 ± 0.18	0.3 ± 0.16	96.3 ± 0.99
	Wax	Sep 16-17	24	82.2 ± 2.41	0.3 ± 0.11	0.9 ± 0.23	95.3 ± 1.17

Table 4. Mean (± SE) recovery of marked boll weevils 24 h after release (25 weevils per release) on top surfaces of cotton modules. Weevils were covered with the module tarp immediately after release. Weslaco (Wes) and Waxahachie (Wax), Texas.

				Recovered (or Missing) at 10-24 h			
Year	**Loc**	**Release Date**	**# Releases**	**# Alive / Release**	**# Dead / Release**	**# Missing / Release**	**# Controls Alive at 10-24 h**
2001	Wes	Aug 6-9	21	0.2 ± 0.11	13.2 ± 0.71	11.6 ± 0.69	--
	Wax	Sep 21-24	30	0.2 ± 0.08	7.0 ± 1.02	17.8 ± 1.05	23.1 ± 0.77
2002	Wes	Aug 12-15	34	0.4 ± 0.30	22.6 ± 0.71	2.0 ± 0.62	23.4 ± 0.45
	Wax	Sep 16-17	36	1.2 ± 0.25	14.8 ± 0.93	9.0 ± 0.93	21.5 ± 1.28

Table 5. Mean (± SE) number of boll weevils (n = 20) recovered alive from sealed pouches after indicated number of days when placed at different relative locations within a cotton module. Lubbock, TX, Oct. 22-29, 2002.

Days	High	Middle	Low	Loc Pooled	Control
1	18.5 ± 0.65	18.3 ± 1.11	18.8 ± 0.95	18.5 ± 0.48a	18.3 ± 1.18a
3	16.3 ± 1.93	14.0 ± 2.71	16.0 ± 2.45	15.4 ± 1.28a	18.3 ± 1.03a
7	0.5 ± 0.50	3.8 ± 2.59	2.3 ± 1.32	2.2 ± 0.98b*	10.5 ± 2.53b

Means ± SE followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD).
* Significantly different than control ($P < 0.05$, two-sample *t*-test).

Table 6. Percent survival of boll weevils of different developmental stages infesting unopened bolls 24 h after passage through a gin trash fan operated at the indicated fan-tip speeds.

Fan-tip	Stage in Unopened Bolls		
Speed (ft/sec)	Adult	Pupa	Larva
183	3.3 ± 3.3	0	0.8 ± 0.83
198	0	0	5.6 ± 5.56
212	3.3 ± 3.3	0	0
226	0	0	0
241	3.6 ± 2.06	0	0
255	3.8 ± 2.21	0	0
269	2.6 ± 2.56	0	0

Table 7. Percent survival of boll weevil adults and immatures infesting bolls that were mechanically cracked before passage through straight or elbowed ductwork to a gin trash fan operated at the indicated fan-tip speeds.

Ductwork	Fan Speed (ft/sec)	% Adults Survived 2 hr	% Adults Survived 24 hr	% Immatures Survived 2 hr
Elbow	183	2.4 ± 2.38	0	0
	198	7.1 ± 2.92	0	0
	212	2.4 ± 2.38	0	0
	226	0	0	0
	241	0	0	0
No Elbow	212	4.8 ± 2.92	0	0
	226	9.5 ± 2.92	0	0
	241	2.4 ± 2.38	0	0
	255	6.0 ± 3.44	0	0

Table 8. Number of recovered free adult and encapsulated boll weevils in subsamples of lint fraction after passage through the gin stand. Marked weevils and capsules were introduced after seed cotton precleaning.

		Free Adults			Encapsulated		
Rep	% of Lint Subsampled	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr
1	21.7	7	0	0	0	0	0
2	19.7	1	1	0	0	0	0
3	20.1	6	0	0	1	0	0
Total		14	1	0	1	0	0

Table 9. Number of recovered free adult and encapsulated boll weevils in seed and mote fractions after passage through the gin stand. Marked weevils and capsules were introduced after seed cotton precleaning.

	Seed						Motes					
	Free Adults			Encapsulated			Free Adults			Encapsulated		
Rep	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr
1	5	2	1	3	3	1	4	2	2	0	0	0
2	4	0	0	3	6	0	0	1	1	0	0	0
3	9	0	0	0	0	0	3	2	1	0	0	0
Total	18	2	1	6	9	1	7	5	4	0	0	0

Table 10. Mean (± SE) free and encapsulated boll weevils found dead or alive in cottonseed after transport through the seed conveyance system.

Weevils	# Reps	Dead	Alive at 2 hr	Alive at 24 hr	% Recovered
Free (n=300/rep)	3	55.3 ± 3.76	225.0 ± 6.51	91.3 ± 12.03	93.4 ± 1.25
Encapsulated (n=100/rep)	3	19.0 ± 6.33	90.0 ± 4.35	35.3 ± 6.49	96.3 ± 1.76

Table 11. Free adult and encapsulated boll weevils recovered (out of 300 and 100 introduced per replication, respectively) after passage through one saw-type lint cleaner in subsamples of cleaned lint and the entire mote fraction. Controls (C) represent weevils and capsules introduced to ginned lint and collected at the bale press, but not passing through an operating seed cleaner.

	Cleaned-Lint Fraction							Mote Fraction					
		Free Adults			Encapsulated			Free Adults			Encapsulated		
Rep	% Lint Sampled	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr	Lb of Motes	Dead	Alive 2 hr	Alive 24 hr	Dead	Alive 2 hr	Alive 24 hr
C	20.4	3	57	52	0	10	10	0	--	--	--	--	--	--
1	21.5	0	0	0	0	0	0	4.4	92	20	4	17	5	2
2	21.1	4	0	0	0	0	0	4.7	155	25	6	38	7	1
3	21.2	3	0	0	1	1	0	4.0	136	10	1	55	0	0

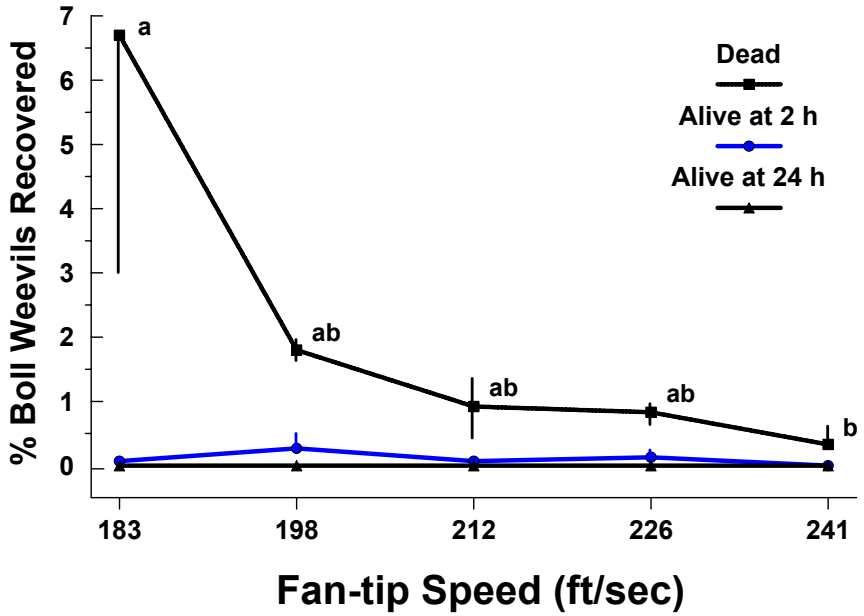

Figure 1. Mean (± SE) recovery of live and dead adult boll weevils (n = 300) after passage with gin trash through a trash fan operating at indicated fan-tip speeds. Means of dead weevils followed by the same letter are not significantly different (Kruskal-Wallis test, $\alpha = 0.05$).