#### Behavioral Studies of the Southern and Tawny Mole Cricket

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Studies were begun using radiographic technology to visualize the movement and feeding patterns of both tawny and southern mole crickets in soil. Tawny mole crickets produce a characteristic 'Y' shaped tunnel that allows two escape routes to the surface and down into the soil, and a long tunnel into the soil profile that most likely aids in thermal and water regulation. Environmental conditions can alter, but do not destroy the stereotypical movement patterns of tawny mole cricket behavior. Predatory southern mole crickets appear to burrow at the thatch/soil interface perhaps searching for food. Studies conducted in this project indicate that prey size is a major determinate in the acceptability of tawny mole crickets as southern mole cricket food.

When disturbed, both mole cricket species discharged a oily, highly odorous substance from their abdomen. Discharges were collected for biological and chemical assays in our laboratory. Radiographic analysis shows a clear avoidance of tawny mole cricket to areas near the discharge. Live tawny mole crickets do no seem to affect the tunnel patterns of their neighbors suggesting that they do not discharge their compounds around other tawny mole crickets. By comparison, live southern mole crickets away move as far from each other as possible when placed together in a chamber. This suggests, but does not conclusively confirm, the presence and activity of a chemically-mediated avoidance behavior in this species. Adult southern and tawny mole crickets were dissected in order to remove anal and protodeal glands. Gas chromatography and mass spectraphotometry of all samples indicated a range of hydrocarbon compounds. Electroantennograms and electropalpograms gave no differential response among the 13 extracts tested. We see no indication of the presence of a long-range male or female sex or aggregation pheromone in tawny or southern mole cricket adults.

Radiographic assays with a synthetic insecticide and the fungal pathogen Beauveria suggests that tawny mole crickets can sense and avoid high concentrations of the product in soil thereby reducing overall insecticide activity. It should also be noted that this behavior did not occur in every insecticide-treated chamber suggesting that the affect may be transient or be in response to only the parent or one or more breakdown products. Radiographic experimental designed so crickets could not escape insecticide suggested a decline in burrow construction and

Field studies in North Carolina have provided significant new information on mole cricket development, dispersal, field behavior, interspecies relationships, and the influence of soil environment on damage and control. The consistently earlier egg hatch and development of tawny mole cricket nymphs is a key to survival in areas also inhabited by southern mole crickets. Since behavior is influenced by nymph size and since the initiation of control strategies is affected by egg hatch the relationship that has been established soil temperatures and degree day accumulation and the occurrence of these events is important new information. This will help target management strategies to those most susceptible stages as well as providing insight into the best timing to diminish the likelihood of mole cricket behavior minimizing the control strategies effectiveness and improved follow-up scouting and management efforts. Additional research on the effect of soil moisture on egg hatch and surface damage helps us determine when visible surface damage is most likely and when environmental conditions favor significant egg survival in nonirrigated areas. This information has also helped us determine preferred areas of egglaying for both species and is providing significant insight into the identification of "high-risk" areas to help reduce scouting time and develop guidelines for targeting the use of new insecticides which are most effective when used in a preventive mode.

### Project Title: Behavioral Studies of the Southern and Tawny Mole Cricket

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### The goals of this project are to:

- improve our understanding of tawny mole cricket and southern mole cricket (southern mole cricket) behavior especially as affected by environmental conditions through radiographic studies.
- 2) isolate and determine the activity of sex, aggregation and alarm pheromones of the tawny mole cricket.
- 3) determine the behavior of tawny mole crickets in the presence of microbial and chemical insecticides.
- 4) initiate field studies to better understand tawny and southern mole cricket behavior as suggested by laboratory studies.

# Improve our understanding of tawny mole cricket and southern mole cricket behavior especially as affected by environmental conditions through radiographic studies.

Before detailed studies can be undertaken on the impact of environmental factors and disease status on mole cricket behavior can be undertaken, a clear picture of 'typical' tawny mole cricket and southern mole cricket behavior must be understood. Studies were begun using radiographic technology (x-rays) to visualize the movement and feeding patterns of both tawny mole cricket and southern mole cricket in the soil matrix. Through the placement of a small lead tag on each cricket, tunnel construction and cricket movement in the tunnel could be monitored over.

A series of time lapse radiographs over a twenty-two day period (figure 1: a through i) shows the stereotypical behavior of a single late instar tawny mole cricket nymph. This nymph produces a characteristic 'Y' shaped tunnel that allows two escape routes to the surface and down into the soil to escape predators including larger southern mole crickets, and a long tunnel into the soil profile that most likely aides in thermal and water regulation. Tawny mole crickets typically forage at the root/soil interface between the Y' arms and are therefore allay near an escape route. As tawny mole crickets grow, their burrows widen and extend further into the soil profile suggesting a possible cause for the difficulty in bringing older crickets to the surface through soap flushes and baits. Crickets also seem to maintain their tunnel system, rebuilding collapsed tunnels over time. When there is more than one tawny mole cricket in an area each

cricket builds its own tunnel system that they maintain over time (as seen in figure 2a). There appears to be little burrow sharing between tawny crickets in the same in the same arenas.

Environmental conditions can alter, but do not destroy the stereotypical movement patterns of tawny mole crickets. For example, a subsoil composed of high density sand does not extinguish the `Y' shaped burrows of the tawny mole crickets but may cause a bending or the burrow (figure 3a) or a termination of the burrow (figure 3b) at the density boundary.

By comparison, the predatory southern mole cricket appears to create less extensive burrows than do tawny mole cricket. Instead, southern mole cricket burrow predominately at the thatch/soil interface perhaps searching for prey items (figure 2b). Since most plant feeding soil insects including tawny mole crickets are found in this soil zone this is a highly adaptive behavior. Studies conducted in this project indicate that prey size is a major determinate in the acceptability of prey for southern mole crickets. In these laboratory studies southern mole crickets were presented tawny mole crickets that were small, of equal size, or larger than themselves. Within one week southern mole crickets had killed and eaten all smaller tawny mole crickets while tawny mole crickets of equal or larger sizes survived. One week later, southern mole crickets preyed upon tawny mole crickets of equal size but did not attack larger tawny mole crickets. Tawny mole crickets that were larger than southern mole crickets caged with them were never attacked by their smaller, hungry cousins.

# Isolate and determine the activity of sex, aggregation and alarm pheromones of the tawny and southern mole crickets

Tawny and southern mole crickets were collected in North Carolina and transported to the NYSAES, Geneva, New York for laboratory analysis. It was noted that when disturbed, both mole cricket species discharged a oily, highly odorous substance from their abdomen. Discharges were collected for biological and chemical assays in our laboratory:

a) preliminary chemical analysis: a small discharge sample from each cricket species was prepared for analysis through the use of gas chromatography. Although there appeared to be basic similarities in the two species discharges as indicated by overlapping peaks in parts of the GC detection strip charts, there were also clear differences in the southern mole cricket & tawny mole cricket discharges indicating unique compound constituents in the discharges for these two species. We are currently working in concert with electroantennogram analysis to determine which peaks are bioactive and therefore should be analyzed further (identification & synthesis).

#### b) Behavioral response of mole cricket nymphs to discharge:

Tawny mole cricket discharge was collected on absorbent cotton and placed in soil arenas along with several tawny mole cricket nymphs. Radiographic analysis shows a clear avoidance of tawny mole cricket to areas near the discharge impregnated cotton further suggesting the

biological activity of the discharge. Interestingly, live tawny mole crickets placed in the same chamber do no seem to affect the tunnel patterns of their neighbors suggesting that they do not discharge their compounds around other tawny mole crickets.

By comparison, live southern mole crickets will move are far away from each other as possible when placed together in a chamber. Figure 4 (a through c) show a time lapse series of the tunneling patterns of two southern mole crickets of approximately equal size. This and other similar series suggest, but do not conclusively confirm the presence and activity of a chemically-mediated avoidance behavior in this species.

1. Biological activity & isolation of sex, alarm and defensive kairmones: Adult southern and tawny mole crickets were dissected in order to remove anal (males and females) and female protodeal glands. crickets were anesthetized with high does of carbon dioxide gas and then chilled for 15 minutes. Dissections were done under sterile saline solution. When carbon dioxide was administered the cricket usually discharged an oily substance that was collected on filter paper for analysis and bioassays. A pair of anal glands and the hind gut were collected from each insect; in addition, the protodeal gland was collected from all females. I glands were soaked in hexane for one hour to extract any bioactive compounds. Gas chromatography and mass spectraphotometry of all samples indicated a range of hydrocarbon compounds. Electroantennograms and electropalpograms gave no differential response among the 13 extracts tested. A double tube bioassay to determine if live crickets showed any behavioral response to the various extracts. There appears to be at least two distinct behaviors exhibited by the crickets. Once they entered a tube, they either moved directly to the end and remained there clawing the end cap, or they exhibited a 'backing' behavior. This behavior consisted of backing up and moving forward, usually in the vicinity of the extract coated filter paper. They also appeared to pause and groom in this region. The strongest initial response was the response of the southern mole cricket male to southern mole cricket female protodeal gland extract but even this response was variable. Additional assays will be conducted to verify or dismiss these preliminary results. Interestingly, when male and female southern mole crickets were place in the tube together, the female often approached the male, after which both the male's and female's behavior was altered, becoming agitated, but the pair had minimal physical contact. The female then flipped on her back and dragged her abdomen over the inner tube, releasing liquid onto the tube and then righting herself. The nature and bioactivity of this extract is being investigated. We see no indication of the presence of a long-range male or female sex or aggregation pheromone in tawny or southern mole cricket adults although we continue to investigate that these compounds do in fact exist.

### Determine the behavior of tawny mole crickets in the presence of microbial and chemical insecticides.

Field studies conducted during 1995 by RLB suggested that biological and chemical insecticides may alter the behavior of mole crickets thereby affecting the performance of these agents in the field. Preliminary radiographic assays with one synthetic insecticide suggests that tawny mole crickets can sense and avoid high concentrations of the product in soil thereby reducing overall activity. Radiographic experimental designs where crickets could not escape insecticide suggested a decline in burrow construction and maintenance. Figure 5 shows the response of two tawny mole cricket nymphs were placed in chambers that contained low density sand in the top third of the chambers and high density sand in the bottom two thirds. Additionally, a mole cricket insecticide was incorporated into the upper left half of the low density sand in each chamber. This treatment was one treatment in a larger study testing the interaction of insecticides and soil compaction on cricket behavior. The radiographs in figure 3 can be considered the control treatment for this study. One can see that tawny mole crickets avoid that portion of the chamber that contains the incorporated insecticide by burrowing into the high density sand (5a) or by remaining in the untreated low density sand at the surface (5b). One should note that the crickets continued to burrow at the root zone in both sides of the chambers. It should also be noted that this behavior did not occur in every insecticide-treated chamber suggesting that the affect may be transient or be in response to only the parent or one or more breakdown products.

Studies suggest that fungal pathogens placed at the soil surface may also repel crickets and cause them to spend reduced time at the surface thereby increasing the possibility that there may be poor overlap of inundative release of fungal pathogens and crickets in the field. Figure 6 shows the behavior of two tawny mole crickets twelve days after their placement in there chambers. Radiograph 6a shows the typical `Y' shaped burrowing pattern of the tawny mole cricket. By comparison figure 6b shows the tunnels of a tawny mole cricket when high concentrations of the fungal pathogen Beauveria were placed at in the root zone. As you can see this cricket has changed its burrowing system and separated its tunnel from contact with the soil containing the pathogen. Similar results were observed with a different fungal pathogen against Japanese beetle grubs using similar chambers.

To further study this phenomenon, a baiting technique was developed to evaluate the possible deterrent effects of the Beauveria against tawny mole crickets. This technique works well in conjunction with radiographic and other nondestructive monitoring techniques. Crickets used in this study were placed on uniform feeding regime prior to the initiation of the study. All food was withdrawn for 24 hours prior to test to clear the cricket's digestive systems.

An attractive bait (laying mash + feeding stimulant) was prepared following Kepner and Yu 1987. Calico Red Dye N-1700 was incorporated into the bait to allow an indirect measure of bait consumption through dissection and spectrophotometric evaluation (Daum et al. 1969). Beauveria conidia was incorporated into half of the baits (treatment) while the other half of the baits were left fungus-free (control). Crickets were individually placed in test arenas containing soil and turfgrass. A known quantity of either control or fungus-treated bait was placed on the surface of each arena. Crickets were to feed for 8 to 12 hours and removed. Half of the crickets were frozen to measure the quantity of bait (and therefore a comparison of feeding between crickets presented with control and Beauveria baits) fed upon during the previous eight hours. The other half of the crickets were removed and placed individually n new arenas containing only soil and turf to evaluate the impact of the fungus on cricket mortality.

To determine the quantity of bait the crickets feed upon the following protocol was followed:

Crickets were dissected and the entire digestive tract was removed (figure 7a shows the dissected that fed on a bait that did not have dye incorporated into it, while figure 7b shows the red digestive tract of a crickets that feed upon diet into which dye had been added. The digestive systems were macerated with forceps and the dye was extracted with acetone. The concentration of red dye in the resulting solution was determined using a spectrophotometer at 517 nm. This technique allowed us to determine that the presence of Beauveria in baits reduced mole cricket feeding by approximately one half when compared to untreated baits. Most, but not all crickets, in both the control and fungus-treated bait arenas fed on some of the bait in their areas but crickets tended to feed on more of the bait if fungus was not present. Some crickets in both treatments did not feed. These results agree with the radiographic studies that suggest that Beauveria can alter tawny mole cricket behavior. Monitoring of the crickets not dissected showed the beginning of cricket mortality due to Beauveria infection approximately 21 days post treatment. The study was terminated at 21 days post treatment due to high control mortality but only crickets exposed to Beauveria in baits were killed by the fungus.

## Initiate field studies to better understand tawny and southern mole cricket behavior as suggested by laboratory studies.

Field studies in North Carolina have provided significant new information on mole cricket development, dispersal, field behavior, interspecies relationships, and the influence of soil environment on damage and control. This labor intensive effort during the past three years have worked in concert with the laboratory studies conducted at Cornell University and provided guidance and insight for these studies as well as serving as field validation of laboratory findings.

Studies have documented coexistence among the two species of mole crickets despite the predatory nature of the southern mole cricket against the tawny. The consistently earlier egg

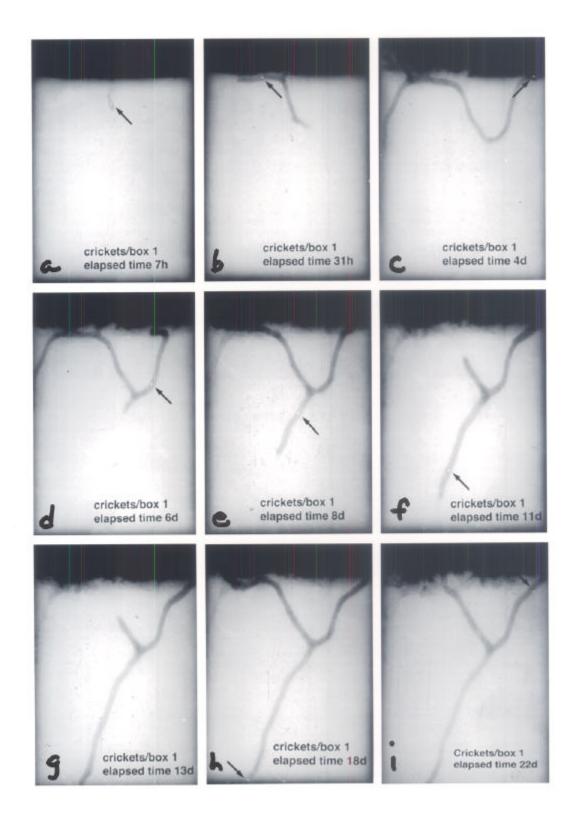
hatch and development of nymphs is a key to survival of the tawny in areas inhabited by southern mole crickets (Figure 8). Southern mole crickets will aggressively feed on tawny mole crickets that are the same size or smaller. The nymph emergence curve for tawny mole crickets is generally two weeks ahead of the same curve for southern mole crickets. However, the nymph emergence curve appears to be truncated due to mortality of late hatching nymphs that are preyed upon by larger southern mole crickets. This overall nymph emergence curve, its timing and duration is critical to effective management of both species of crickets.

Since behavior is influenced by nymph size and since the initiation of control strategies is affected by egg hatch the relationship that has been established soil temperatures and degree day accumulation and the occurrence of these events is important new information (Figure 9). This will help target management strategies to those most susceptible stages as well as providing insight into the best timing to diminish the likelihood of mole cricket behavior minimizing the control strategies effectiveness. In addition, the duration of the egg hatch can be predicted and lead to improved follow-up scouting and management efforts. A manuscript documenting this research and its application is planned within the next year.

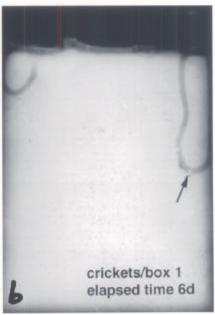
Additional research on the effect of soil moisture on egg hatch and surface damage helps us determine when visible surface damage is most likely and when environmental conditions favor significant egg survival in nonirrigated areas. Research data taken under various soil moisture and soil type regimes and in conjunction with and without pesticide use provides us with circumstantial evidence on mole cricket behavior and the response to insecticide use which can be confirmed in the laboratory (Table 1).

This information has also helped us determine preferred areas of egglaying for both species and is providing significant insight into the identification of "high-risk" areas to help reduce scouting time and develop guidelines for targeting the use of new insecticides which are most effective when used in a preventive mode (e.g. Merit (imidacloprid) and Chipco Choice (fipronil)). These data, in conjunction irrigation studies on the effectiveness of control strategies, is allowing us to document the expected effectiveness of these strategies under a range of field conditions. This is essential information for targeting control efforts to those conditions that encourage maximum effectiveness and minimize overall pesticide use. This is particularly useful for the effective use of biological control materials.

However, research during the past two years and field observations throughout the Southeast indicate that even performance of the "state of the art" product, Chipco Choice, is influenced by irrigation, nymph development, and cricket behavior and this has resulted in numerous instances of customer complaints. Our research and a review of the site data from the manufacturer has allowed us to pinpoint those time periods when mole cricket behavior reduces, at least in the short-term, the effectiveness of this product.







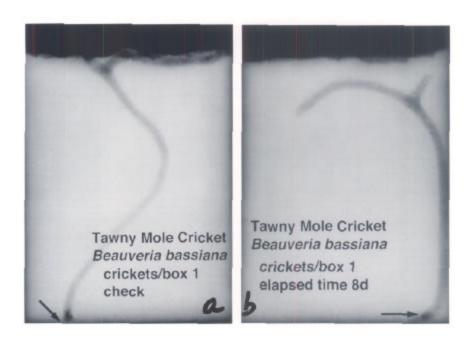








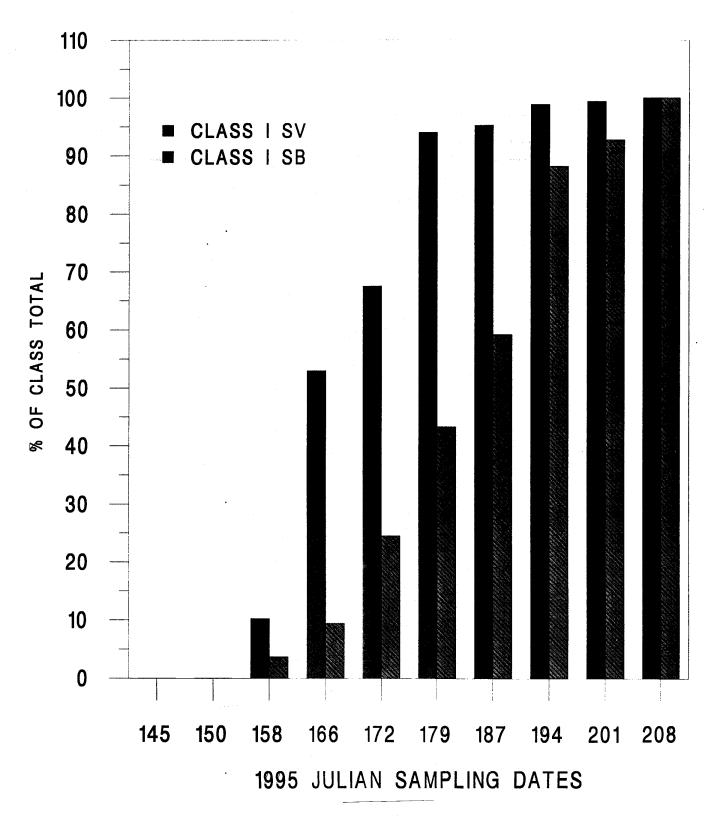








Class I nymphs of both tawny and southern mole crickets for 1995 sampling dates, Fox Squirrel C C, Brunswick Co. NC.



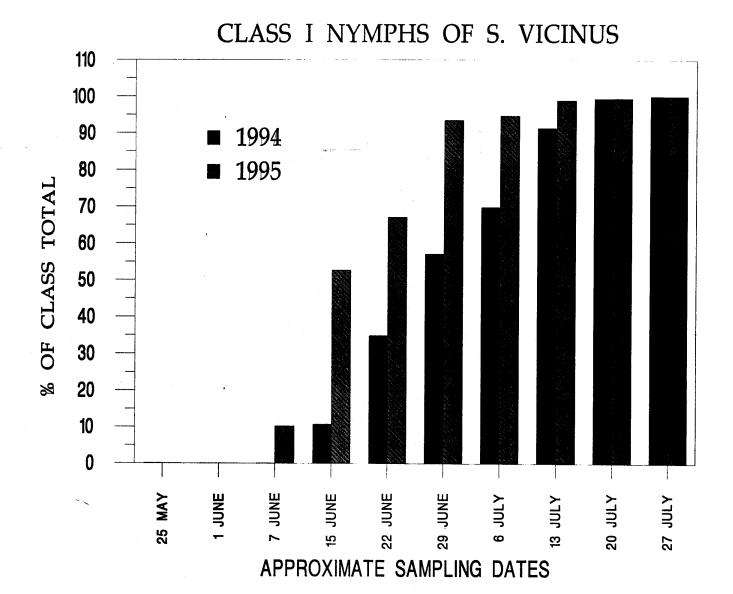


Table 1. Effects of irrigation on the efficacy of Lambda-cyhalothrin applied for the control of mole cricket nymphs, Brierwood Golf Club, Brunswick Co., N.C., evaluation of 14 August 1996 (6 DAT), 5 damage grid ratings (0 - 9) per replicate (0 = no damage, 9 = severe damage).

Treatment	Rate (form)	Irrigation Schedule	Mole oricket damage ratings ave. of 5 damage ratings/rep.				Average
	oz/A)		I	11	Ш	IV.	
Simitar GC	10	Pre/No post	0.2	0.8	0.8	0.4	0.55
Simitar GC	10	No Pre/Post at 2 hr	2.2	3.0	1.4	1.6	2.05
Simitar GC	. 10	No irrigation	2.6	0.8	1.0	2.2	1.65
Simitar GC	10	Pre/Post imm.	0.4	1.0	2.4	2.4	1.55
Untreated		Pre/Post	5.4	1.4	0.4	2.4	2.40