Respiration and Behavior of a Sugarcane Grub, *Ligyrus Subtropicus* (Coleoptera: Scarabaeidae) Under Flooded Conditions[1]

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J. Entomol. Sci. 27(1): 71-77 (January 1992)

ABSTRACT The sugarcane grub, *Ligyrus subtropicus* (Blatchley) encounters both natural field flooding and controlled field flooding by Florida sugarcane growers. Third instar larvae (grubs) of *L. subtropicus* were tested in various flood tests. After a five day (120 h) simulated flood, 68% of grubs survived at 18° C versus 0% at 28° C. Respiration as indicated by CO_2 production was significantly higher in grubs at 28° C in both flooded and unflooded treatments than in grubs at 18°C under similar conditions. These data indicate that grubs drown faster at higher temperatures at least partly due to increased respiration. In behavior tests conducted at water temperatures of ca. 24° - 25°C, grub populations moved upward in response to flooded conditions including 21.7% of the grubs which emerged from the soil to settle on the soil surface. Grubs became sluggish after 2 h underwater. Within 24 h underwater, all grubs were comatose (non-responsive to probing) and remained in this condition until death.

KEY WORDS *Scarabaeidae, flooding, sugarcane, Ligyrus subtropicus,* respiration, behavior

Sugarcane, Florida's most valuable field crop, is primarily grown in the Everglades area of southern Florida. Since 1971, several species of Scarabaeidae have caused substantial damage to sugarcane in southern Flordia. Of these pests, the white grub, *Ligyrus subtropicus* (Blatchley), is of primary economic importance (Gordon and Anderson 1981). This grub reduced sugar per hectare in Florida by 39% in areas of high infestation (Sosa 1984). Currently, no chemical control is known for this pest in sugarcane.

One method of grub control in sugarcane that has been used is controlled flooding of sugarcane fields. A normally abundant water resource and water control expertise have created an ideal situation in the Everglades for water use in the control of certain diseases, nematodes, and subterranean insects (Genung 1970). Flooding the highly organic muck soils of southern Florida is also sound soil conservation, because flooding reduces microbial oxidation of soil organic matter (Tate 1979). Flooding mortality to *L. subtropicus* has been reported by Genung (1976) Summers (1977), Cherry (1984a), and Cherry et al. (1990).

Field flooding for controlling soil insect pests has been noted in several studies for species other than *L. subtropicus*. These studies include wireworms in Florida (Genung 1970; Hall 1990) and in California (Campbell and Stone 1938) and scarabs

[1] Accepted for publication 2 January 1992.

in India (Avasthy 1967). Various studies have also reported that higher water temperatures are more effective in causing flooding mortality of soil insect pests (Campbell and Stone 1938; Cherry 1984a; Hall 1990). However, the reason(s) for increased flood mortality at higher temperatures is currently unknown. In addition, behavior of most soil insect pests under flooded conditions is anecdotal with little quantitative data. The objective of our study was to determine physiological and behavioral responses of *L. subtropicus* grubs to flood conditions. These data should provide a better understanding of *L. subtropicus* responses to flooding, and may also give insight into responses of soil insects under flood conditions noted in other studies.

Materials and Methods

Flooding mortality. *L. subtropicus* third instars were collected by digging in commercial sugarcane fields during January, 1990. After collection, grubs were stored in large plastic pans filled with muck soil and carrots for food and held at room temperature (approximately 25° C) in a laboratory. The effect of temperature on flood mortality of the grubs was determined at 18° C and 28° C in temperature cabinets. These two temperatures were used since they approximate the extremes of seasonal temperatures in flooded fields in southern Florida (Cherry 1984a). Ten grubs were placed into each 31 by 23 by 9 cm covered plastic pan which was one half filled with water. Five pans with grubs were flooded at each temperature for a five day period. In this study, "day" is defined as 24 continuous hours (i.e. 5 days = 120 h). The five day flood period is based on the earlier recommendation of Summers (1977) who stated that flooding standing cane for five to seven days from August to November gave control of *L. subtropicus*. A control pan containing 10 grubs in muck soil with carrots was also held at each temperature. After the 5 day flood period, grubs were removed from the water and held 48 h in pans containing muck soil and survival was determined by gently prodding insects. Grubs not moving were considered dead. Mortality was checked after 48 h because grubs were all comatose when first removed from the water, but may recover later. Flooding tests were conducted from Feb. 1, 1991 to Feb. 9, 1991.

Respiration. During November - December, 1990, *L. subtropicus* third instars were collected and stored as described previously. During this time, respiration as indicated by CO_2 production was determined under unflooded and flooded conditions at 18° and 28° C. A total of 40 grubs were tested with 10 grubs being tested at flooded and unflooded conditions and at each temperature. Grubs tested were selected in the weight range of 3.5 to 4.5 g/grub to minimize grub weight differences among the four treatments. Grubs used in all tests weighed an average of 4.05 g and mean grub weights among the four treatments ranged from 4.00 to 4.13 g/grub. Respiration of each grub was determined as total CO_2 produced by each grub in each flask during 24 h. Analysis of variance was used to determine differences in means of total CO_2 among the four treatments.

Unflooded conditions were simulated by placing 1 grub into a 125 ml Erlenmeyer flask containing 50 mls of moist sand. Moist sand provided grubs a medium to dig into to simulate field conditions. Sand was chosen since no CO_2 would be produced unlike an organic soil such as the muck in which the grubs are normally found. Each grub was rinsed with water, gently dried with a paper towel, weighed, and placed into a flask. The flask was immediately sealed with a septum stopper

and sampled for an initial CO_2 measurement. Thereafter, each flask was put into a temperature cabinet at 18° or 28° C and held undisturbed in darkness, for 24 h at which time another CO_2 measurement was performed. To measure CO_2 in each flask, 1.0 ml of air was withdrawn through the sealed stopper with a lockable gas syringe. These samples were injected directly into the inorganic carbon port of a Dohrman DC-90 carbon analyzer which had been calibrated with gas samples having precisely known CO_2 contents.

Flooded conditions were simulated by placing 1 grub into a 125 ml Erlenmeyer flask filled with water. Grubs were previously gently washed, dried, and weighed, and then dropped directly into the water filled flasks. The larvae initially thrashed about apparently in distress, but became motionless after 60 to 90 min. After the grub was dropped into the water, a septum stopper was added immediately and the initial CO_2 reading determined. Thereafter each flask was put into a temperature cabinet at 18° or 28° C and held in darkness, undisturbed, for 24 h at which time another CO_2 reading was performed. Carbon dioxide samples were taken by inserting a glass liquid syringe through the stopper, withdrawing 200 microliters of water and injecting this sample into the inorganic carbon port of the previously described carbon analyzer. The carbon analyzer was calibrated with standard sodium carbonate samples.

Behavior. During March - April, 1991, *L. subtropicus* third instars were collected and stored as described in flooding mortality tests. Vertical movement of grubs in response to flooding was measured in plastic buckets. Each bucket was 30 cm high by 30 cm in diameter and filled with 20 cm deep with muck soil along with a small sugarcane plant to simulate field conditions. From 0 to 20 cm deep approximates the depth at which most *L. subtropicus* third instars normally occur in Florida sugarcane fields (Cherry 1984b). To simulate natural light conditions buckets were wrapped in black plastic except on top. Tests were conducted during March - April, 1991 and buckets were held in a screenhouse. Five grubs were placed into each bucket at 15 cm deep by dropping each grub into one of five holes 15 cm deep made by a 2 cm diameter pipe pushed into the soil to create the hole. After placement of these grubs into the holes, the holes were quickly filled with soil and the procedure repeated at 5 cm deep with 5 new grubs. Buckets were paired during tests with one bucket remaining unflooded and one bucket being flooded. Immediately after grubs were placed into the designated flood bucket, flooding commenced by pouring 500 ml of water into the bucket on the soil surface. Thereafter, 500 ml of water was added to the flood bucket at approximately eight h intervals so that after 48 h, the bucket was flooded about 2 cm deep with only sugarcane leaves rising above the water level. The 48 h interval for gradual flooding was selected to simulate the occurrence of natural field flooding which occurs frequently in southern Florida, especially during the summer-fall "rainy season." After the buckets were flooded, they were held another five days (=120 h) in this flooded condition. The five day period was used since we wanted to fully stress the grubs to elicit their reponse to flooding and previous research (Cherry 1984a) indicated that the five day period would kill most grubs in the buckets. Soil temperatures taken during the test averaged 24° C in all buckets.

At the end of the seven day flood, grubs were recovered in all buckets by slowly sorting through the soil in the unflooded buckets or mud in flooded buckets. The seven day flood period refers to the two days of rising water levels plus five days where the water level was 2 cm above the soil surface. Sorting

consisted of visually examining the mud or soil for the grubs which are large and white and easily seen in the black muck. Sorting was conducted from top to bottom and the position of each grub recovered was noted in reference to vertical zonation such as surface, 0 to 10 cm deep, or 10 to 20 cm deep. Survival of grubs in unflooded buckets was noted during sorting and survival of flooded grubs was determined after 48 h due to their comatose condition at recovery. Soil in unflooded buckets remained moist during the experiment, thus approximating soil moisture conditions in which the grubs are normally found. Six pairs of unflooded versus flooded buckets were tested during March - April 1991. Vertical zonation of grubs in buckets was compared between buckets before flooding, after 7 days unflooded, and after the 7 day flood test by using Chi-square contingency tables to test for homogeneity (Dixon and Massey 1969).

We conducted an additional test to determine how long third instars of *L. subtropicus* are active underwater. On April 4, 1991, ten grubs were placed into 500 ml Erlenmeyer flasks (1 grub/flask) filled with water. Flasks were placed into a container (cooler) where they could be maintained undisturbed in darkness. Each grub was checked hourly for activity during the first 8 hours and then at 8 hour intervals thereafter. Grubs were first visually examined for movements in the flasks. If movement was not observed, grubs were gently probed in the flasks to discern if comatose (= no movement). Water temperatures in the flasks were recorded and grubs were flooded for five days (120 h). Based on data from Genung (1976) and Cherry et al. (1990), it was estimated that most grubs would die after 3 to 5 days under water at the temperatures in the flasks. Five grubs were removed from the water after 2 days (48 h) and the survival noted 48 h later. The remaining 5 grubs were removed from the water after 5 days and survival was again noted 48 h later.

Results and Discussion

Flooding Mortality. Lane and Jones (1936) reviewed flooding for insect control and were the first entomologists to stress the importance of temperature in affecting flood mortality of insects. In their study, flooding was more effective in controlling wireworm populations in California when soil temperatures were higher. Campbell and Stone (1938) essentially corroborated Lane and Jones (1936) data by again showing that warmer soil temperatures were more effective in controlling wireworm populations in California. Cherry (1984a) showed flood mortality to *L. subtropicus* grubs was greater at 25° C than 20° C. Hall (1990) in a laboratory study on flooding to control the wireworm, *Melanotus communis* (Gyl.) found greater flood mortality at 26° C than at 18° C. In this study we selected 18° C and 28° C to test for flood mortality to *L. subtropicus* third instars since these temperatures approximate the extremes of seasonal temperatures in flooded fields in southern Florida (Cherry 1984a). Consistent with previous studies, we also found greater flood mortality at the higher temperature. Sixteen (32%) of the 50 grubs flooded for five days (120 h) at 18° C died versus 100% mortality for the 50 grubs at 28° C. The mean number ± SE of grubs surviving in pans at 18° C was 6.80 ± 0.38 versus 0 ± 0 at 28° C. These data show that with equal time intervals flooding sugarcane during warmer months results in a greater mortality of *L. subtropicus* grubs than flooding in cooler months. The importance of the correct

timing of flooding for controlling *L. subtropicus* including such factors as seasonal feeding activity, flood duration, insect stage, and weather conditions has been discussed by Cherry (1991).

Respiration. Lane and Jones (1936) noted that oxygen deficiency, carbon dioxide accumulation, and osmotic reactions were possible explanations for increased flood mortality at higher temperatures in wireworms in California. However, these authors presented no data to support these explanations. Cherry (1984a) suggested that increased flood mortality to *L. subtropicus* observed at higher temperatures was probably due to increased respiration, but no data were provided to support this conjecture. Respiration as indicated by CO_2 production of third instars of *L. subtopicus* under flooded and unflooded conditions at two temperatures is given in Table 1. Respiration was significantly higher in grubs at 28° C in both flooded and unflooded treatments than in grubs at 18° C under similar conditions. These data are expected since insects as a class are pokilothermic; i.e. their metabolism varies directly with fluctuations in the environmental temperature (Edwards 1953). Insects have evolved numerous adaptations for aquatic respiration such as cutaneous respiration, tracheal gills, etc. (Wigglesworth 1972). Currently, however, we do not fully understand how *L. subtropicus* grubs cope with aquatic conditions when flooded or why the grubs die more quickly at higher water temperatures. However, our data do suggest that the increased flood mortality at higher temperatures is at least partly explained by increased respiration which causes the insects to drown more quickly. Hopefully, future experiments will more clearly resolve the reason(s) for increased flood mortality observed at higher temperatures in this study and in other flooding studies such as Lane and Jones (1936), Campbell and Stone (1938), and Hall (1990).

Table 1. Respiration as indicated by CO_2 production of third instars of *L. subtropicus* under flooded and unflooded conditions.

	Means ± SE of mg CO_2 produced/grub/24h*	
Temperature	Flooded	Unflooded
18° C	1.36 ± 0.18	10.7 ± 1.0
28° C	2.64 ± 0.15	17.3 ± 0.66

* Ten grubs used in each of the four treatments. Analysis of variance showed all four means were significantly different from each other at the 0.05 P level.

Behavior. Little data exist on the behavioral response of grubs to flooding. Avasthy (1967) observed that in flooded sugarcane fields in India, few grubs of *Holotrichia consanguinea* Blanch were in the top soil layers and most grubs were found deeper, although data were not provided. Genung (1970) reported that white grubs were observed at the surface where birds gathered in flooded pastures in southern Flordia. However, the grub species or percentage of grubs at the surface was not reported. The percentage of third instars of *L. subtropicus* found at different soil levels before and after flooding is shown in Table 2. The frequency distribution of grubs 7 days after flooding was significantly different from the distribution of the grubs before flooding and significantly different from the distribution of the unflooded grubs after 7 days. Overall, there was an upward movement of grub populations in response to flooding including 21.7% of the grubs

Table 2. Percentage of third instars of *L. subtropicus* found at different soil levels before and after flooding.

Depth	After 7 days (168 h)		
	Before flooding*	Unflooded†	Flooded
Surface	0	0	21.7
0 - 10 cm	50	18.3	43.3
10 - 20 cm	50	81.7	35.0

* Frequency distributions of grubs before flooding and 7 days after flooding are significantly ($P < 0.005$) different as analyzed by a Chi-square contingency table to test for homogeneity (Dixon and Massey 1969). Chi-square = 24.6 with 2 degrees of freedom.

† Frequency distributions of grubs in unflooded versus flooded buckets are significantly ($P < 0.005$) different as analyzed with a Chi-square contingency table to test for homogeneity (Dixon and Massey 1969). Chi-square = 50.4 with 2 degrees of freedom.

which came out of the soil to settle on the soil surface. These latter grubs moved to the surface approximately 15 minutes to 2 h after the water level was raised above the soil surface. These grubs became inactive within 2 h on the soil surface and remained inactive the remainder of the flood duration. These grubs made little attempt to climb or lodge in the sugarcane plant. The flood mortality in the test was 100%.

In the test to determine how long grubs are active underwater, most grubs became sluggish after 2 h underwater, although 100% of the grubs were still responsive to probing. Within 24 h underwater, all grubs were comatose (non-responsive to probing) and remined in this condition the duration of the five day flood. The five grubs removed after 2 days all recovered indicating that grubs at this time were comatose, but not dead. The water temperature during the experiment averaged 25° C. At this temperature, most grubs would be expected to die after 3 to 5 days (Genung 1976; Cherry et al. 1990). The 5 grubs withdrawn from the water after 5 days were all dead. The preceding data on length of grub activity under water are consistent with unquantified observations in the flooding mortality tests, respiration tests, and bucket tests. In all these tests, grubs generally became inactive within 2 hours underwater, and remained inactive thereafter until they eventually drowned.

Acknowledgment

We are grateful to Lydia Polyanchek for her assistance in conducting the laboratory portion of this research. Florida Agricultural Journal Series Number R-01690.

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