MORTALITY INDUCED BY *BACILLUS POPILLIAE* IN *CYCLOCEPHALA PARALLELA* (COLEOPTERA: SCARABAEIDAE) HELD UNDER SIMULATED FIELD TEMPERATURES

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Abstract

The bacterium, *Bacillus popilliae* Dutky, causes milky disease in numerous species of scarabs around the world. *Bacillus popilliae* induced mortality in naturally infected grubs (third instars) of *Cyclocephala parallela* Casey was measured when held under simulated field temperatures. Our data show that visual examination in the field underestimates the percentage of grubs actually infected by *B. popilliae*. 5.6 to 8.2 times as many milky disease infected grubs died during the first 60 days of incubation under simulated field temperatures than did uninfected grubs. These data show that the widely used prevalence value underestimates the total mortality which this bacterium ultimately causes to *C. parallela*.

Key Words: white grubs, milky disease, sugarcane pests, natural infection

Resumen

La bacteria *Bacillus popilliae* Dutky, causa la enfermedad lechosa en numerosas especies de escarabajos alrededor del mundo. La mortalildad inducida por *B. popilliae* en larvas (tercer instar) de *Cyclocephala parallela* Casey fue medida bajo condiciones simuladas de temperatura de campo. Nuestros datos muestran que el examen visual en el campo subestima el porcentaje de larvas realmente infestadas por *B. popilliae*. De 5.6 a 8.2 veces más larvas infestadas que no infestadas murieron durante los primeros 60 dias de incubación bajo temperaturas de campo simuladas. Estos datos muestran que el valor aplilamente usado de prevalencia subestima la mortalidad total que esta bacteria causa a *C. parallela*.

White grubs of the family Scarabaeidae are important insect pests of agricultural crops, horticultural plants, and turf world wide. The bacterium, *Bacillus popilliae* Dutky, causes milky disease in many scarab species and is one of the most widely known pathogens in biological control of insects (Klein & Jackson 1992). Infection with milky disease is synonymous with eventual grub death (Warren & Potter 1983). Prevalence of a disease in insect populations is the most commonly used parameter in epizootiology and is defined as the number of hosts afflicted with that disease at a given point in time (Fuxa & Tanada 1987). Many studies such as those of Harris (1959), Hutton & Burbutis (1974), Boucias et al. (1986), Kaya et al. (1992, 1993), and Redmond & Potter (1995) have reported on the prevalence of *B. popilliae* in different grub species. In many of these studies, it is either stated or implied that a high prevalence of infection indicates that *B. popilliae* is effective in controlling grub populations. Conversely, a low prevalence suggests that the bacterium is ineffective. However, grubs infected with *B. popilliae* would be expected to die more quickly under field conditions and be removed from future samples. Hence, prevalence data will underestimate the cumulative mortality of the insects over time. This would especially be true in a warm climate where milky diseased grubs will die more quickly due to faster development of *B. popilliae* at high temperatures (Milner et al. 1980; Cherry & Boucias 1989), and thus these grubs will be removed from prevalence estimations. Numerous studies have been conducted on *B. popilliae* in different grub species (Klein 1992). However, no studies have attempted to determine if prevalence data underestimate the impact of *B. popilliae* in controlling grub populations. In this study, we report on *B. popilliae* induced mortality in naturally infected grubs of *Cyclocephala parallela* Casey held under simulated field temperatures. The relevance of these data to the use of prevalence data of *B. popilliae* in grub populations is discussed.

Materials and Methods

Third instar larvae (grubs) of *C. parallela* were collected by digging under sugarcane plants in commercial fields in southern Florida. *Cyclocephala parallela* larvae were collected from October to March when the predominant life stage in Florida sugarcane fields is the third instar (Cherry 1985). Approximately 70 grubs were collected each month from October 1993 to March 1994 (354 grubs) and October 1994 to March 1995 (326 grubs). Grubs were collected in the morning and held 2 to 3 h in plastic buckets filled with soil. Physical damage to grubs caused by digging became apparent during the 2 to 3 h period after collection and these bruised grubs were removed from the tests. After the damaged grubs were discarded, milky appearing grubs were noted, and the grubs were placed individually into petri dishes. Each petri dish (9 cm diam) contained a piece of raw carrot for food and moist soil. The soil was obtained from a field which had been in rice production for several years. This soil was selected since we believed it would contain few, if any, *B. popilliae* spores due to the absence of scarab populations in this cropping system. Hence, we felt that we were evaluating naturally infected grubs in all stages of infection directly from the sugarcane fields and not infecting them while they were being held in the lab.

Grubs were held in the petri dishes in a temperature cabinet in constant darkness and at simulated field temperatures. Simulated field temperatures were obtained by holding the grubs at the mean monthly soil temperatures (Cherry 1991) at 10 cm, which is where most grubs are found (Cherry 1984). Daily temperatures at that depth in Florida sugarcane fields do not fluctuate greatly during the time the tests were conducted. The temperature was changed each month to match the field temperature of that month. Cabinet temperatures were 26.0, 26.0, 22.8, 18.3, 20.2, 21.3, and 23.3°C for the October through April test period. Grub survivorship was checked every 3-4 days by opening the petri dish to examine the grub. This procedure also allowed fresh carrot and/or water for soil moisture to be added. Grubs which were inactive and did not move when prodded were considered dead and were frozen for later examination for *B. popilliae* spores. All grubs still alive 60 days after the start of the tests were killed by freezing and held for later examination. Since these grubs were alive at 60 days, and they would have lived longer than 60 days if we had not frozen them, they were recorded as dying at >60 days. Frozen grubs were thawed out later and bled mid-dorsum onto individual microscope slides. These slides were examined with phase contrast microscopy for the presence of *B. popilliae* spores. Mortality data were grouped into four time intervals of 0-20, 21-40, 41-60, and >60 days. Thereafter, these data were put into a 4×2 contingency table (Steel & Torrie 1980) using Chi-square analysis to determine if the mortality rate was independent of *B. popilliae*.

Results and Discussion

A total of 354 grubs were observed from October 1993 to March 1994. Four of these grubs appeared milky at field collection and hemolymph examination later showed *B. popilliae* present in all four. *B. popilliae* was found in a total of 23 grubs. Hence, only 17.4% of the grubs with *B. popilliae* were actually seen as milky at field collection.

A total of 326 grubs were observed from October 1994 to March 1995. Fourteen of these grubs appeared milky at field collection and hemolymph examination later showed *B. popilliae* present in all 14. *B. popilliae* was found in a total of 39 grubs during the second year. Hence, 35.9% of the grubs with *B. popilliae* were actually seen as milky at field collection.

Harris (1959) used a visual examination for milky appearing grubs in the field to estimate the disease incidence of *B. popilliae* in *C. paralella* populations. Our data show that visual examination in the field for milky grubs seriously underestimates the percentage of grubs actually infected by *B. popilliae*. These data support the findings of Kaya et al.(1992, 1993) for *B. popilliae* in *C. hirta* LeConte in California turf.

Mortality data for *C. parallela* grubs held from October 1993 to March 1994 are shown in Table 1. Chi-square analysis showed that mortality rate was dependent (Chi-square = 106.3, $P < 0.005$) upon the presence of *B. popilliae*. During 0 to 59 days after field collection, 69.6% of grubs with *B. popilliae* died. In contrast, 8.5% of grubs without *B. popilliae* died during that time frame. Hence, in this test, 8.2 times more grubs which were infected with *B. popilliae* died during the first 59 days under simulated field temperatures than did uninfected grubs.

Mortality data for *C. parallela* grubs held from October 1994 to March 1995 are also shown in Table 1. Chi-square analysis again showed that mortality rate was dependent (Chi-square =74.6, $P < 0.005$) upon the presence of *B. popilliae*. During 0 to 59 days after field collection, 64.1% of grubs with *B. popilliae* died. In contrast, 11.5% of grubs without *B. popilliae* died. Hence, in this test, 5.6 times more milky disease in-

TABLE 1. MORTALITY OF THIRD INSTAR *CYCLOCEPHALA PARALLELA* AFTER FIELD COLLECTION FROM OCTOBER 1993 TO MARCH 1994, AND OCTOBER 1994 TO MARCH 1995.

	Number Dying During Interval (Days)[a]			
	0-20	21-40	41-60	>60
October 93-March 94				
+*Bacillus popilliae*	3(1.4)	8(1.0)	5(0.5)	7(20.2)
−*B. popilliae*	18(19.6)	7(14.0)	3(7.5)	303(289.9)
October 94-March 95				
+*B. popilliae*	10(3.2)	5(1.8)	10(1.9)	14(32.2)
−*B. popilliae*	17(23.8)	10(13.2)	6(14.1)	254(235.8)

[a]Number in parentheses = Expected via Chi-square analysis. The contingency table (Steel and Torrie 1980) shows that the mortality rate is dependent (Chi-square = 106.3, $P < 0.005$ [93-94], and = 74.6, $P < 0.005$ [94-95]) upon the presence of *Bacillus popilliae*.

fected grubs died during the first 59 days under simulated field temperatures than did uninfected grubs.

C. parallela has a one year life cycle with the third larval instar being the predominant stage during nine months of the year (Cherry 1985). *B. popilliae* is found in all three larval instars (Cherry and Boucias 1989). Data in Table 1 show that percent prevalence taken at any one time underestimates the total mortality to *C. parallela* caused by *B. popilliae*. This underestimation is simply due to *B. popilliae* infected grubs dying more rapidly under conditions in the fields than healthy grubs and hence infected grubs are removed from future samples.

In summary, our data show that the impact of *B. popilliae* upon *C. parallela* may be underestimated for two reasons. First, field observation of visually obvious milky appearing grubs indicates only a proportion of the total infected grubs. Second, and more important, the widely used percent prevalence method underestimates the total mortality which the bacterium ultimately causes to *C. parallela* over time.

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