Feeding Ecology of Pest Mole Crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) and Associated Damage to Turfgrass

by

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Abstract

Two omnivorous species of mole crickets (Orthoptera: Gryllotalpidae), *Scapteriscus vicinus* Scudder and *S. borellii* Giglio-Tos, are introduced subterranean pests that cause damage to turfgrass by feeding and tunneling in the southeastern United States. Unlike strict herbivores, the impact of omnivorous pests on host plants can vary according to the availability of plant and animal prey. After a series of greenhouse experiments investigating the influence of earthworm (*Eisenia fetida* Savigny) prey on hybrid bermudagrass by adult *S. vicinus* and *S. borellii*, it was shown that alternative prey, when present, may result in a negative impact on turfgrass roots from foraging omnivorous mole crickets. In laboratory experiments, the feeding preference of both mole cricket species was determined using choice and no-choice tests. In choice tests, *S. borellii* preferred an animal diet and plant diets were preferred by *S. vicinus* supporting the previous studies on gut contents. Additionally, further investigation about the impact of diet on survival and nymphal development provided insight into the relative benefits of each dietary choice on these omnivores. Overall, *S. borellii* provisioned with animal diet had less mortality, greater body mass, and faster development compared to the ones fed on plant diet. Even though *S. vicinus* is primarily a herbivorous species, nymphs fed on animal diet gained more weight and developed faster than the ones fed on plants. This work suggests that *S. vicinus* and *S. borellii* have the capability of nutrient regulation and adaptability on various diets, and that an animal diet benefits both species in terms of survival and nymphal development.

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CHAPTER 1

GENERAL INTRODUCTION

Biology of Mole Crickets

There are three species of mole crickets (Orthoptera: Gryllotalpidae) present in Alabama. The northern mole cricket, *Neocurtilla hexadactyla* (Perty), is a native species found throughout the eastern United States that rarely causes damage in turf (Potter 1998). The tawny mole cricket, *Scapteriscus vicinus* Scudder, and the southern mole cricket, *S. borellii* Giglio-Tos are invasive species and among the most significant turf pests in the southeastern United States (Walker and Nickle 1981). They were inadvertently introduced from South America in ship's ballast into ports of Georgia, South Carolina, Alabama, and Florida in the early 1900's (Walker and Nickle 1981). The spread of these two species was rapid due to the ability of flight and in the absence of natural enemies. Although colder temperatures and arid conditions would probably restrict additional spread, these pests have occupied most of the southern region, extending northward to North Carolina and westward to eastern Texas (Brandenburg 2002) and even an isolated location in Arizona (Potter 1998).

Morphological characters are usually used in separating the species of *Scapteriscus* mole crickets. In the forelegs of *S. vicinus*, there is a narrow V-shaped space (Figure 1.1) between two tibial dactyls; while in *S. borellii*, the tibial dactyls are widely separated to form a U-shaped space (Figure 1.2; Hayslip 1943, Potter 1998). Another distinguishing characteristic concerns the

dorsal pronotum. Generally, the pronotum of *S. vicinus* is broader and more robust than that of *S. borellii*. Moreover, *S. vicinus* is golden brown colored with a central band on the pronotum, whereas *S. borellii* is gray colored with four spots or a mottled pattern on its pronotum (Walker 1984, Potter 1998).

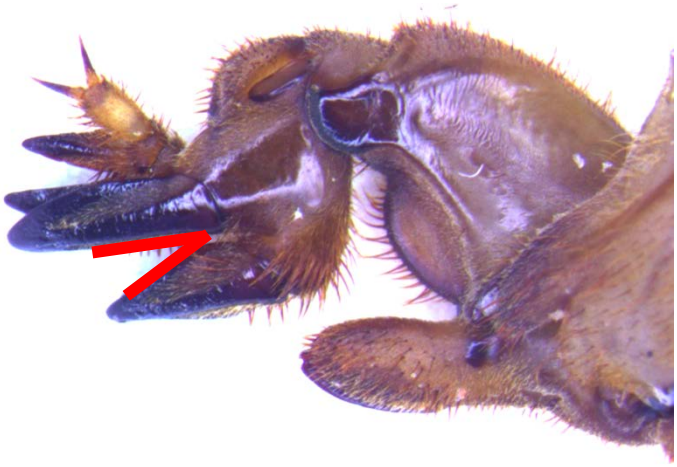

Figure 1.1. V-shaped dactyl of adult *S. vicinus*.

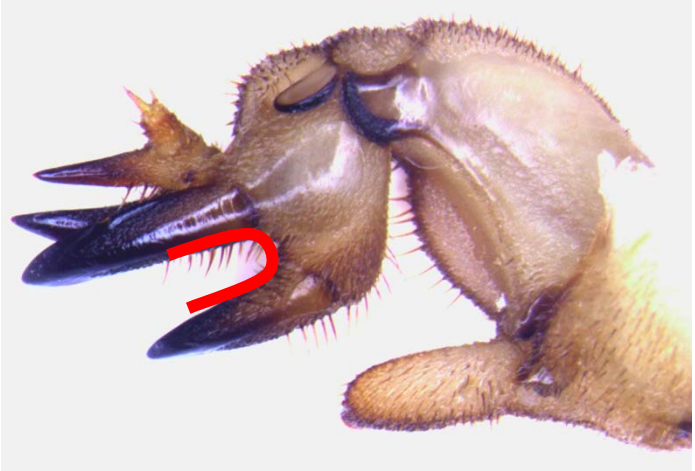

Figure 1.2. U-shaped dactyl of adult *S. borellii*.

In central Florida and northward, both *S. vicinus* and *S. borellii* have one generation per year (Potter 1998). They seem to have a similar life cycle, except that the majority of *S. vicinus*

overwinter as adults, whereas most *S. borellii* overwinter as large nymphs, maturing by the following spring (Hayslip 1943). Mole crickets spend nearly their entire lives underground in excavated tunnels. However, the adults also fly for local searching for mates and new egg-laying sites or for long range dispersal (Walker 1984). Larger spring flights and minor fall flights occur for each mole cricket species in most of the southeastern United States. Peak spring flights of adult *S. vicinus* begin by mid-late March, whereas large flights of *S. borellii* occur in May or June. Generally, fall flight activities of both species are concurrent with minor peaks in October (Braman and Hudson 1993).

Like most other crickets, male mole crickets of both species produce a loud calling song to attract flying or walking females. The noisy song is produced by opening and closing the forewings, which have stridulatory organs. Externally, forewings of the male have a pair of harp-shaped large cells and a dark spot resulting from the coalescence of wing veins. These characters distinguish this sex from the female (Figure 1.3; Hayslip 1943, Walker 1984). Calling songs of *S. vicinus* and *S. borellii* are species-specific trills that differ in the tone and pulse rate. However, calling generally begins 10 to 30 min after sunset and continues for about one hour for both species (Walker 1984). Louder and larger males attract more females and probably have better chances for copulation. Immediately after being fertilized, females have dispersal flights for finding suitable oviposition sites. Eggs are laid in ovate shaped clutches located from one inch to one foot beneath the soil surface in an egg chamber (Hayslip 1943). The oviposition period of female *S. borellii* typically is 30 d longer than that of *S. vicinus* (Hayslip 1943). *Scapteriscus borellii* generally oviposits more eggs than *S. vicinus* (Braman 1993). The period of egg incubation is influenced by temperature, but generally is 3 wk (Hayslip 1943). The peak of hatching occurs in June for both species (Hayslip 1943). The newly hatched nymphs often eat the

chorion and cannibalize siblings in the egg clutches, and construct a small tunnel straight up to the soil surface for escape (Hayslip 1943, Walker 1984). Nymphs develop underground through the fall with at least six or seven stages (Walker 1984) and continuously expand the tunnel system using their powerful forelegs. Along with the rapid growth of nymphs, their damage to turf increases linearly with time of year (Hertl and Brandenburg 2002).

Figure 1.3. Forewings of male (left) and female (right) *S. vicinus*.

Damage by Mole Crickets to Turfgrass and Management

Turfgrass is considered the most widely grown ornamental crop in the United States. In the southern region, turfgrass and sod production are in the top five in horticultural sales (Pettis 2004). The estimated economic impact of turfgrass and sod in Alabama was $150.37 million in 2007 which is an increase of 50.5% since 2003 (Alabama Agricultural Experiment Station 2009). Turfgrass contributes substantially to the national economy and provides environmental, aesthetic, and recreational benefits (Potter 1998).

Mole Cricket Damage

Mole crickets can damage or kill turfgrass by direct feeding on roots and stems. Among species of turfgrass, hybrid bermudagrass, bahiagrass, St. Augustinegrass, and centipedegrass are the most severely damaged (Potter 1998). When night temperatures are warm and the soil is moist, mole cricket adults and nymphs typically move up to the soil surface and feed on grass (Hayslip 1943). Heavy feeding damage can result in severe turf loss during late summer and fall in Alabama (Cobb 1998). Another important factor in injury to turfgrass is subsurface tunneling created by both mole cricket species. Extensive tunneling uproots the plants, dehydrating the roots, and eventually killing the whole plant (Potter 1998). Generally, *S. borellii* appears to do much more surface burrowing, especially in sandy soil, compared with the mainly herbivorous *S. vicinus* (Hayslip 1943, Walker 1984). Mole crickets primarily create vertical tunnels and horizontal galleries in the top 20 to 25 cm of soil and occasionally tunnel as deep as 75 cm (Walker 1984). Their tunneling activity is correlated to soil moisture (Hayslip 1943, Ulagaraj 1975, Hertl and Brandenburg 2002), soil type (Reinert 1983, Villani et al. 2002), and time of year (Adjei et al. 2003, Hertl and Brandenburg 2002). Mole crickets generally prefer moist, sandy soil conditions and their surface activities peak in June–July. Soil radiographs clearly indicated that the tunnels made by *S. vicinus* branch more readily at the surface and have a Y-shaped pattern as opposed to the meandering type produced by *S. borellii*. Castings of tunnels from the two species suggest that their tunneling patterns are related to their respective feeding behaviors (Villani et al. 2002).

Mole cricket damage and cost of control in Florida alone are estimated at $170 million per year in total (Xia and Brandenburg 2000). Though only the southern half of Alabama is considerably affected by mole crickets, still over $12 million is spent annually to control them

(Cobb 1998). Several market research studies have indicated that golf course managers usually spend $5,000 to $25,000 each year for mole cricket management (Shaw 1993).

Sampling Techniques

Effective management of mole crickets requires monitoring their seasonal development and following a systematic approach to control. Generally, attempts at field sampling of mole cricket populations have included such methods as soap flushing, linear pitfall trapping, light traps, and acoustic trapping. Soap flushing is the easiest and most convenient method of field sampling for mole crickets. It is most effective when the soil is warm and moist (Hudson 1985, Potter 1998). Pitfall traps capture both adults and nymphs mole crickets but extensive manpower is needed to operate the traps, which capture many other irrelevant insects (Hudson 1985). Both *S. vicinus* and *S. borellii* can be captured at light traps. However, lights are restricted to the adults during flight at night. Acoustic trapping broadcasts the real or imitation calling song of the male and attracts flying mole crickets of both sexes (Walker 1988). This method has made it possible to acquire large numbers of living mole crickets for research on chemical and biological control.

Biological Control

In the last 50 years, extensive research related to non-chemical and more permanent controls of *Scapteriscus* mole crickets has been conducted. Until the mid to late 1980's, biological control agents were imported from the southern South America. From the histogram of 25 years of pest mole cricket data (Frank and Walker 2006), the releases of the ectoparasitoids *Larra bicolor* F. (Hymenoptera: Crabronidae) and *Ormia depleta* (Wiedemann) (Diptera: Tachinidae), and the beneficial nematode *Steinernema scapterisci* Nguyen & Smart (Rhabditida:

Steinernematidae) have worked successfully to regulate mole cricket population in Gainesville, Florida. The geographical expansion of *O. delpeta* is limited to the peninsular Florida (Walker et al. 1996). *Larra bicolor* currently is widespread in Florida (Frank and Walker 2006) and reported from coastal Mississippi and Georgia (Held 2005), as well from golf courses in the southern and coastal Alabama (Abraham et al. 2008). Recent works (Arévalo and Frank 2005, Abraham et al. 2010) explored nectar sources as attractants for *L. bicolor* to increase their impact on pests in turf habitats. These papers documented the successful establishment of this effective parasitic wasp and its natural expansion in the southern United States.

Another desirable method of mole cricket management would be the development of genetic resistance (mole crickets do not eat the grass under any circumstances), or at least non-preference (mole crickets prefer other grasses) in turfgrass cultivars (Hanna et al. 2001, Held and Potter 2012). However, these turfgrass hybrids could not eliminate mole crickets in practice due to the differences in tunneling activity and feeding behavior of the two species. As previously reported (Braman et al. 2000), nymphal survival of *S. vicinus* and *S. borellii* was not influenced by turfgrass type. Moreover, genotypes, as Hanna et al. (2001) suggested, probably would place selection pressure on the insects to adapt to the new grass hybrid, and might narrow the selectable range in turf establishment. To turfgrass managers and golfers, turfgrass agronomic characteristics and aesthetics are generally more significant factors in turfgrass selection than pest resistance (Held and Potter 2012). These studies (Braman et. al. 2000, Xia and Brandenburg 2000, Hanna et. al. 2001) suggested a need to better understand the biology and ecology of pest mole crickets to improve practical management programs.

Chemical Control

Conventional insecticide applications are most effective when they target the vulnerable life stages of mole crickets (Shaw 1993). Applications of insecticides such as fipronil and bifenthrin in June, July, and even early August on the more vulnerable nymphs are more effective than later applications on larger mole crickets (Cobb 1998). Mapping infested areas in spring and targeting these sites for treatment may save labor and reduce insecticide applications and costs (Cobb 1998). The soap flush technique can be used from spring to fall to verify the presence of newly hatched nymphs and to monitor their development. Thus, the factors that influence nymphal development should be better understood. Baits are another important insecticide management strategy for mole cricket control. Commercial products such as Advion Mole Cricket Bait, active ingredient indoxacarb, are formulated on diet and applied to the surface. Bait products must be consumed to be effective. Therefore, investigating the feeding preference and dietary implication for nymphal development is essential to better understand this practical management strategy.

Known Feeding Ecology of Mole Crickets

Although commonly considered as turfgrass pests, *S. vicinus* and *S. borellii* are omnivores feeding on both plant material and animal tissues (Potter 1998). Specifically, they feed largely on decomposing organic matter in the soil and a wide variety of plants including peanut kernels, ripening strawberries, young seedlings of vegetables (Hayslip 1943), sugarcane, underground tubers of potato, carrot, sweet potato (Walker 1984), pastures and turfgrass (Potter 1998). Mole crickets have also been reported to consume hamburger meat (Abate 1979) and soil-inhabiting animals including other mole crickets (Hayslip 1943).

Previous studies (Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and

Brandenburg 2011) used dissections of the alimentary tract to contrast the feeding habits of the two mole cricket species. Nearly 90% of materials observed in *S. borellii* were animal tissue with some plant parts. *Scapteriscus vicinus* appeared to be principally herbivorous based on primarily plant parts in their alimentary tracts. They concluded that *S. vicinus* should be accorded a greater pest status than *S. borellii*, due to its high level of herbivory. Though these studies have given some insight into the feeding habits of mole crickets, they provide limited insight into the dietary choices and consequences for mole crickets. Many external factors may contribute to differences in the contents of the digestive systems of mole crickets. For instance, each of the above referenced studies also collected nymphs and adults of mole crickets that were found to have nothing or unidentifiable remains in their alimentary tracts. It has been suggested that flight (Ulagaraj 1975) and different rates of digestion (Taylor 1979) may be associated with these phenomena. Therefore, observation on the feeding behaviors in choice and no-choice experiments will either refute or support these findings.

Even though we know that both mole cricket species are omnivores (Taylor 1979, Potter 1998), little is understood about their feeding behavior. In the experiments with omnivorous European mole cricket, *Gryllotalpa gryllotalpa* L., individuals raised on an herbivorous diet showed a significantly slower development, a reduction in longevity, and no reproduction (Godan 1964). This supports the idea that omnivorous mole crickets require a balance of both plants and prey to successfully live and reproduce. Omnivory provides flexibility in adjusting to variable resource supplies and better utilization of different food sources at different life stages (Coll and Guershon 2002). Generally, variation in the quantity or quality of a diet can have significant effects on insect development (Chapman 1998). Typically, there is a significant increase in food-intake in the penultimate instar. However, there is only limited information

about the diets of *S. vicinus* and *S. borellii* at different nymphal stages.

As described by optimal foraging theory, dietary self-selection by insects is a continuous regulation of food intake that involves frequent shifts between foods. Unlike the simple diet switching in food shortage, when food is sufficient, omnivorous insects can self-select the intake that is most efficiently utilized for growth and metabolism (Waldbauer and Friedman 1991). Because of the ability to adapt to plant and animal diets, dietary self-selection behavior potentially influences the impact to host plants by omnivorous pests. Further work is needed to examine feeding ecology of these omnivorous mole crickets and their damage to turfgrass.

Objectives

Although mole cricket management is not a new issue, the feeding ecology of *S. vicinus* and *S. borellii* is still poorly understood. This work investigated dietary preference of these mole crickets and the interaction between their feeding and the damage caused to turfgrass through a series of laboratory and greenhouse experiments, including 1) investigating the interaction between feeding ecology of mole crickets and their damage to turfgrass, 2) determining feeding preference of adult *S. vicinus* and *S. borellii* in laboratory feeding assays, 3) determining the influence of diet on survival and development of these mole crickets.

CHAPTER 2

POTENTIAL NEGATIVE EFFECTS OF EARTHWORM PREY ON DAMAGE TO TURFGRASS BY OMNIVOROUS MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE)

Abstract

The severity of damage to host plants by omnivorous pests can vary according to the availability of plant and animal prey. Two omnivorous mole crickets, *Scapteriscus vicinus* Scudder and *S. borellii* Giglio-Tos, were used to determine if the availability of prey influences damage to hybrid bermudagrass by adult mole crickets. Experiments were conducted in arenas with either grass alone (control), grass plus one mole cricket, grass plus earthworms (*Eisenia fetida* Savigny), or grass with earthworms and a mole cricket. Root growth variables (e.g., volume, dry weight) after 4 wk and weekly measurements of top growth were compared among the treatments. Surprisingly, bermudagrass infested with either mole cricket species caused no significant reduction in root growth and a minimal reduction on top growth with *S. vicinus* compared to controls. Survival of earthworms with *S. borellii* was significantly lower than survival in the earthworm only treatment suggesting predation. Survival of earthworms with *S. vicinus*, however, was not different from the earthworm only treatment. The addition of earthworm prey with mole crickets did not significantly impact bermudagrass root or shoot growth relative to grass with only mole crickets. Despite no negative impacts from earthworms or mole crickets separately, earthworms plus mole crickets negatively impact several root

parameters (e.g., length) suggesting an interaction between these two soil-dwelling invertebrates. Increased use of more target-selective insecticides in turfgrass may increase available prey. This work suggests that alternative prey, when present, may result in a negative impact on turfgrass roots from foraging omnivorous mole crickets.

Introduction

The tawny mole cricket, *Scapteriscus vicinus* Scudder, and the southern mole cricket, *S. borellii* Giglio-Tos are among the most significant turf pests in the southeastern United States (Walker and Nickle 1981). These inadvertently introduced species can cause severe damage to turfgrass by direct feeding and subsurface tunneling (Hayslip 1943, Potter 1998). Several studies (Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and Brandenburg 2011) have used dissection to identify the gut contents and to interpret dietary patterns of field-collected mole crickets. Based on these studies, *S. vicinus* was considered herbivorous due to primarily plant material in their alimentary tracts, whereas *S. borellii* was deemed carnivorous because animal tissues were more prevalent. However, both species feed on plant material and animal tissues and should therefore be considered omnivorous.

Both species are considered destructive because their excessive subsurface activity uproots plants, dehydrates the roots, and may destroy a stand of turf (Hayslip 1943, Potter 1998). Their tunneling activity is related to soil moisture (Hayslip 1943, Ulagaraj 1975, Hertl and Brandenburg 2002), soil type (Reinert 1983, Villani et al. 2002), and time of year (Adjei et al. 2003, Hertl and Brandenburg 2002). Mole crickets generally prefer moist, sandy soil conditions and their surface activities peak in June–July. The tunneling activity is also hypothetically related to their dietary needs (Villani et al. 2002). Specifically, the more intensive tunneling at the root-

soil interface is attributed to herbivory from *S. vicinus* whereas the extensive branching of the tunnel below ground allegedly indicates a carnivorous habit of *S. borellii* (Villani et al. 2002).

The impact of omnivorous pests on the host plants can vary according to the availability of plant and animal prey (Coll and Guershon 2002, Rosenheim et al. 2004). During periods of limited prey, omnivores typically switch to herbivory (Gillespie and McGregor 2000). Likewise, when prey is abundant, omnivores may reduce consumption of plant material (Agrawal et al. 1999). Using *Scapteriscus* mole crickets as a model system, we determined how the availability of prey would impact subsequent damage to turfgrass by omnivorous mole crickets.

Materials and Methods

Sources of Insects. Adult female *S. borellii* were collected from 1–5 Jun 2011 using a modified acoustic trap (Thompson and Brandenburg 2004) from the driving range at Grand National Golf Course in Opelika, AL. Adult *S. vicinus* were collected by soap flush (30 ml of Joy liquid detergent in 8 l of water) on 25 Oct 2011 on tee boxes at Great Southern Golf Club in Gulfport, MS. All collected individuals were immediately washed with fresh water upon emergence, and then were transferred into separate 473 ml plastic cups (Dart, Mason, MI). Cups contained autoclaved, moistened sand and had ventilated lids. They were provided a mixed diet of organic carrot strips (Inter-American Products, Cincinnati, OH) and freeze-dried mealworms (Coleoptera: Tenebrionidae; Fluker Farms, Port Allen, LA), and held in a growth chamber (Percival Scientific Inc., Perry, IA) at 27 °C with a 14:10 (L:D) photoperiod prior to the experiments.

Source of Turfgrass. Hybrid bermudagrass 'Tifway 419', *Cynodon dactylon* (L.) Pers × *C. transvaalensis* Burtt-Davy, plugs were harvested from the Auburn University Turf Research

Unit, Auburn, AL and transplanted to PVC arenas (15 cm diameter × 38 cm tall) in Apr 2010 for the experiment with *S. borellii* and in Jul 2011 for the experiment with *S. vicinus*. Arenas were designed similarly to those used by Braman et al. (2000). Each arena was covered at the bottom with a plastic Petri dish lid (15 cm diameter × 1 cm tall, VWR International, Radnor, PA), open on the top, and filled with the same volume of fine sand. Grasses were watered daily, fertilized weekly with a solution containing 250 ppm of Peters 20N-10P-20K (Scotts-Sierra Horticultural Products, Marysville, OH), and cut weekly to a height of 5 cm.

Experiment Setup. Greenhouse experiments were conducted with *S. borellii* and *S. vicinus* adults from 23 Jun–14 Jul 2011 and from 14 Dec 2011–11 Jan 2012, respectively. For each assay, the following treatments were tested: grass only (the control), grass with earthworms, grass with mole crickets, and grass with earthworms plus mole crickets. Arenas with mole crickets were infested with one adult mole cricket. For treatments with earthworms, 10 living *Eisenia fetida* Savigny (Haplotaxida: Lumbricidae; approximately 4–6 cm long, 1.5 mm thick; Uncle Jim's Worm Farm, Spring Grove, PA) were initially added to each arena. Weekly, three more worms from the same source were added to each arena to supplement prey. Each treatment was replicated six times. All arenas were covered with aluminum insect screen (Phifer, Tuscaloosa, AL) to prevent the escape of mole crickets (Figure 2.1). The daily temperature was monitored with a temperature data logger (HOBO U23 Pro v2, Onset Computer, Bourne, MA). Watering and fertilization regimes were maintained as described previously.

Impact of treatments on damage to turfgrass was assessed using top growth and root growth measurements similar to Braman et al. (2000). Top growth of bermudagrass was weekly clipped to a height of 5 cm, beginning 1 wk after mole crickets were introduced into the arenas. Clippings were collected into labeled glass Petri dishes and oven dried at 70 °C for 4 h, then

weighed, and weights recorded. After 4 wk, each arena was sampled to assess the survival of mole crickets, percentage of survival of earthworms, and the growth and mass of roots. The intact root profile was harvested by removing the Petri dish lid at the bottom, gently lifting the PVC cylinder, and washing away the sand. The above ground parts of bermudagrass were removed and the remaining roots were placed in plastic zipper bags (SC Johnson, Racine, WI). Freshly harvested roots were immediately transported to the Phytobacteriology Laboratory, Auburn, AL for scanning and measuring using WinRHIZO system (Regent Instrument, Canada; Figure 2.2). This image analysis software determines total root length, surface area, volume, and other architectural characteristics. After this process, roots were oven dried at 70 °C for 4 h, then weighed.

Figure 2.1. PVC arenas established with hybrid bermudagrass plugs in greenhouse.

A repeated measures ANOVA was performed using the MIXED procedure to determine the effect of different treatments on dry weight of bermudagrass clippings produced within 4 wk, and treatment means within each week were separated using LSmeans. Root measurement analysis was conducted using the GLM procedure with LSmeans for pairwise mean comparisons.

Percentages of survival of earthworms were compared between treatments by two-sample t-tests. Data for each species were analyzed separately. All statistical analyses were conducted using SAS 9.2 (SAS Institute 2008).

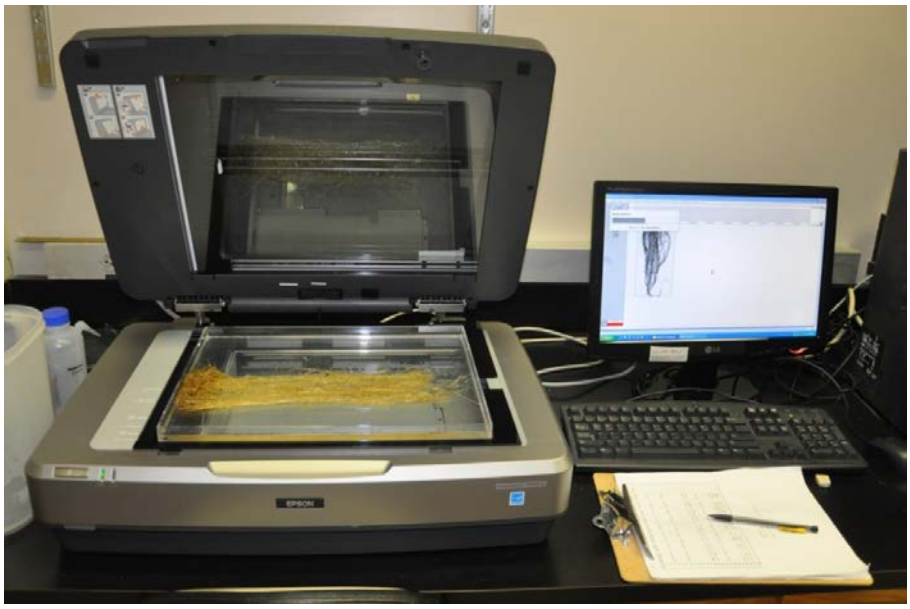

Figure 2.2. EPSON Scanner and WinRHIZO software were used to measure architectural characteristics of bermudagrass roots.

Results

Experiment with *S. borellii*. All *S. borellii* adults remained alive after 4 wk. In the earthworm only treatment, 23.68 ± 5.73% of the earthworms were recovered compared to 6.14 ± 2.11% in the earthworm plus mole cricket treatment (t = 2.87; df = 10; P = 0.0165). The minimum and maximum air temperatures within 4 wk were 22 and 33 °C, respectively.

Top growth of hybrid bermudagrass decreased weekly in all treatments (Figure 2.3). There were significant main effects of time (F = 45.2; df = 3, 60; P < 0.0001), treatment (F = 4.79; df = 3, 20; P = 0.0113), and a significant treatment × time interaction (F = 3.52; df = 9, 60;

$P = 0.0015$). No treatment resulted in significantly less top growth than the controls. At 2 and 4 wk, top growth was significantly greater in the earthworm only treatment. At 1 and 3 wk, top growth in the earthworm treatment was significantly greater than the control and the earthworm plus mole cricket treatment, respectively (Figure 2.3).

Overall, the root growth of bermudagrass infested with adult *S. borellii* was not influenced by the presence or absence of earthworm prey (Table 2.1). However, root length, surface area, projecting area, and number of forks were reduced significantly in the mole cricket plus earthworm treatment relative to the controls (Table 2.1). Within each affected parameter, the earthworm treatment was significantly greater than either treatment with mole crickets, but not different from the control.

Experiment with *S. vicinus.* At 3 wk, one *S. vicinus* adult died on the surface of the arena in the earthworm plus mole cricket treatment. This observation was excluded before data analysis. At the end of the experiment (week 4), 46.49 ± 5.16% of the earthworms were recovered in the earthworm only treatment compared to 47.37 ± 7.81% in the earthworm plus mole cricket treatment ($t = -0.09$; df = 7.2; $P = 0.9278$). The minimum and maximum air temperatures within 4 wk were 20 and 30 °C, respectively.

Weekly top growth of hybrid bermudagrass was reduced after 4 wk (Figure 2.4; $F = 17.40$; df = 3, 59; $P < 0.0001$ ANOVA for repeated measures). There was a significant treatment effect ($F = 6.27$; df = 3, 20; $P = 0.0036$) but not significant time × treatment interaction ($F = 0.89$; df = 9, 59; $P = 0.5364$). There was no significant difference in top growth until after 2 wk when top growth in the controls was significantly greater than either treatment with mole crickets. At 3 and 4 wk, top growth in control was only significantly greater than the earthworm plus mole cricket treatment (Figure 2.4).

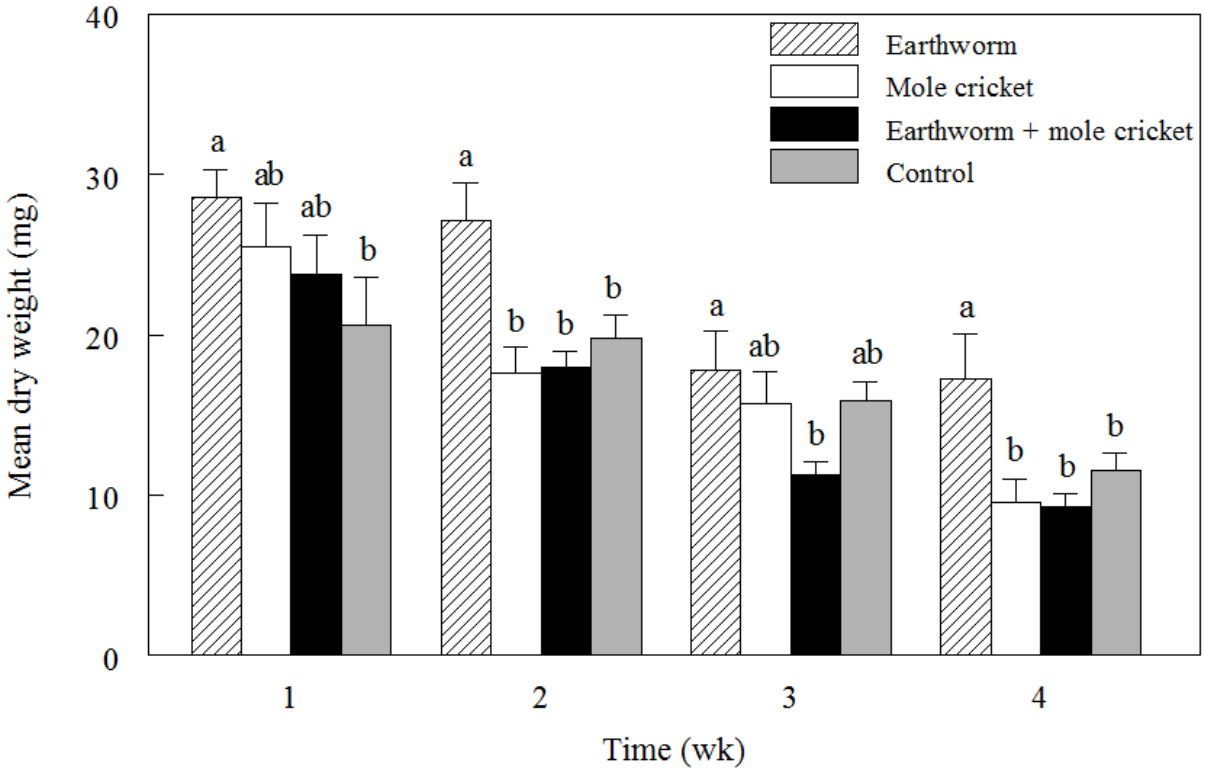

Figure 2.3. Mean dry weight of hybrid bermudagrass clippings produced over a 4 wk period when infested with adult *S. borellii* in the presence and absence of earthworm prey. There were six replicates with four treatments (24 total). Means followed by the same letter are not significantly different from each other within each week ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

Table 2.1. Bermudagrass root measurements after 4 wk exposure to adult *S. borellii* and earthworm prey treatments

Treatment	Mean (± SEM) values of root measurements (N=6)								
	Length (cm)	Avg diam (mm)	Surface area (cm^2)	Projecting area (cm^2)	Vol (cm^3)	No. tips	No. forks	No. cross	Dry wt (g)
Earthworm	3011.61±275.56a	0.7±0.03a	671.78±78.2a	213.84±24.89a	12.07±1.92a	15212.17±2274.93a	50227.67±6177.63a	7330±1166.44a	1.78±0.38a
Mole cricket	2213.2±232.79bc	0.75±0.05a	505.51±21.66bc	160.91±6.9bc	9.4±0.41a	15367±1919.11a	36932.33±2966.54bc	5549±767.97ab	1.27±0.11ab
Earthworm + mole cricket	1864.94±126.59c	0.79±0.1a	452.81±45.98c	144.13±14.63c	9.47±2.27a	12842.83±1456.31a	31886.67±2027.94c	4681.5±447.93b	1.07±0.17b
Control	2699.65±142.4ab	0.77±0.05a	641.88±29.03ab	204.32±9.24ab	12.37±1.12a	14762±1733.8a	48431.83±2908.06ab	7542.67±665.45ab	1.41±0.06ab
F value	4.97	0.29	3.92	3.92	0.87	0.35	4.38	2.42	1.93
P value	0.0137	0.833	0.03	0.03	0.4783	0.7908	0.0211	0.1069	0.1687

Means presented are actual means. Within a column, means ± SEM followed by the same letter are not significantly different from each other ($P < 0.05$; GLM; LSmeans [SAS Institute 2008]).

Overall, root growth of bermudagrass infested with adult *S. vicinus* was not influenced by the absence of earthworm prey (Table 2.2). All root measurements, except average diameter, were reduced approximately in half (relative to controls) when earthworms and mole crickets were present but not when mole crickets alone were present. The number of forks and cross were significantly affected by treatment. There were marginally significant differences ($P \geq 0.06$) in root length, surface area, projecting area, and volume (Table 2.2).

Discussion

Previous studies have reported omnivory, consumption of plant and soil-dwelling prey, by *S. borellii* and *S. vicinus* (Hayslip 1943, Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and Brandenburg 2011). They also implied that *S. vicinus* should have greater pest status, due to its more herbivorous feeding habit, compared with the principally carnivorous *S. borellii*. Although *S. borellii* prefers a habitat of bare sand (Reinert 1983, Schuster and Price 1992) and primarily feeds on animal material even in grass habitat (Matheny 1981), there is potential for severe damage to turfgrass by its excessive tunneling activity if few or no alternative food sources are available (Schuster and Price 1992). In the present study, predation of earthworms by either species of mole crickets could not be verified. Survival of earthworms, however, was similar when confined with *S. vicinus* but was significantly reduced when confined with *S. borellii*. A large *S. borellii* nymph (with wingpads) is able to cut off a living earthworm using its mouthparts and immediately consume the prey (Y. Xu, personal observations; Figure 2.5). We are therefore reasonably certain that *S. borellii* consumed the alternate prey but this isn't likely for *S. vicinus*.

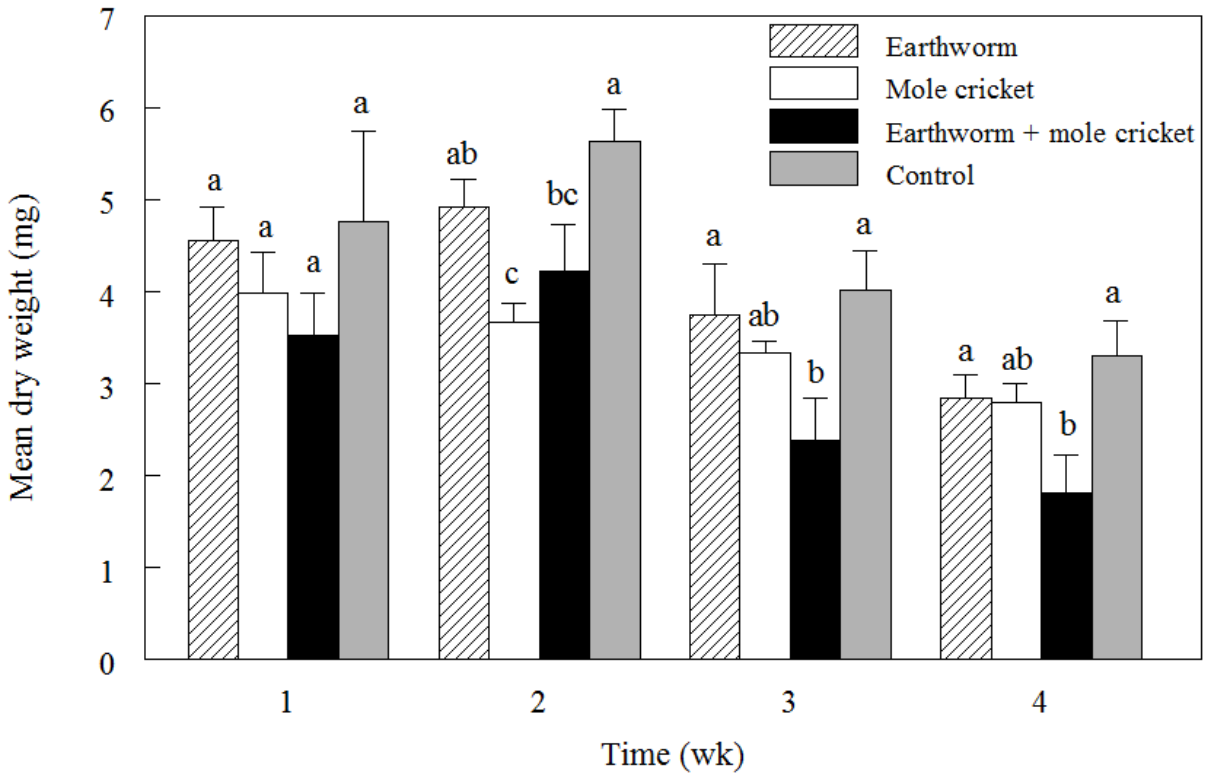

Figure 2.4. Mean dry weight of hybrid bermudagrass clippings produced over a 4 wk period when infested with adult *S. vicinus* in the presence and absence of earthworm prey. There were six replicates with four treatments (24 total). One mole cricket in the earthworm plus mole cricket treatment died after 3 wk, and this observation was not used at 4 wk. Means followed by the same letter are not significantly different from each other within each week ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

Table 2.2. Bermudagrass root measurements after 4 wk exposure to adult *S. vicinus* and earthworm prey treatments

Treatment	Mean (± SEM) values of root measurements (N=6)								
	Length (cm)	Avg diam (mm)	Surface area (cm^2)	Projecting area (cm^2)	Vol (cm^3)	No. tips	No. forks	No. cross	Dry wt (g)
Earthworm	2057.98±222.4ab	0.46±0.03a	292.96±32.22a	93.25±10.26a	3.37±0.44a	13671.5± 3338.82ab	29926±3301.16a	5921.5±713.08a	0.52±0.05a
Mole cricket	1612.95±297.88ab	0.45±0.02a	231.05±47.33ab	73.55±15.06ab	2.65±0.61ab	11747.67±1838.51ab	23675.33±4515.23ab	4490.17±834.92ab	0.39±0.09ab
Earthworm + mole cricket[a]	1259.87±177.35b	0.4±0.02a	156.8±21.49b	49.91±6.84b	1.57±0.24b	7752.6±1974.06b	16526.6±2052.44b	3408.2±394.6b	0.27±0.04b
Control	2238.04±250.65a	0.43±0.02a	300.05±29.23a	95.51±9.3a	3.24±0.34a	15382.33±2584.97a	31139.83±2466.13a	6122.5±476.78a	0.52±0.06a
F value	3.02	1.21	3.19	3.19	2.86	1.89	4.06	4.19	2.37
P value	0.0652	0.3421	0.0565	0.0565	0.0749	0.1782	0.0287	0.026	0.1143

Means presented are actual means. Within a column, means ± SEM followed by the same letter are not significantly different from each other ($P < 0.05$; GLM; LSmeans [SAS Institute 2008]).

[a] Only five observations were used (one observed mole cricket died after 3 wk in this treatment).

Figure 2.5. A large *S. borellii* nymph held in a plastic Petri dish consuming a living earthworm.

The results of the present study indicate that the presence of prey does not affect top growth or root parameters of bermudagrass relative to mole crickets alone. Walker and Dong (1982) conducted similar tests to quantify the damage to coastal bermudagrass (variety unnamed) and bahiagrass (*Paspalum notatum* Flueggé 'Pensacola') by adult *S. borellii* and *S. vicinus* over a 3-month period. Neither adult *S. borellii* supplemented with dog food (21% protein) or starved (no alternative food provided) had significant effects on stand or forage production in either grass, even though extensive tunneling was observed in the coastal bermudagrass. Conversely, adult *S. vicinus* reduced the yield of bermudagrass slightly especially when starved. With bahiagrass, however, 19% of plugs were lost when *S. vicinus* was provided supplemental food compared with 88% loss when no supplemental food was provided (Walker and Dong 1982). In the present study, however, neither mole cricket species had measurable effects on top growth of bermudagrass. Braman et al. (2000) observed similar results with *S. vicinus*. Under experimental conditions similar to our study, there was no significant reduction in top growth of various cultivars of bermudagrass.

Compared with the control plants, neither species of mole cricket negatively affected the various measured root parameters (Tables 2.1 and 2.2). This was unexpected considering that mole crickets confined with turfgrass under similar conditions have been shown to reduce root dry weight relative to non-infested controls, particularly with *S. vicinus* (Braman et al. 2000). Also, bermudagrass in the southeastern United States is commonly damaged by mole crickets on golf courses and in home lawns (Potter 1998). *Scapteriscus borellii* and *S. vicinus* in captivity complete most of the excavation for their underground tunnel structure within about 18 d. After that time, the tunnel structure was typically not expanded (Villani et al. 2002). When the grasses were harvested in the present study, both species had well-developed tunnels (Y. Xu, personal observations). Root data were not collected over time to be able to determine if there were short-term reductions during the excavation of the tunnel system. However, a reduction in root growth may explain the significant reduction in top growth 2 wk after *S. vicinus* were introduced into the arenas, but not thereafter (Figure 2.4).

The earthworm with mole cricket treatment interestingly had lower top growth than the earthworm only treatment at week 3 and 4 for *S. vicinus* and from week 2–4 for *S. borellii* (Figures 2.3 and 2.4). However, this decrease in top growth cannot be attributed only to mole crickets since infestation with mole crickets can result in an increased top growth (Braman et al. 2000). On most of those same dates, top growth in the mole cricket treatment was not significantly different from the earthworm treatment. Coupled with this, the earthworm only treatment was often numerically or even significantly greater than the grass only controls. Likewise, several root parameters (length, surface area, number of forks, number of cross roots) were significantly reduced in grasses with mole crickets and earthworms compared with those with only earthworms. The difference may be exaggerated by the increase in root and shoot

growth in the earthworm only control or perhaps there is an interaction when earthworms and mole crickets are combined. This synergy may have resulted from increased subsurface activity of mole crickets in the presence of another soil organism. Earthworms (Edwards 2004) and mole crickets (Potter 1998) both cause soil disturbance through their subterranean digging, and earthworm castings are known to increase growth responses of turfgrass (Potter 1998).

Since the late 1990's, new classes of turf insecticides have been released that are reduced-risk and have less impact on non-target invertebrates including earthworms (Potter et al. 1990, Kunkel et al. 1999). These new soil insecticides have more favorable toxicological profiles (Held and Potter 2012) resulting in a more active and diverse microfaunal community that potentially sustains alternative prey for mole crickets. Earthworms can be locally abundant in greens and tees especially in the spring and fall (Potter 1998) and mole crickets may forage into these areas to access earthworm prey. The overlap of these two organisms in turfgrass can be completely coincidental, however, this study suggests a possible negative interaction. Although speculative, earthworm populations in bermudagrass may influence the abundance of mole crickets and the severity of root damage.

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CHAPTER 3

DIETARY CHOICES AND THEIR IMPLICATION FOR SURVIVAL AND DEVELOPMENT OF OMNIVOROUS MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE)

Abstract

Omnivory is a common feeding strategy in terrestrial arthropods, and usually is beneficial for survival, development, and fecundity. We determined the feeding palatability and preference of omnivorous mole crickets, *Scapteriscus vicinus* Scudder and *S. borellii* Giglio-Tos, using choice and no-choice experiments in the laboratory. Among various plants, both *S. vicinus* and *S. borellii* adults had significantly greater consumption of carrots than bermudagrass parts (blades, roots, and rhizomes). When given animal and plant diets, *S. vicinus* tended to feed preferentially on plant tissues whereas *S. borellii* preferred an animal diet. The nutritional profiles of diets suggested dietary self-selection by these mole crickets. Further investigation concerning the impact of diet on survival and nymphal development provided insight into the relative benefits of each dietary choice on these omnivores. *Scapteriscus borellii* provisioned with an animal diet had less mortality, greater body mass, and faster development compared to the ones fed on a pure plant diet. Even though *S. vicinus* is primarily herbivorous, nymphs fed on animal diet gained more weight and developed faster than the ones fed on plant diet. This work suggests that *S. vicinus* and *S. borellii* have the capability of nutrient regulation and adaptability on various diets, and that an animal diet benefits both species in terms of survival and nymphal development.

Introduction

Omnivory is characterized by an animal that consumes both plant and prey resources in nature (Coll and Guershon 2002). The benefits of omnivory include acquiring the well-balanced nutrients (Behmer 2009), decreasing sensitivity to limitations in food resources (Pearson et al. 2011), and reducing interspecific competition (Coll and Guershon 2002). In a natural habitat, omnivorous insects usually have opportunities to regulate intake and nutritional needs for growth and metabolism through dietary self-selection, which is more beneficial for survival, weight gain, development rate, and fecundity (Waldbauer and Friedman 1991, Coll and Guershon 2002, Behmer 2009). For insects, water and nitrogen content are considered essential nutritional values of determining the food quality (Shoonhoven et al. 2005). When the nutritional environment changes (i.e., food shortage and poor-quality food), physiological and behavioral mechanisms associated with nutrient regulation can evolve (Behmer 2009). Dietary self-selection behavior has been demonstrated in many Orthopteroids including grasshoppers (*Melanoplus differentialis* Tomas), locusts (*Schistocerca gregaria* Forsskål), and cockroaches (*Supella longipalpa* Fabricius) that non-randomly select nutritionally suitable meals when given a choice (Waldbauer and Friedman 1991).

In general, the literature on feeding ecology of subterranean omnivores has few examples of non-social insects. Mole crickets can be a useful model system to increase understanding of the feeding behavior of hypogeal omnivores. The tawny mole cricket, *Scapteriscus vicinus* Scudder (Orthoptera: Gryllotalpidae), and the southern mole cricket, *S. borellii* Giglio-Tos, are two introduced species that cause damage to turfgrass in the southeastern United States (Potter 1998). They can consume a variety of plants, ranging from above-ground grass leaves (Walker 1984) and seedlings of vegetables (Hayslip 1943, Schuster and Price 1992) to below-ground

roots and developing peanuts (Hayslip 1943). However, investigations showed mole crickets also feed on earthworms (Hudson 1987) and soil arthropods (Fowler et al. 1985), and have cannibalistic behavior (Hayslip 1943). Numerous studies (Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and Brandenburg 2011) have used dissection to identify the gut contents of field-collected mole crickets. Based on gut contents, *S. borellii* feeds on both plant material and animal tissues with animal tissue dominant in the gut (Taylor 1979), whereas gut contents of *S. vicinus* are mainly plants. Thus, *S. vicinus* was considered a greater pest due to its high level of herbivory (Fowler et al. 1985) than *S. borellii* which was deemed carnivorous (Taylor 1979).

Gut dissection studies have been the only insight into mole cricket feeding ecology. However, they are limited because the results may simply reflect abundance of a particular food in the habitat or differential rates of digestion (Taylor 1979, Matheny 1981), and not necessarily dietary preference made by self-selection. Long-term feeding studies (Godan 1964) of omnivorous European mole cricket, *Gryllotalpa gryllotalpa* L., indicated that the nymphs continuously fed an animal protein diet (larvae of *Galleria*) had lower mortality, reduced cannibalism, a shorter time for development, and higher fecundity compared to the ones reared with a vegetable diet (i.e., carrots, potatoes, lettuce). Such comparisons have not been done for pest *Scapteriscus* species. As a subterranean omnivore that is highly mobile, mole crickets have access to multiple foods in the natural world. They come to the surface, cut off plants, and pull them down into their burrows to feed (Hayslip 1943). Also, they tunnel in soil and prey on earthworms and dead arthropods. However, answers to questions, “What happens when omnivores cannot mix diets?” and “Does the diet influence the disparity in development of mole crickets?” are generally absent from the literature. Notably, no published studies have addressed the dietary choices of *Scapteriscus* mole crickets by quantifying the consumption of defined diet.

Using adult *S. borellii* and *S. vicinus*, we determined the feeding preferences of omnivorous mole crickets in no-choice and choice laboratory feeding assays. Furthermore, the effects of diets of animal, plant, or a rotation of both on survival and development of mole cricket nymphs were compared.

Materials and Methods

Sources of Insects. Adult *S. borellii* were field-collected from 16 May–6 Jun 2011 using a modified acoustic trap (Thompson and Brandenburg 2004) from the driving range at Grand National Golf Course in Opelika, AL. Adult *S. vicinus* were collected by soap flush (30 ml of Joy liquid detergent in 8 L of water) on 14 Mar 2012 from the driving range at Shell Landing Golf Club in Gautier, MS. All collected individuals were immediately washed with fresh water upon emergence, and then placed into 473 ml of plastic cups (Dart, Mason, MI). The cups contained autoclaved, moistened sand and had ventilated lids. They were provided a mixed diet of organic carrot strips (Inter-American Products, Cincinnati, OH) and freeze-dried mealworms (Coleoptera: Tenebrionidae; Fluker Farms, Port Allen, LA), and held in a growth chamber (Percival Scientific Inc., Perry, IA) at 27 °C with a 14:10 (L:D) photoperiod prior to the experiments.

Relative Palatability among Plants. Laboratory no-choice tests with different plant diet treatments were conducted using *S. borellii* and *S. vicinus* adults on 31 Aug 2011 and 6 Apr 2012, respectively. Mole crickets were provisioned with 100 mg of carrot strips (Godan 1964), bermudagrass blades, bermudagrass rhizomes, or bermudagrass roots. Grass parts (blades, rhizomes, and roots) were hybrid bermudagrass ‘Tifway 419’ harvested from the Auburn University Turf Research Unit, Auburn, AL. Cores of grass were washed free of soil and were

separated into blades, rhizomes and roots (each individual part was approximately 3 – 5 cm long) before exposing to mole crickets (Figure 3.1). Tests using *S. borellii* were conducted in a plastic container (34.2 × 20.9 × 11.8 cm; Kis, Brampton, Canada) with one half (lengthwise) of the container filled with autoclaved, moistened sand for the mole crickets to reside, whereas *S. vicinus* were placed in a paper food container (1.89 L; Solo, Highland Park, IL) filled with 470 ml of sand. Before each assay, one mole cricket was introduced to the sand in the test arena and starved for 24 h in the growth chamber. The diet treatment was placed on a 5 × 5 cm piece of filter paper (Whatman, Piscataway, NJ) and added into each container. It was placed on the other side of the plastic container for *S. borellii*, on the surface of sand for *S. vicinus*. The containers were placed in a dark growth chamber at 27 °C. After 12 h (one feeding bout), diets were reweighed, then corrected for background weight change using a set of untreated diets that were held in the same condition without mole crickets. Each diet treatment was replicated seven times for each species. Insects were tested with only one diet treatment. The amounts (mg) of consumed food were analyzed using PROC GLM with Tukey's HSD test (SAS 9.2) for mean separations to determine the relative palatability among different diet treatments. Data for each species were analyzed separately.

Figure 3.1. 100 mg of each following plant diet treatments: roots, rhizomes, blades, and carrot strips (from left to right) were provisioned in no-choice tests.

Feeding Preference between Plant and Animal Diets. Laboratory choice and no-choice tests were conducted using *S. borellii* and *S. vicinus* adults from 19 May–9 Jun 2011 and from 18–29 Mar 2012, respectively. Choice tests provided mole crickets with 145 mg of a plant and an animal diet, whereas no-choice tests determined consumption of each diet treatment (290 mg) independently (Figure 3.2). The plant diets were organic carrot strips and animal diets were freeze-dried mealworms. The experimental procedure and test arenas for determining plant preference was the same as previously described for feeding palatability tests.

Choice tests were replicated with 30 mole crickets and no-choice tests were replicated with ten mole crickets per diet treatment. Mole crickets that did not consume diet in choice tests were excluded before analysis. The amount (mg) of consumed food in choice tests were analyzed using PROC TTEST (SAS 9.2; SAS Institute 2008) for paired t-tests to determine adult mole cricket feeding preference. The amount (mg) of consumed food in no-choice tests were analyzed using PROC TTEST for two-sample t-tests to determine the acceptability or palatability of the diets to adult *S. borellii* and *S. vicinus*. Data for each species were analyzed separately.

Figure 3.2. Choice test using *S. borellii* was conducted in a plastic container (left), whereas *S. vicinus* was placed in a paper food container (right).

Nutritional Analyses. Samples consisting of 0.5 g organic carrot strips, hybrid bermudagrass blades, roots, or mealworms were collected from five different individuals (replicates). Samples were weighted freshly, oven dried at 60 °C for 24 h, and then re-weighed to calculate water loss. Nitrogen content was determined from a 0.1 g sample of each tissue by modified Kjeldahl analysis (McKenzie and Wallace 1954) in Auburn University Soil Testing Laboratory. Water (%) and nitrogen content (%) data of each tissue were square root transformed prior to statistical analyses, and compared by PROC ANOVA with Tukey's HSD test for mean separations using SAS 9.2.

Effects of Diet on Survival and Development. Laboratory experiments were separately conducted to evaluate how different diet treatments affect survival and nymphal development of *S. borellii* and *S. vicinus* nymphs from Jul 2011–May 2012 and from Apr–Jun 2012, respectively. For each species, field-collected males and females were paired randomly in the laboratory and each pair was placed into separate 473 ml transparent plastic cups containing approximately 450 ml of autoclaved, moistened sand until females oviposited (see Appendices for more details on this method). Each cluster of newly laid eggs was transferred into a 473 ml plastic cup filled with moistened vermiculite (Sun Gro Horticulture, Bellevue, WA) and incubated in a growth chamber at 27 °C until eggs hatched. Newly hatched nymphs were transferred to separate 473 ml plastic cups with ventilated lids containing approximately 150 ml of autoclaved, moistened sand and reared separately in growth chamber at 27 °C with a 14:10 (L:D) photoperiod. Every 2 d, each nymph was provisioned with a comparable amount (80 mg) of either a plant (organic carrot strips), animal (mealworms), or rotational diet (alternating plant and animal diets every 2 d). The amount of diets was increased as mole crickets developed such that they were being provided 160 mg of each diet as 6^{th} instars. Starved nymphs were a negative control. Each of the four

treatments was replicated eight times for each species. Nymphs in each replicate were hatched from the same egg cluster. To determine the impact of diet treatments on longevity and growth, survival of mole cricket nymphs was recorded every 2 d and they were weighed weekly. To determine the number of molts, nymphs were marked with white fingernail polish (Bari Cosmetics, Greenwich, CT) on the pronotum. After each molt, the pronotal length (Hudson 1987) and pronotal width were also measured using a digital caliper (Absolute Digimatic, Mitutoyo, Aurora, IL).

Survival analysis was conducted using the Kaplan-Meier method (PROC LIFETEST; SAS Institute 2008). Pairwise comparisons of the survival distribution function between treatments were made using the log-rank test. Statistical significance was determined with the Bonferroni adjustment for multiple comparisons ($\alpha = 0.05/6 = 0.0083$). A repeated measures ANOVA was performed to determine the effects of diet treatments on body weight of *S. borellii* for weeks 0-40 and *S. vicinus* for weeks 0-8. Body weight (mg), pronotal length and width (mm), and molting duration (days) of instars of mole crickets raised on various diets were analyzed using the PROC MIXED (SAS 9.2) with LSmeans test for mean separations. Body weight and molting duration data were log 10 transformed prior to statistical analysis. Data for each species were analyzed separately.

Results

Relative Palatability among Plants. There was no consumption of bermudagrass rhizomes by adult *S. borellii* within 12 h. Among four plant diet treatments, *S. borellii* adults consumed significantly more weight of carrot strips (Figure 3.3; $F = 12.98$; df = 3; $P < 0.0001$) compared to the others. The consumption of blades and roots was statistically similar. For adult

S. vicinus, the consumptions of three grass parts were similar to each other. However, *S. vicinus* also consumed significantly more weight of carrot strips (Figure 3.3; $F = 9.96$; df = 3; $P = 0.0002$).

Feeding Preference between Plant and Animal Diets. Out of 30 individuals per species, three *S. borellii* and two *S. vicinus* did not consume either plant or animal diet within 12 h in choice tests. Adult *S. borellii* consumed similar amounts of plant and animal diets in no-choice tests (Satterthwaite's test; Figure 3.4; $t = –1.10$; df = 11.681; $P = 0.2942$). However, when given a choice, *S. borellii* consumed about two times more weight of animal than plant tissue in 12 h (Figure 3.4; $t = 3.03$; df = 26; $P = 0.0054$). Adult *S. vicinus* consumed four times more weight of plant tissue than animal diet in no-choice tests (Satterthwaite's test; Figure 3.4; $t = –4.41$; df = 9.8332; $P = 0.0014$). However, when given a choice, *S. vicinus* consumed similar amounts of animal and plant diets in 12 h (Figure 3.4; $t = –1.82$; df = 27; $P = 0.0797$).

We also separately performed analyses on the consumption of males and females for both mole cricket species, and found that there was no difference in dietary preference between sexes. For *S. borellii*, 7 females and 3 males consumed similar amounts of animal (mean ± SE: 45.71 ± 7.88 and 81.67 ± 16.9 mg, respectively; $t = –2.24$; df = 8; $P = 0.0552$) and plant tissue (mean ± SE: 73.14 ± 26.26 and 106.67 ± 49.72 mg, respectively; $t = –0.66$; df = 8; $P = 0.5299$) in no-choice tests. When given a choice, 22 females and 5 males also had similar consumption of animal (mean ± SE: 56.32 ± 7.74 and 48.2 ± 8.43 mg; $t = 0.48$; df = 25; $P = 0.6354$) and plant tissue (mean ± SE: 24.5 ± 7.61 and 21.8 ± 16.29 mg; $t = 0.15$; df = 25; $P = 0.8803$). For *S. vicinus*, the sexes (5 females and 5 males) did not differ in the consumption of animal (mean ± SE: 41.8 ± 9.43 and 48 ± 10.02 mg, respectively; $t = –0.45$; df = 8; $P = 0.6641$) or plant tissue (mean ± SE: 203 ± 53.75 and 161.6 ± 32.83 mg, respectively; $t = 0.66$; df = 8; $P = 0.5294$) in no-

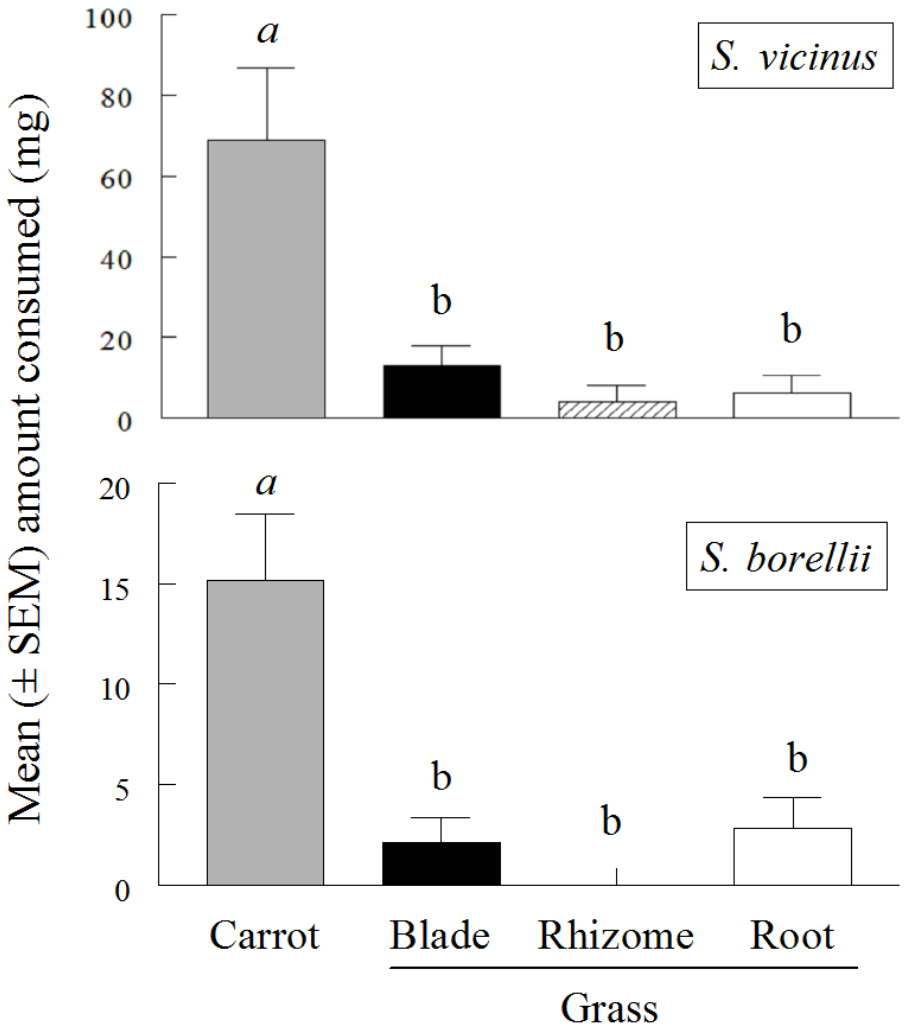

Figure 3.3. Adult *S. borellii* and *S. vicinus* consumption of four different plant materials (carrot strips, bermudagrass blades, rhizomes, roots) within 12 h. There was no consumption of bermudagrass rhizome by *S. borellii*. Bar graphs with different letters are significantly different within a species ($P < 0.05$; GLM; Tukey's HSD [SAS Institute 2008]). Each diet treatment was replicated seven times for each species.

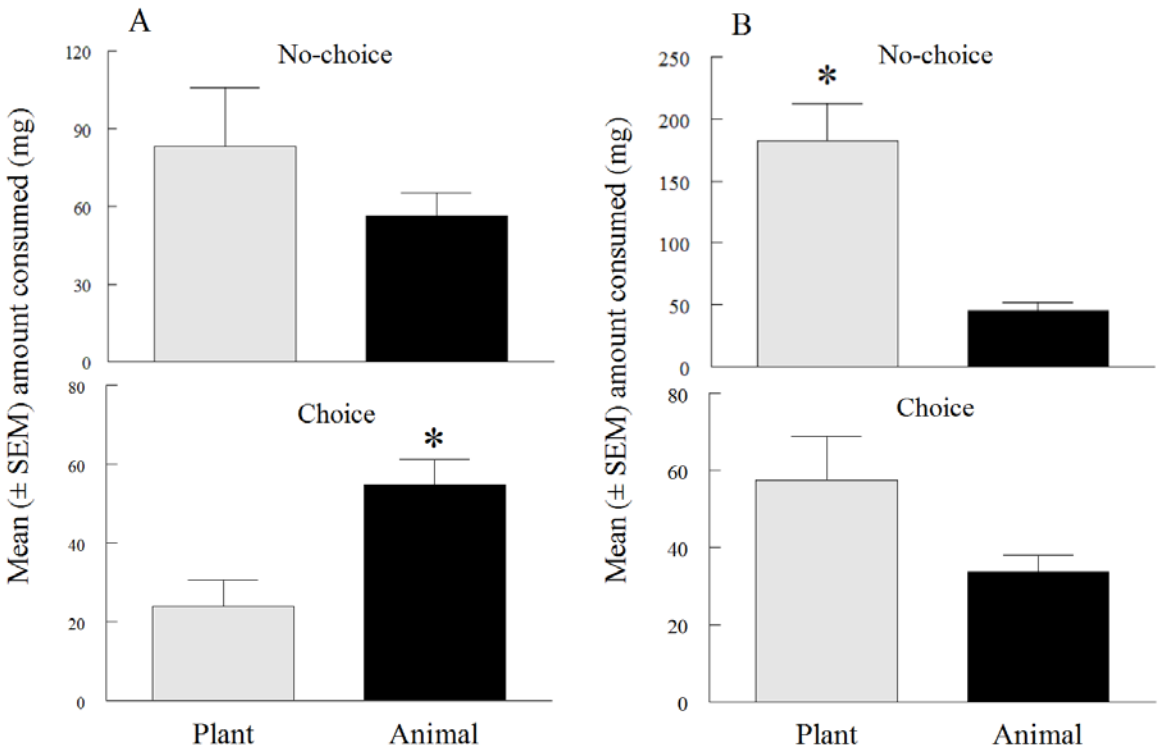

Figure 3.4. Consumption of plant and animal diet by adult *S. borellii* (A) and *S. vicinus* (B) within 12 h in no-choice and choice tests. Asterisk over the bar denotes that significantly different consumption of diet ($P < 0.05$; TTEST; [SAS Institute 2008]). There were 30 replicates (5 males and 25 females *S. borellii*; 20 males and 10 females *S. vicinus*) in choice test and ten replicates (3 males and 7 females *S. borellii*; 5 males and 5 females *S. vicinus*) in no-choice test. Three *S. borellii* (females) and two *S. vicinus* (1 male and 1 female) did not consume either diet treatment within 12 h in choice tests were excluded before analysis.

choice tests. When given a choice, both sexes (9 females and 19 males) consumed similar amounts of animal (mean ± SE: 34.89 ± 8.81 and 33.16 ± 5.06 mg, respectively; $t = 0.18$; df = 26; $P = 0.8570$) and plant tissue (mean ± SE: 63.11 ± 19.52 and 54.84 ± 14.43 mg, respectively; $t = 0.33$; df = 26; $P = 0.7427$).

Nutritional Analyses. Carrot strips had the greatest water content ($P < 0.0001$) but lower nitrogen content compared to the other diets (Table 3.1). Bermudagrass blades were intermediate in both nitrogen and water content. Bermudgrass roots had similar amount of water content with blades but the lowest nitrogen content. Nitrogen content of mealworms was approximately nine times higher than that of carrot strips and bermudagrass roots (Table 3.1).

Effects of Diet on Survival and Development of *S. borellii*. Nymphs fed on animal ($\chi^2 = 14.4164$; $P = 0.0009$) lived significantly longer than starved individuals (Figure 3.5). Nearly half of *S. borellii* could survive for 4 wk without food provisioned (starved) but died eventually at the end of 5 wk. At the termination of the experiment (week 40), 50% of *S. borellii* nymphs developed to adults in the animal diet group. Forty percent of nymphs developed to adults in the rotational diet group, however, none of the nymphs fed the plant diet developed to adults during the 40 wk experiment (Figure 3.5).

Body weight of *S. borellii* increased weekly in all diet provisioned treatments (plant, animal, and rotation; Figure 3.6). Weight of *S. borellii* nymphs was significantly influenced by diet treatment ($F = 112.29$; df = 3; $P < 0.0001$), time ($F = 75.65$; df = 40; $P < 0.0001$), and diet × time interaction ($F = 6.15$; df = 84; $P < 0.0001$). Overall, nymphs fed diets included animal tissue (animal and rotation treatments) gained significantly more weight over time than nymphs fed plant only diet ($t = 17.45$; df = 28; $P < 0.0001$ and $t = 14.09$; df = 28; $P < 0.0001$, respectively). Although the growth curve of *S. borellii* fed on rotational diet intersected the one

Table 3.1. Mean (± SEM) values of nitrogen and water content of different diet treatments for *S. borellii* and *S. vicinus*

Diet treatment	Nitrogen (mg/g dry wt)	Water (%)
Mealworms	8.96 ± 0.24a	3.60 ± 0.40c
Bermudagrass blades	1.41 ± 0.13b	71.20 ± 0.49b
Bermudagrass roots	0.76 ± 0.04c	70.00 ± 1.10b
Carrot strips	1.09 ± 0.01b	90.80 ± 0.49a
Test statistics	F = 781.50; df = 3; P < 0.0001	F = 2501.19; df = 3; P < 0.0001

Means presented are actual means. Within a column, means ± SEM followed by the different letters are significantly different from each other (P < 0.05; ANOVA; Tukey's HSD [SAS Institute 2008]).

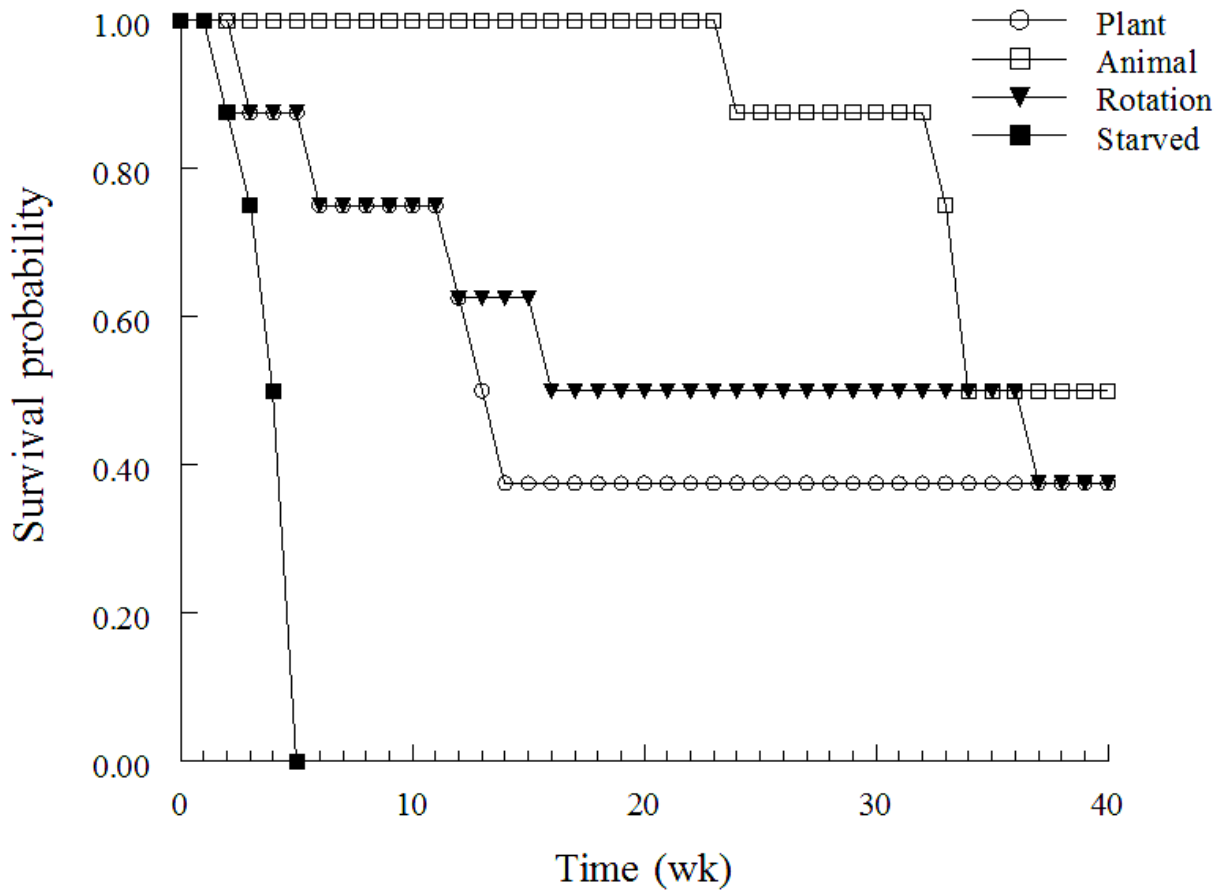

Figure 3.5. Observed survival probabilities of *S. borellii* raised on different diet treatments in the laboratory over a 40 wk period. There was a significant heterogeneity among the survival curves of *S. borellii* provisioned with different diet treatments (χ^2 = 26.4634; df = 3; $P < 0.0001$).

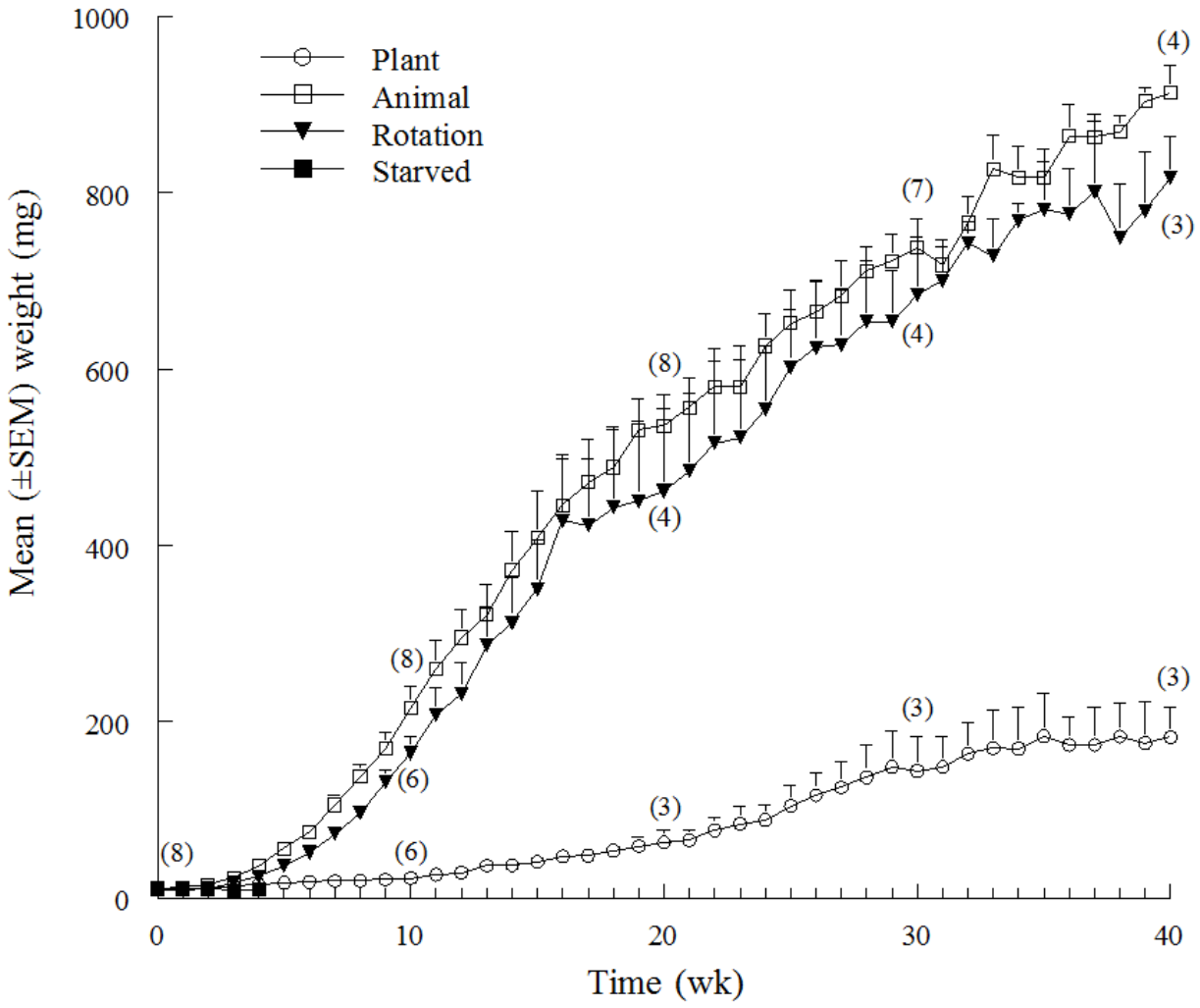

Figure 3.6. Growth rate for *S. borellii* raised on different diet treatments in the laboratory over a 40 wk period. Means presented are actual means. There were initially eight replicates with each diet treatment. The numbers in the brackets represent the number of mole crickets analyzed at 10, 20, 30, and 40 wk.

of nymphs in animal diet group at 16 wk (Figure 3.6), the body weight of nymphs fed on animal diet was significantly greater (t = 2.50; df = 28; P = 0.0185).

All starved *S. borellii* nymphs remained in 1st instar stage and died without molting (Figure 3.7). Thus, these observations were excluded before data analysis. The main effect of diet treatment on *S. borellii* developmental time (time to 40 wk) was significant (Figure 3.8; F = 10.44; df = 2; P = 0.0010). Developmental times for 1st instars were not significantly different among three treatments. From the 2nd – 5th instar stage, however, developmental rates were significantly lower for the plant diet group than for the other groups. The duration of 2nd – 5th instars in the plant diet treatment was generally twice as long as nymphs in the animal and rotational diet treatments (Figure 3.8). Generally, 50% of *S. borellii* fed on animal diet reached wing pad stage at 7th instar and developed to adults at 9th instar. In contrast, only 12.5% of nymphs in plant diet group presented wing pad at 9th instar and never developed to adults at the end of 40 wk.

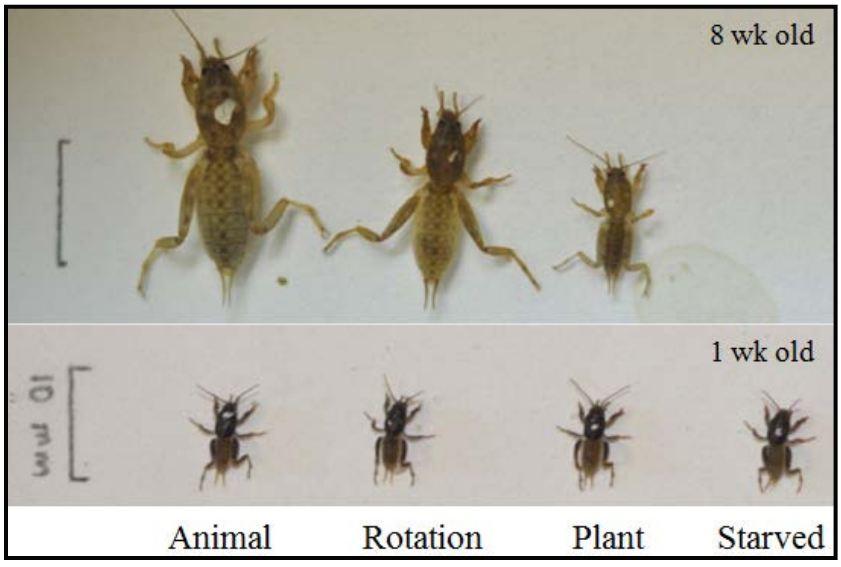

Figure 3.7. *Scapteriscus borellii* nymphs raised on different diet treatments over a 1 wk and 8 wk period. The starved nymphs died at 5 wk.

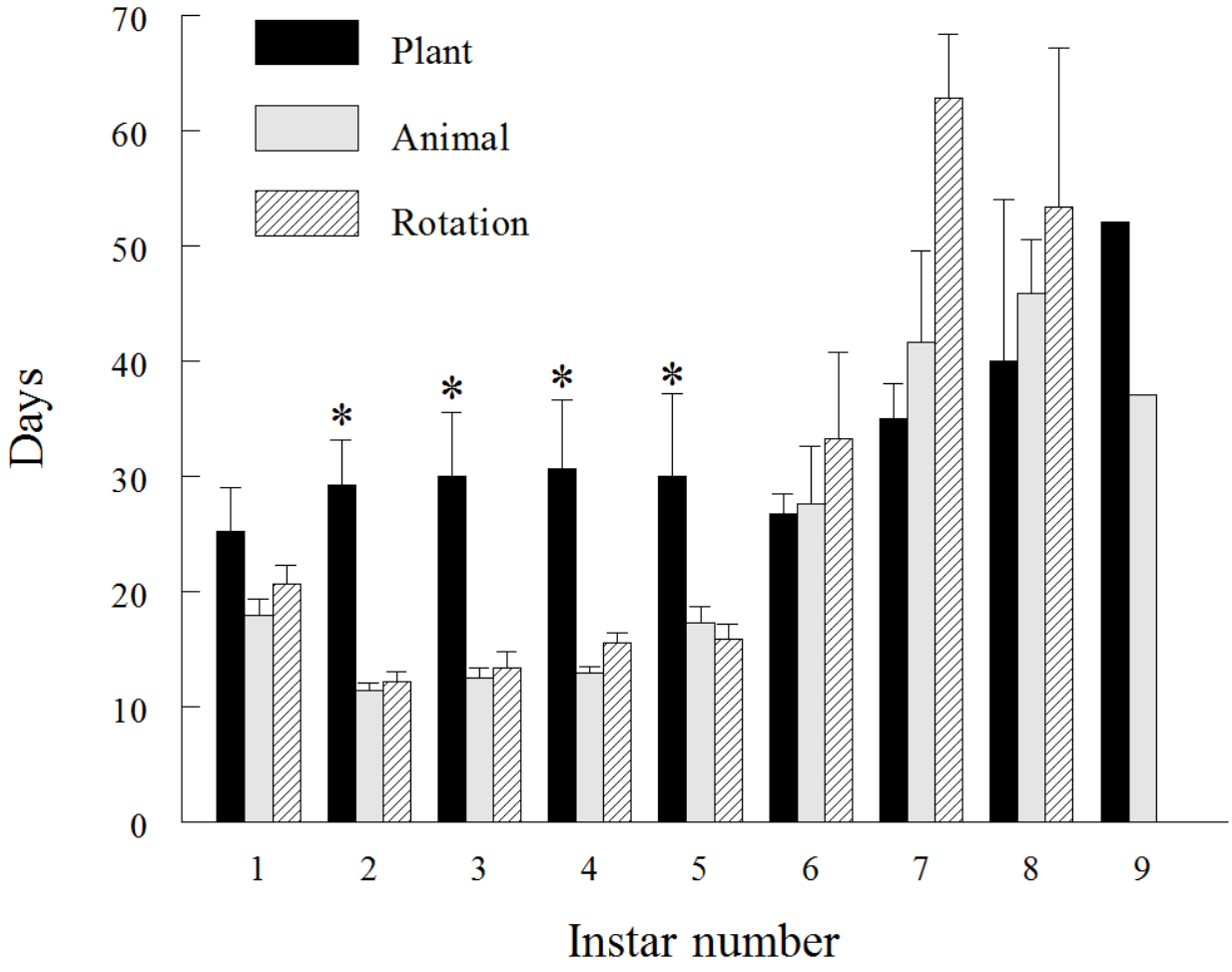

Figure 3.8. Development of *S. borellii* provisioned with either a plant, animal, or rotational diet (alternating plant and animal diets every 2 d) in the laboratory. Asterisks, significant differences among treatments at each instar stage ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

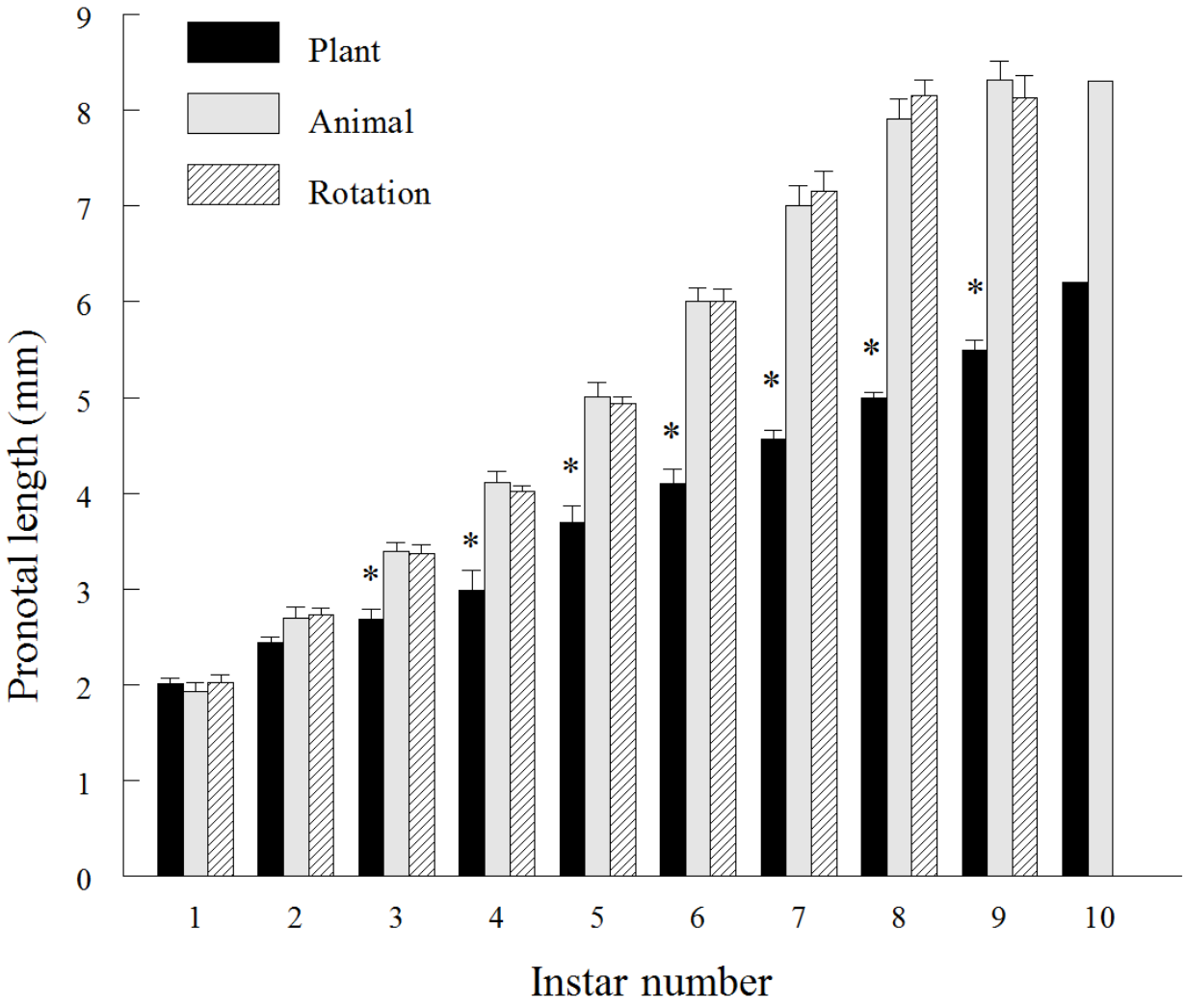

Figure 3.9. Pronotal length of *S. borellii* provided with either a plant, animal, or rotational diet (alternating plant and animal diets every 2 d) in the laboratory. Asterisks, significant differences among treatments at each instar stage ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

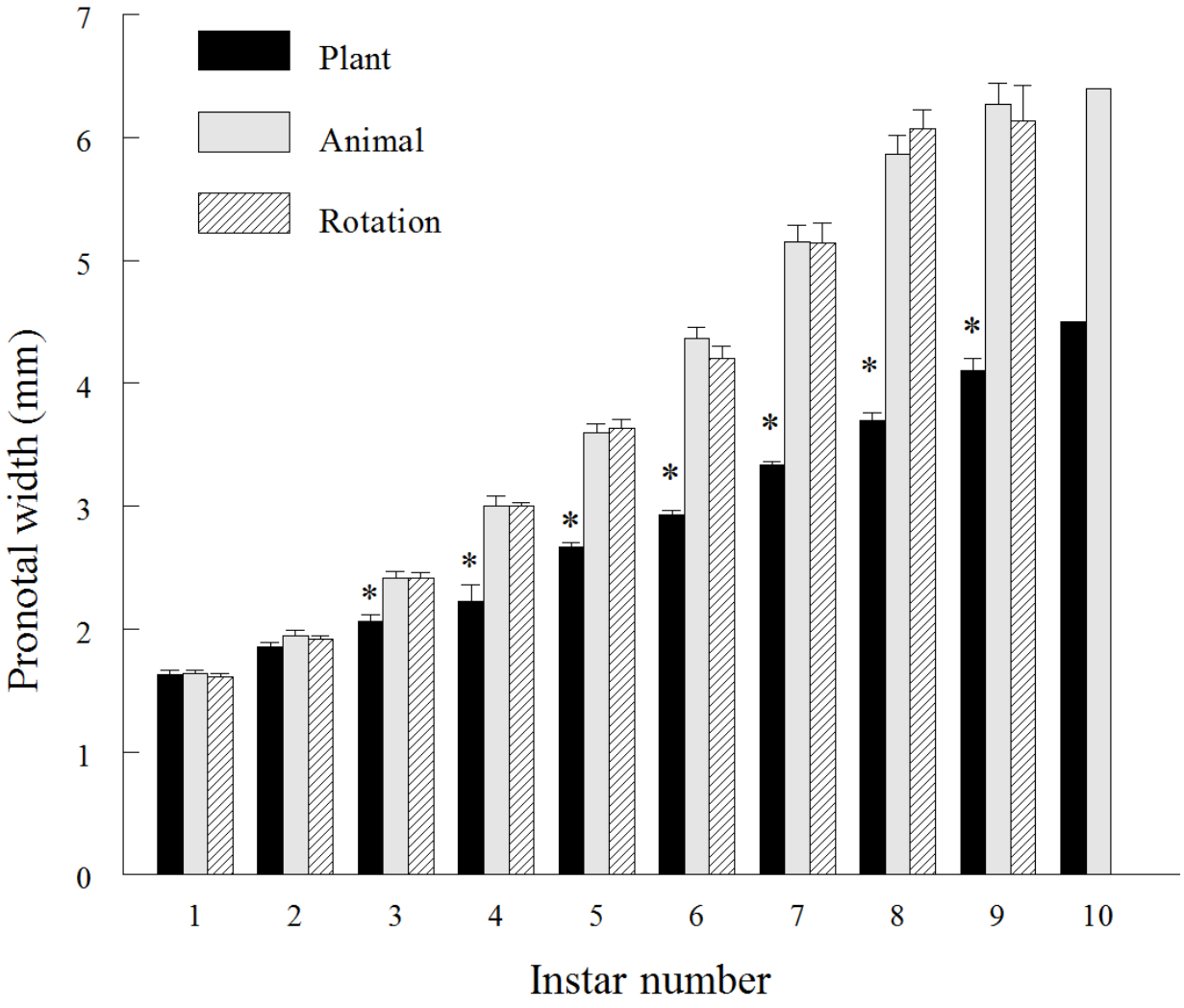

Figure 3.10. Pronotal width of *S. borellii* provided with either a plant, animal, or rotational diet (alternating plant and animal diets every 2 d) in the laboratory. Asterisks, significant differences among treatments at each instar stage ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

Pronotal length and width were significantly influenced by diet treatment ($F = 93.29$; df = 2; $P < 0.0001$ and $F = 101.09$; df = 2; $P < 0.0001$, respectively). They were also influenced by instar stage ($F = 290.07$; df = 9; $P < 0.0001$ and $F = 295.16$; df = 9; $P < 0.0001$, respectively) and diet × stage interaction ($F = 11.00$; df = 17; $P < 0.0001$ and $F = 12.19$; df = 17; $P < 0.0001$, respectively). After the 1st and 2nd instar stages, pronotal length and width were significantly shorter for the plant diet group than for the other two diet groups (Figures 3.9 and 3.10); the differences increased with each instar.

Effects of Diet on Survival and Development of *S. vicinus*. Survival of *S. vicinus* was significantly influenced by diet treatment ($\chi^2 = 11.7897$; df = 3; $P = 0.0081$). Nearly half of *S. vicinus* could survive for 3 wk without food provisioned (starved) but died eventually at the end of 5 wk (Figure 3.11). Overall, nymphs in the rotation diet group died faster than starved individuals from 0-4 wk. At the termination of the experiment (week 8), 37.5% of *S. vicinus* nymphs survived in both plant and animal diet groups.

Body weight of *S. vicinus* increased weekly only in the animal and rotational treatments (Figure 3.12). Weight of *S. vicinus* nymphs was significantly influenced by diet treatment ($F = 18.33$; df = 3; $P < 0.0001$), time ($F = 31.08$; df = 8; $P < 0.0001$), and diet × time interaction ($F = 5.44$; df = 20; $P < 0.0001$). Overall, nymphs fed diets that included animal tissue (animal and rotation treatments) started to gain weight consistently at 2 wk, whereas nymphs fed plant only diet had negligible (average 3.7 mg) weight gain over 8 wk. Starved nymphs lost approximately 2 mg of weight on average at 3 wk, compared to initial weight (Figure 3.12).

All starved *S. vicinus* nymphs and most (7/8) nymphs in rotation treatment remained in 1st instar stage and died without molting (Figure 3.13). Thus, these observations were excluded before data analysis to only compare the plant and animal diet treatments. The main effect of diet

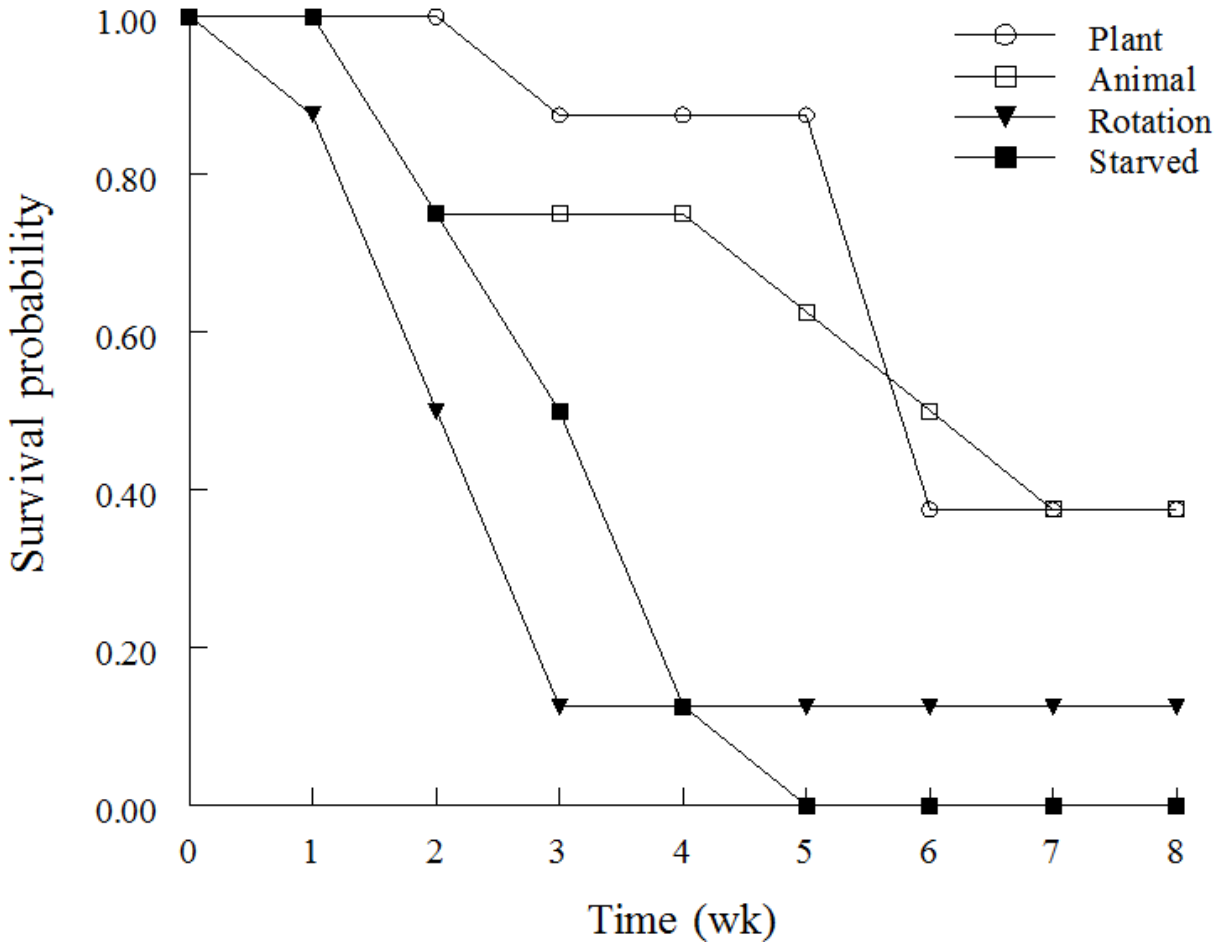

Figure 3.11. Observed survival probabilities of *S. vicinus* raised on different diet treatments in the laboratory over an 8 wk period. There was a significant heterogeneity among the survival curves of *S. vicinus* provisioned with different diet treatments (χ^2 = 11.7897; df = 3; *P* = 0.0081).

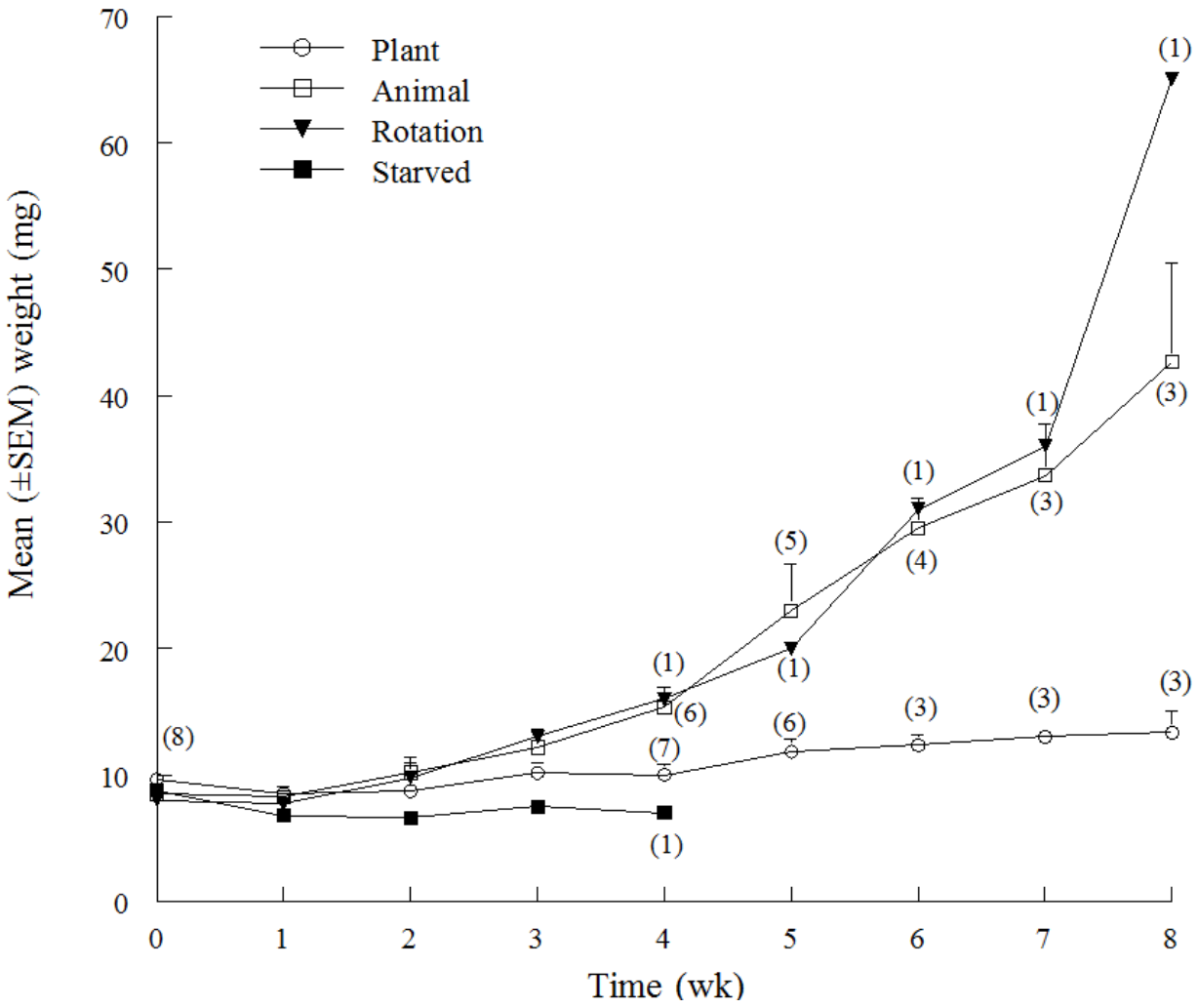

Figure 3.12. Growth rate for *S. vicinus* raised on different diet treatments in the laboratory over an 8 wk period. Means presented are actual means. There were initially eight replicates with each diet treatment (at 0 wk). The numbers in the brackets represent the number of mole crickets analyzed from 4-8 wk.

treatment on *S. vicinus* developmental time (time to 8 wk) was significant (F = 25.24; df = 1; P = 0.0015). Developmental times for 1st instars were not significantly different between the plant and animal treatments. However, the duration of 2nd instars in the plant diet treatment was approximately twice as long as nymphs in the animal diet treatment (Figure 3.14). Out of eight, three *S. vicinus* fed on animal diet reached the 4th instar stage, whereas only two nymphs in plant diet group reached 3rd instar stage after 8 wk (Figures 3.15 and 3. 16).

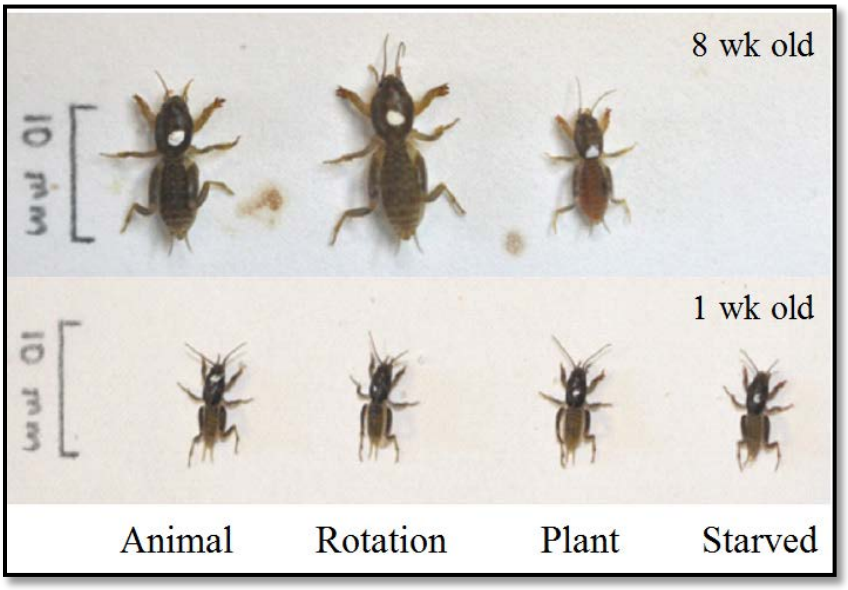

Figure 3.13. *Scapteriscus vicinus* nymphs raised on different diet treatments over a 1 wk and 8 wk period. The starved nymphs died at 5 wk.

Over an 8 wk rearing period, pronotal length and width were significantly influenced by diet treatment (ANOVA for repeated measures; F = 8.29; df = 1; P = 0.0121 and F = 21.45; df = 1; P = 0.0004, respectively). They were also influenced by instar stage (F = 82.19; df = 3; P < 0.0001 and F = 213.62; df = 3; P < 0.0001, respectively) and diet × stage interaction (F = 8.84; df = 2; P = 0.0038 and F = 12.41; df = 2; P = 0.0010, respectively). From the 1st – 3rd instar stage, pronotal length and width were not significantly different between the plant and animal treatments for each instar stage (Figures 3.15 and 3.16).

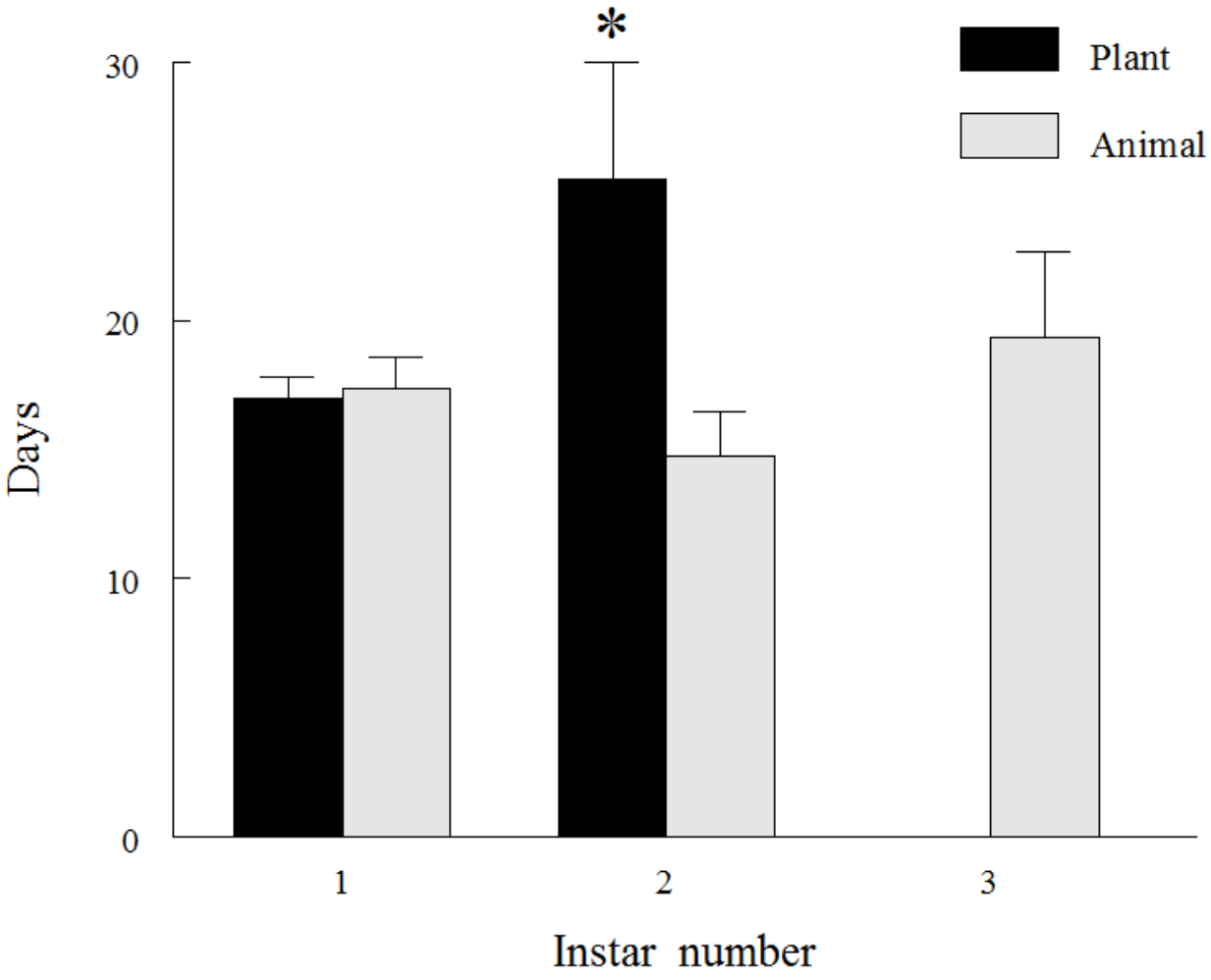

Figure 3.14. Development of *S. vicinus* provided with either a plant or animal diet in the laboratory over an 8 wk period. Asterisks, significant differences between treatments at each instar stage ($P < 0.05$; MIXED; LSmeans [SAS Institute 2008]).

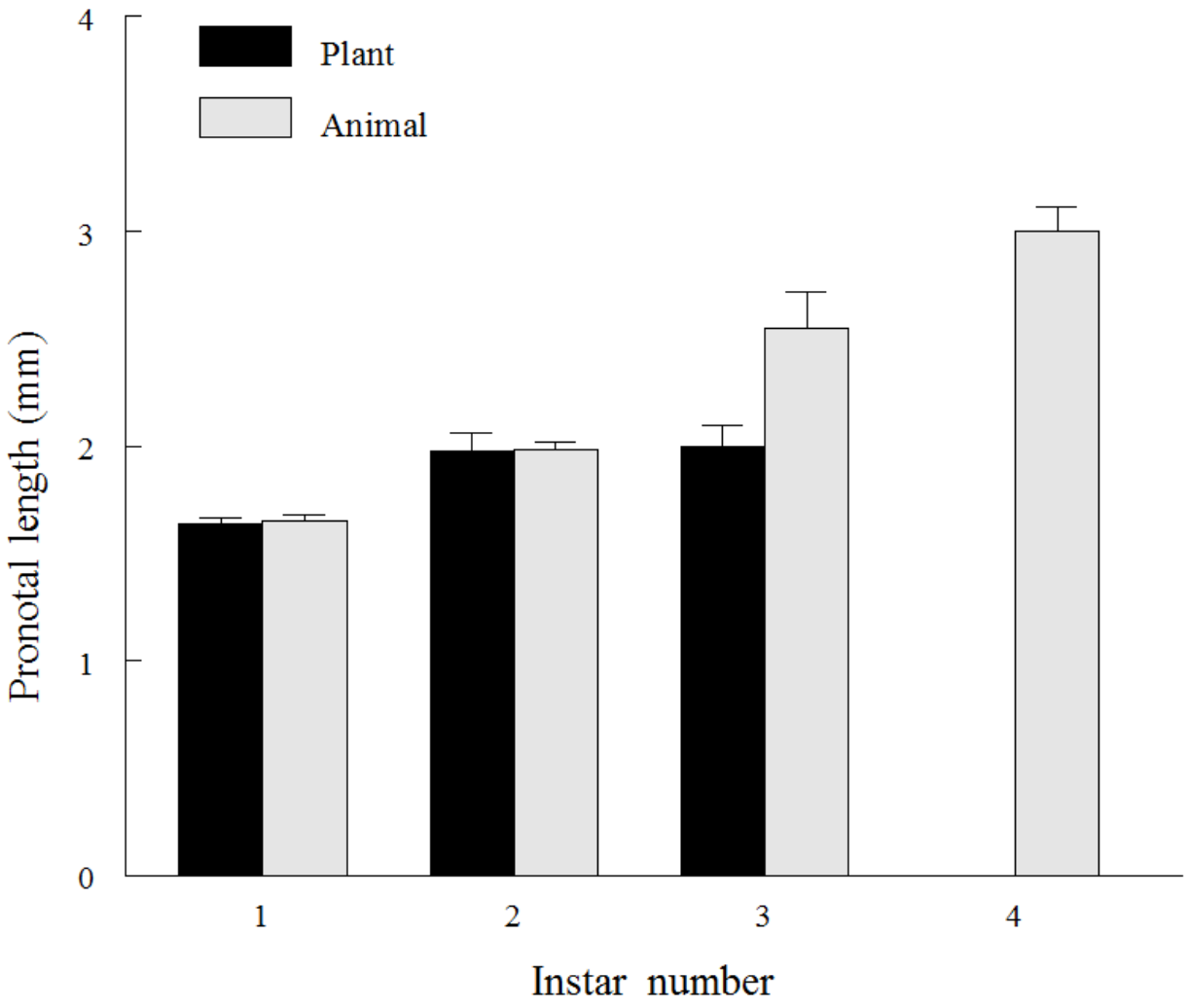

Figure 3.15. Pronotal length of *S. vicinus* provided with either a plant or animal diet in the laboratory over an 8 wk period.

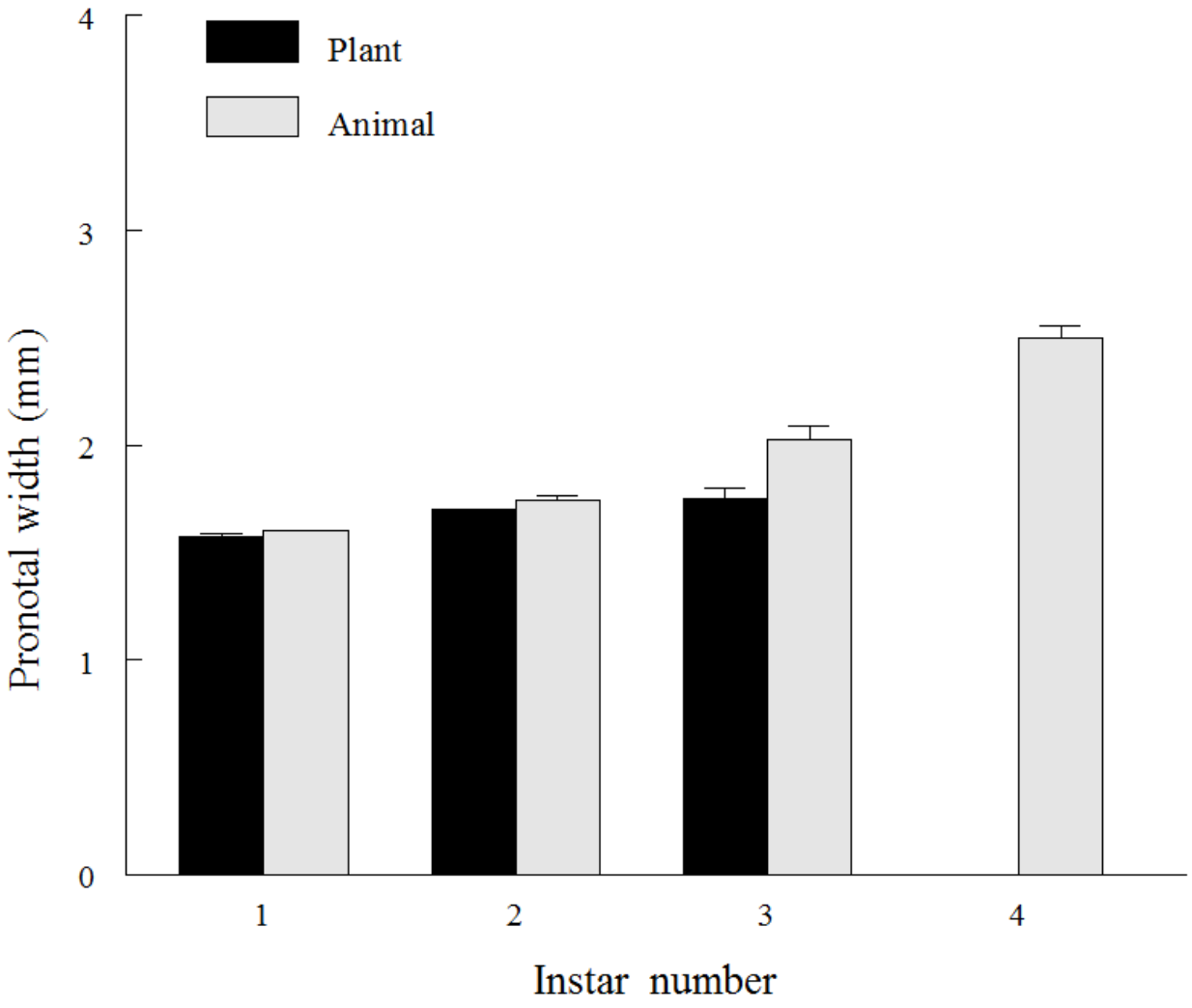

Figure 3.16. Pronotal width of *S. vicinus* provided with either a plant or animal diet in the laboratory over an 8 wk period.

Discussion

Our studies demonstrated a preference for animal diet by *S. borellii* and plant diet by *S. vicinus* adults (Figure 3.4). They also supported previous studies on gut contents (Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and Brandenburg 2011). As omnivores, both plant and animal diets were acceptable to *S. vicinus* and *S. borellii*. However, a preference for either diet likely reflected the dietary self-selection that enables insects to approach their nutrient target (Waldbauer and Friedman 1991, Behmer 2009) when given a choice. For instance, significantly greater water content probably is the determined nutrient in the carrot which was preferred by *S. vicinus* compared to mealworms (Table 3.1). The amount of water in the food of many herbivorous insects provides an important index of its nutritional value (Schoonhoven et al. 2005, Behmer 2009). For *S. borellii*, adults selectively ingested carrots and chose the relatively richer quality animal food. It is possible that nitrogen content is the determined nutrient in the animal tissue. Particularly for adult insects, animal protein supply is essential for reproduction (Schoonhoven et al. 2005). Additionally, 22% of primarily carnivorous *S. borellii* consumed both plant and animal diets in choice tests, whereas 43% of herbivorous *S. vicinus* consumed both diets. This also suggested that herbivores need more alternative diet to reach their intake target by dietary self-selection (Behmer 2009). Although foraging differences between the sexes are reported (Schoonhoven et al. 2005), our feeding preference tests did not reveal dietary divergence between the male and female mole crickets.

In no-choice tests, mole crickets were restricted to single foods significantly differing in water and nitrogen content. Although it is common that herbivorous (Lee et al. 2002) and carnivorous (Jetton et al. 2010) insects consume more of one diet than another in no-choice experiments, few studies have demonstrated this phenomenon with omnivorous insects. We

documented here that *S. vicinus* adults consumed significantly more of plant tissues than animal diet in the no-choice tests (Figure 3.4). Similarly, *S. vicinus* consumed more plants (Pensacola Bahiagrass) when no alternative food sources (i.e., lettuce, grapenut cereal, and hamburger meat) were available, whereas *S. borellii* did not (Abate 1979). Interestingly, although roots and stems of grass are anecdotally considered the primary plant food sources for mole crickets in the field (Hayslip 1943, Potter 1998), both *S. borellii* and *S. vicinus* consumed more carrots, which had the greatest moisture (Table 3.1), in the relative palatability among plants test (Figure 3.3). This possibly was caused by induction of feeding preference that occurs in many insect orders, including Orthoptera (Schoonhoven et al. 2005). Both *S. borellii* and *S. vicinus* adults were fed carrot strips and mealworms in the laboratory environment prior to the feeding tests, and they may have more readily accepted carrot strips as a conditioned response. Alternatively, a 12 h exposure to the diets may not have been enough time to force mole crickets to feed on the grass parts (Abate 1979).

Further investigation concerning dietary effects on survival and nymphal development provided insight into the relative benefits of each dietary choice on these omnivorous mole crickets. *Scapteriscus borellii* provisioned with diets containing animal protein supply (animal and rotational diets) had less mortality, greater body mass, and faster development compared to the ones fed on a pure plant diet. Animal diet was also a preferred option self-selected by *S. borellii* adults in the choice experiments. For *S. vicinus*, the lowest mortality of nymphs fed on plant diet confirmed their herbivore tendencies (Figure 3.11). However, relatively greater body mass and faster development of *S. vicinus* in animal diet group suggested that animal diet was more advantageous than plant diet for nymphal development. *Scapteriscus* mole crickets were observed to feed on a variety of food in the field (Hayslip 1943, Walker 1984, Schuster and Price

1992), and mainly carnivorous *S. borellii* also flexibly accepted pure plant diet to maintain survival and growth in laboratory rearing studies. Perhaps when energy is limiting (e.g., prey items are scarce), plants provide a readily available energy source, mostly in the form of digestible carbohydrates. This also implied that *S. borellii*, which may be a facultative omnivore (Coll and Guershon 2002), has a great capability of nutrient regulation and adaptability to diets to optimize development.

For primarily carnivorous *S. borellii*, the effect of absolute animal diet on its performance was not significantly different from that of more balanced rotational diet (Figures 3.6 and 3.9). Unexpectedly, *S. vicinus* nymphs fed on rotational diet did not perform well as *S. borellii* did; they even died faster than the starved ones (Figure 3.11). There is no likelihood that animal diet was hazardous to *S. vicinus*, because the nymphs in the animal treatment performed well over time. Thus, one reasonable explanation is the potential handling effects. Even though all nymphs were handled in the same way and disturbed with the same frequency by meal replacement, we were unable to avoid the mechanical disturbance to young nymphs. This also suggested that *S. vicinus* is likely more sensitive to mechanical disturbance, compared to *S. borellii*. Just like the survivorship, less starved *S. vicinus* nymphs (12.5%) survived at 4 wk compared to starved *S. borellii* nymphs (50%). Although nymphs were provisioned with dead prey which benefited them in the laboratory trials, *S. vicinus* performed as grazers and unlikely preyed on living creatures in the greenhouse assays (see details in Chapter 2). The study of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) determined that omnivores can feed on prey only as a result of random encounters on the host plant (Coll and Guershon 2002). Perhaps, the herbivory of *S. vicinus* determines its foraging behavior showing that they are opportunistic carnivores rather than predators.

Our experiment is unique in that we evaluated feeding behaviors of *S. vicinus* and *S. borellii* and quantified consumption of assigned diets when all diets were equally abundant. This is the first report of a laboratory choice test with *Scapteriscus* mole crickets. Furthermore, the nymphal development test is the first systematic study in the laboratory related to development of *Scapteriscus* mole crickets as influenced by diet. In the field, monitoring the population of mole crickets and scoring the age distribution can be essential to determine the timing of insecticide applications (Potter 1998). Previous rearing studies (Hudson 1987, Braman 1993) revealed the varieties in development duration and pronotal length of nymphal *S. borellii* and *S. vicinus*. Our studies also suggested that the variation in nymphal development may be extended due to the diet, especially when the pronotal length is $\geq$ 2.5 mm (older than 3rd instars; Figure 3.9). For example, at 7th instar stage, *S. borellii* nymphs fed on animal and rotational diets had longer pronotum (nearly 7 mm; Figure 3.9) than the ones in plant diet (approximately 4.5 mm). However, the 7th instars fed on cricket chow had 5.65 mm of pronotal length on average (Hudson 1987). Since the influences of diet on nymphal development are clearly visible, pronotal characters derived from the laboratory colony fed on single diet may be misleading when applied to the field population, which has relatively diverse diets (Taylor 1979, Matheny 1981, Fowler et al. 1985, Silcox and Brandenburg 2011). Our data may help to interpret the disparity in development of field populations of mole crickets (DWH unpublished data). As a result, this may enable turfgrass managers, homeowners, and sod producers to make better-informed decisions regarding the timing of mole cricket management, which is based on nymphal development (Potter 1998).

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APPENDICES

NOTES ON LABORATORY REARING AND INCUBATION OF MOLE CRICKETS

Objectives

A series of laboratory rearing trials were conducted to obtain eggs and neonate of mole crickets using field-collected adults. Materials and methods documented here were more elaborate compared to those in Chapter 3 of this thesis. Many previous experiments we normally conducted using living mole crickets failed due to high mortality and unsuccessful incubation of mole crickets. Therefore, we attempted different methods and summarized them as a final report. The reported methods should be a helpful reference for future students establishing a mole cricket colony under laboratory conditions.

Materials and Methods

Adult *S. vicinus* and *S. borellii* were collected using either the soap flush technique or acoustic trapping from golf courses in AL or MS. They were sorted by species and gender (Hayslip 1943), paired randomly, and each pair was placed into separate 473 ml transparent plastic cups (Dart, Mason, MI) with ventilated lids (with punched holes using scissors) containing approximately 450 ml of autoclaved, moistened sand. They were provided a mixed diet of organic carrot strips (Inter-American Products, Cincinnati, OH) and freeze-dried mealworms (Coleoptera: Tenebrionidae; Fluker Farms, Port Allen, LA) every 2 d, with dead

Popillia japonica Newman (Coleoptera: Scarabaeidae; collected locally using traps baited with a food-type lure) added occasionally, and held in a growth chamber (Percival Scientific Inc., Perry, IA) at 27 °C with a 14:10 (L:D) photoperiod.

After 2 weeks of pairing, each cup was checked every 2 d to determine egg laying. Mostly, females created an egg chamber at the bottom of the cup and laid eggs, which could be observed through the transparent cup. A male mole cricket was held with a female until one cluster of eggs was laid and then the male was removed. A male would be paired with more than one female if there were more collected females. After laying each cluster of eggs, the female was held with another male within the plastic cup for more progeny production (Walker 1984). Eggs were transferred using a plastic spatula (VWR International, Radnor, PA) into a 473 ml plastic cup with a ventilated lid containing 8 cm of moistened vermiculite (Figure A.1; Sun Gro Horticulture, Bellevue, WA), then incubated in a growth chamber at 27 °C with a 14:10 (L:D) photoperiod, and monitored weekly for eclosion. Number of clusters produced per female, number of eggs per cluster, and number of nymphs hatched per cluster were documented.

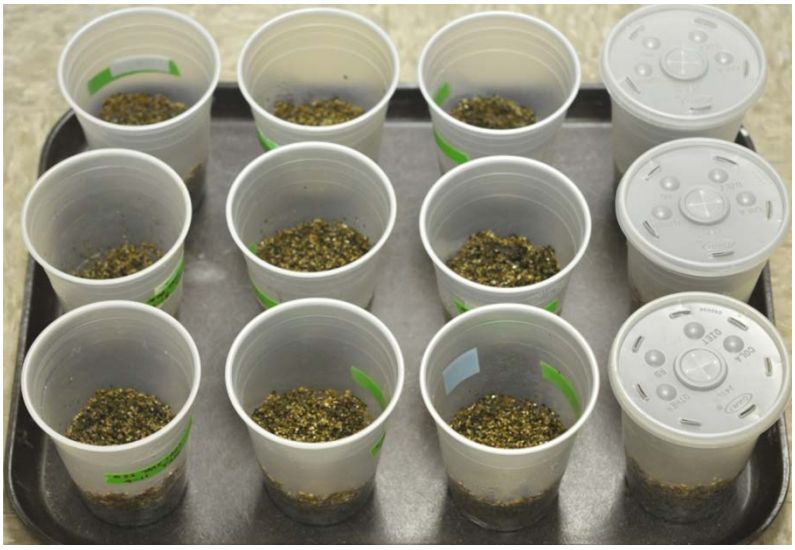

Figure A.1. Plastic cups with ventilated lids containing moistened vermiculite for incubation.

Results and Discussion

Photographs were taken to document the process of incubation of *S. borellii* (Figure A.2). A total of 72 egg clusters was obtained from 28 *S. borellii* females (73.68% of total field-collected females) that collected from four different sites on various dates. These clusters contained from 1 to 52 eggs, averaging 24.65 eggs per cluster. The peak of ovipostion of *S. borellii* was reached in August 2011 (Table A.1). A total of 60 egg clusters was obtained from 33 *S. vicinus* females (80% of total field-collected females) that collected from two sites on the same date. The clusters contained from 3 to 47 eggs, averaging 17.92 eggs per cluster. The peak of *S. vicinus* oviposition was reached in April 2012 (Table A.2). The number of eggs per cluster for each species is much smaller than was obtained in field, which ranged from 10 to 59 with an average of 35.6 eggs (Hayslip 1943).

Typically, nymphs hatched within 4 wk for both species as investigated in previous studies (Hayslip 1943, Braman 1993). Only 100 *S. borellii* nymphs (5.63%) and 68 *S. vicinus* nymphs (6.33%) hatched successfully (Tables A.1 and A.2). The rest of eggs were null without visible progress in development during 4 wk, and turned into dark gray color rather than milky white or light brown with appendages visible through the chorion (Hayslip 1943; Figure A.2). Although modified rearing methods guaranteed the number of mole cricket nymphs used in the development tests (see details in Chapter 3), the hatching rate was much lower than that presented in previous field study (Hayslip 1943; 47 and 42% for *S. borellii* and *S. vicinus,* respectively). This may be caused by multiple factors, such as unfertilized eggs and artificial environment of incubation (i.e., vermiculite) rather than natural egg chamber created by females under natural environment (Hayslip 1943).

Almost all females died immediately following the ovipostion of the last cluster of eggs. The peak of mortality occurred in September and October 2011 for *S. borellii* females, and in April 2012 for *S. vicinus* females. Similarly, the oviposition period of *S. borellii* females was longer than that of *S. vicinus* both in the laboratory environment and in the field (Hayslip 1943). This probably was caused by the different types of development or temperature (Hayslip 1943) in different sites. According to the life cycle of *S. borellii* and *S. vicinus* summarized by Hayslip (1943), all *S. vicinus* females collected from in current study probably were the fall adults. However, it was not convincing to conclude the types of development for *S. borellii* females due to the various sites and dates. Perhaps, the relation between biolocation and oviposition should be investigated for future study.

Figure A.2. Photographs illustrate the incubation process of *S. borellii* under laboratory conditions, from newly laid eggs (a) to 1st instar nymphs (f).

Table A.1. Female *S. borellii* collected from four locations and their ovipostion information

Location	Collection Date	SMC #	Oviposition Date	No. of Eggs	No. of Nymphs
Shoal Creek Country Club, Birmingham, AL	03-14-2011	1	06-16-2011	26	2
			07-11-2011	23	4
			07-22-2011	31	0
			08-01-2011	37	3
			08-29-2011	15	0
		2	05-07-2011	33	0
Mr. Turf Sod Farm, Union Springs, AL	05-03-2011	3	08-09-2011	14	0
			08-22-2011	14	0
			09-01-2011	22	0
			09-17-2011	26	1
			09-28-2011	33	0
		4	06-03-2011	24	0
			07-03-2011	32	0
			08-01-2011	31	1
			09-26-2011	51	0
	04-21-2011	5	07-11-2011	3	0
Grand National Golf Course, Opelika, AL	05-16-2011	6	08-24-2011	14	2
		7	08-03-2011	38	0
	05-24-2011	8	08-24-2011	33	0
			09-21-2011	17	0
	05-25-2011	9	08-05-2011	17	0
			08-16-2011	15	0
			08-26-2011	17	0
			09-07-2011	26	0
			09-28-2011	19	0
			11-24-2011	10	0
	06-01-2011	10	08-07-2011	27	0
			10-04-2011	31	1
		11	07-02-2011	15	12
			08-09-2011	25	0
			08-26-2011	26	0
	06-02-2011	12	07-11-2011	39	0
			08-19-2011	19	0
			08-29-2011	4	0
			09-07-2011	33	0
			09-16-2011	33	0
			10-17-2011	10	0
	06-03-2011	13	07-03-2011	1	0
			08-07-2011	17	0
			08-19-2011	21	0
		14	06-27-2011	31	0

			07-22-2011	17	0
			08-11-2011	10	0
			09-16-2011	52	0
		15	08-03-2011	32	0
			08-24-2011	33	2
			09-12-2011	28	0
		16	06-27-2011	30	0
	06-04-2011	17	07-01-2011	22	18
		18	07-02-2011	18	0
			08-16-2011	39	2
	06-05-2011	19	08-09-2011	43	11
			10-14-2011	49	1
		20	08-09-2011	19	0
		21	07-12-2011	22	0
		22	07-01-2011	39	15
		23	07-01-2011	22	10
			08-14-2011	23	0
	06-13-2011	24	08-03-2011	5	0
			08-29-2011	43	0
			09-09-2011	23	0
			09-26-2011	20	1
			10-04-2011	14	0
Great Southern Golf Club, Gulf Port, MS	10-24-2011	25	01-06-2012	22	11
			01-13-2012	21	0
		26	01-27-2012	11	0
			02-08-2012	32	0
			02-28-2012	44	0
			03-07-2012	21	0
		27	02-27-2012	27	3
			03-07-2012	30	0
		28	01-23-2012	11	0

Table A.2. Female *S. vicinus* collected from two locations and their ovipostion information

Location	Collection Date	TMC #	Oviposition Date	No. of Eggs	No. of Nymphs
Gulf Hills Golf Club, Ocean Springs, MS	03-14-2012	1	04-13-2012	15	0
		2	04-11-2012	28	0
			04-23-2012	17	0
		3	03-28-2012	4	0
			05-02-2012	6	1
			05-23-2012	9	0
		4	04-23-2012	20	0
		5	04-06-2012	36	0
		6	03-30-2012	21	0
		7	04-11-2012	20	5
			04-18-2012	34	0
Shell Landing Golf Club, Gautier, MS	03-14-2012	8	04-04-2012	11	0
		9	03-23-2012	6	0
		10	04-25-2012	27	8
		11	04-16-2012	30	1
			05-04-2012	6	0
		12	04-04-2012	25	0
			04-11-2012	18	2
			04-18-2012	8	0
			04-25-2012	6	4
		13	04-11-2012	20	0
			04-20-2012	18	0
		14	04-06-2012	15	0
			04-30-2012	22	9
		15	04-04-2012	10	0
		16	04-16-2012	26	0
			04-23-2012	22	0
		17	04-06-2012	28	0
			04-16-2012	9	0
		18	04-04-2012	22	0
			04-18-2012	21	2
		19	04-04-2012	18	0
		20	04-04-2012	12	0
		21	04-18-2012	34	8
		22	04-18-2012	47	0
		23	04-10-2012	25	5
		24	04-06-2012	25	1
		25	03-21-2012	4	0
		26	03-21-2012	15	1
			03-30-2012	15	4
		27	03-28-2012	3	0

	28	03-23-2012	9	4
		03-30-2012	27	0
		04-09-2012	15	2
	29	04-06-2012	19	1
		04-16-2012	22	0
	30	03-20-2012	9	0
		04-06-2012	22	0
		04-18-2012	24	1
		04-30-2012	17	0
	31	03-21-2012	5	1
		04-11-2012	21	3
		04-27-2012	47	0
	32	04-16-2012	19	2
		04-23-2012	10	3
		05-04-2012	12	0
	33	04-11-2012	9	0
		04-18-2012	19	0
		04-30-2012	7	0
		05-07-2012	4	0