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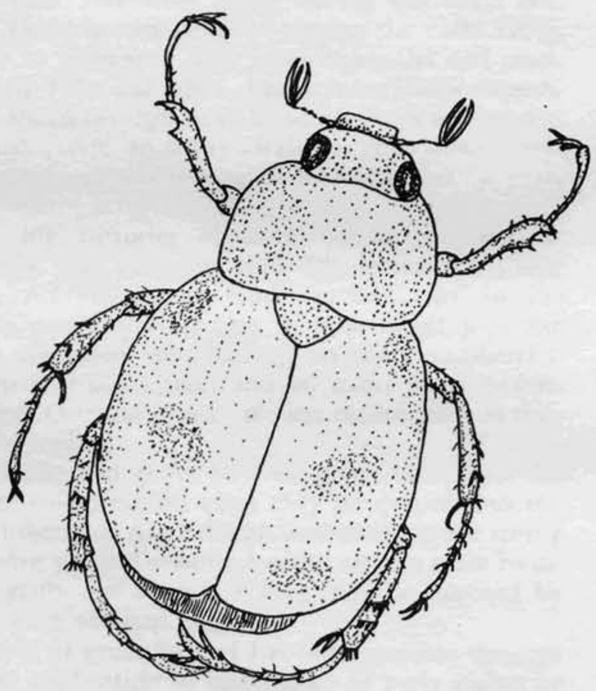

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The Oriental beetle, *Anomala orientalis* Waterhouse, was found in Connecticut in New Haven in 1920 (Friend 1929), and the Japanese beetle, *Popillia japonica* Newman, was first discovered in the state in Stamford in 1926 (Britton 1927). Grubs of both species caused severe damage to turf throughout Connecticut and other states during the 1930s and 1940s. These beetles declined during the early 1950s, and for 20 years they were inconsequential turf pests.

But in 1973 and 1974, heavy infestations demonstrated that a resurgence of these beetles is well underway in the state. In many locations, grub density and damage to turf was similar to that observed 25 years ago.

The life histories of both beetles are similar. Generally, they complete their life cycles in a single season. Adults emerge from infested turf in the summer, mate, and lay eggs in turf. Adult Japanese beetles are often seen feeding on roses, raspberries, sassafras and plum trees and on many other plants. But adult Oriental beetles are less conspicuous as they do little feeding.

Newly-hatched grubs feed on grass roots until the onset of cold weather when they go deeper into the soil to hibernate. After the ground thaws in the spring they move up and resume feeding on the grass roots. When grubs are abundant they may be exposed by rolling back the turf (Fig. 1).

Control of grubs in turf has been possible through periodic application of insecticides in early spring or late summer, or by distribution of spore dust of the bacterium, *Bacillus popilliae* Dutky, causal agent of milky disease. This report summarizes field and laboratory studies which evaluate the current status of disease and insecticides in controlling Japanese and Oriental beetle grubs in Connecticut.

Fig. 1. Rolling back the turf shows heavy infestation of Japanese beetle grubs. Many grass roots have been destroyed. A similar infestation of Oriental beetle grubs would have much the same appearance.

MILKY DISEASE

During 1939-51, Experiment Station personnel in cooperation with the USDA distributed approximately 5000 lbs of spore dust of the milky disease bacterium over 2374 acres at 2996 sites in Connecticut (Fleming 1968). The disease became established at many of the sites, and spread naturally to other areas. In 1945, 41.5% of all Japanese beetle grubs were found to be diseased at sites where spore dust was distributed (Schread 1946). Beard (1964) found the incidence of milky disease in 1962 so high among Japanese beetle grubs at several locations that he concluded that the beetle would become a problem only in infested areas where disease or other natural control agents were absent.

No further systematic survey of milky disease was made until 1974 (Dunbar and Beard 1975). Grubs were collected from 53 sites in spring and 62 sites in fall for laboratory examination. The incidence of disease was remarkably low for both Japanese and Oriental beetle grubs. Sixteen locations where spore dust of milky disease was distributed during 1939-43 were included in our fall survey. Although the bacterium was found at a number of the 16 locations, the incidence of disease, and hence control, was not as great as that once observed.

Laboratory experiments (Dunbar and Beard 1975) determined that spores of *B. popilliae* have become less infective. This appears to be associated with decreased productivity of spores in infected hosts. Field inocula are inadequate to check rising grub populations. In addition, induced infection is well below that expected from earlier reports (Beard 1944, 1945; Fleming 1968). Thus grubs appear to have developed some resistance to the bacterium.

If genetic selection for resistance is starting to have an effect, it may accelerate with further increase in beetle populations. But, on the other hand, with more host material available, the bacterium may be favored by a natural selection for greater infectivity and productivity.

INSECTICIDES

Lead arsenate was used during the late 1930s and early 1940s to control Japanese and Oriental beetle grubs in turf in Connecticut (Johnson 1943). DDT and chlordane came into use in the late 1940s (Schread 1948, 1953). Because of its special effectiveness, chlordane became the most widely used insecticide for grub control in Connecticut. A single application often protected turf for several years.

A recent report from Yale Golf Course, New Haven (hereafter called Yale), that repeated applications of chlordane were no longer controlling Japanese beetle grubs led us to suspect that resistance to this insecticide may be developing in Connecticut. This would not be surprising in view of the recent discovery of chlordane-resistant Japanese beetle grubs in Ohio (Niemczyk and Lawrence 1973) and in New York (Tashiro and Neuhauser 1973).

The field and laboratory studies reported below were conducted during 1974 to determine if chlordane resistance had developed in Connecticut and to evaluate the effectiveness of insecticides that might replace chlordane.

Development of Chlordane Resistance

Field Test. Chlordane was last applied at Yale in spring, 1973, at a rate of 8 lbs A.I./acre. Acceptable grub control was not achieved. Our field test was initiated in spring, 1974, to determine if higher rates of chlordane would give control of Japanese beetle grubs.

On April 20, liquid and granular formulations of chlordane were each applied to two plots 10 x 10 ft at rates of 10 and 20 lbs A.I./acre. Diazinon was applied to two plots at a rate of 5.5 lbs A.I./acre. Two plots were left untreated. The liquid formulations were mixed with 3 gals. water and applied with a sprinkling can. Granular chlordane was mixed with sifted sand and applied with a lawn fertilizer spreader. Plots were thoroughly irrigated to wash the insecticides from the vegetation.

Control was evaluated on May 24 by counting the living grubs in 1.38 ft^2 of turf excavated in six random core samples (Fig. 2) per plot. The data (Table 1) clearly show that diazinon was highly effective, but chlordane, even at two and four times the normal rate (5 lbs. A.I./acre), was ineffective. Development of a chlordane-resistant strain of Japanese beetle at Yale seemed likely.

Fig. 2. Core sampler used in examining soil for Japanese beetle grubs; diam. = 6.4 inches.

Laboratory Test. A dose-mortality test was conducted to compare chlordane susceptibility in field

Table 1. Effectiveness of diazinon and chlordane against Japanese beetle grubs at Yale Golf Course, New Haven, during spring, 1974.[1]

Insecticide and formulation	lbs. A.I./acre	Mean no. grubs/ six 0.23 ft.² samples/plot[2]
Diazinon (AG 500)	5.5	1
Chlordane (74% EC)	10.0	23
	20.0	24.5
Chlordane (10% G)	10.0	27
	20.0	18
Untreated	—	26

1/ Two replicates/treatment, 10 x 10 plots.
2/ All grubs were 3rd-instar.

collected 3rd-instar Japanese beetle grubs from the resistance-suspected population at Yale and from a susceptible population from East Rock Park, New Haven. The locations are about 3.5 miles apart. Third-instar grubs of the Oriental beetle also from East Rock Park were included in the same dose-mortality test.

Chlordane (74% EC) was incorporated into moist soil to produce equivalent rates of 10, 5, 2.5, 1.25, 0.625 and 0 lbs. A.I./acre. Grass seeds (a rye-fescue mixture) were added to the soil to provide food for the grubs. Treated soil was then placed in metal trays (10 x 10 in.) which were partitioned into 100 cells. One grub was placed in each cell. Thirty-six to 130 grubs were observed/treatment. Trays were covered and held at 78°F. Test grubs were checked after 11 days. Corrections for check mortality were made by applying Abbott's formula (Abbott 1925).

Mortality of grubs from Yale was low, while Japanese and Oriental beetle grubs from East Rock Park experienced high mortality (Table 2). Although the Oriental beetle grubs from East Rock Park were less susceptible to chlordane than the Japanese beetle grubs from the same area, there is no reason to suspect resistance to chlordane in that population. By examining the data in Table 2 by probit analysis (Litchfield and Wilcoxon 1949) and plotting on log-probit scales to determine LD_{50} values, we estimate resistance in the Yale population to be 80-fold higher than in East Rock Park (Fig. 3).

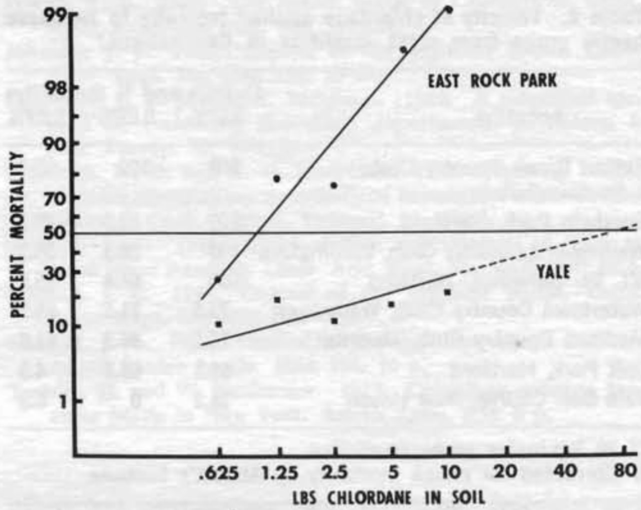

Fig. 3. Dose-mortality responses of Japanese beetle grubs from Yale and East Rock Park held in chlordane-treated soil for 11 days.

Geographic Distribution of Resistance

Having established resistance to chlordane, we evaluated other grub populations. Because the soil test method used above was not practical to evaluate several populations simultaneously, a quicker technique of topically applying chlordane in dosage series was used. In a preliminary test where grubs from Yale and East Rock Park were compared, this method produced results similar to the soil test.

Third-instar Japanese beetle grubs were collected from eight locations in the state. Solutions of 0.87, 0.27, 0.09 and 0% chlordane in acetone were applied topically to 30 grubs/dose/location. Approximately 3.1 μl of solution were applied between the legs and head of each grub. Grubs were held in soil containing grass seeds in 8 oz. plastic containers (15 grubs/container) for 7 days.

Although grubs from different locations varied in their response to chlordane (Table 3), all, except those from Yale, were susceptible. The response of grubs from Yale further supports the conclusion that they are resistant to chlordane. The low mortality observed in grubs from Watertown, Madison, and

Table 2. Susceptibility of grubs to chlordane-treated soil.[1]

	Source of Grubs					
	Yale		East Rock Park			
	Japanese Beetle		Japanese Beetle		Oriental Beetle	
lbs. A.I./acre	no. observed	% mortality[2]	no. observed	% mortality[2]	no. observed	% mortality[2]
10	117	19.7	57	100	52	77.7
5	100	15.9	33	100	60	65.0
2.5	99	10.1	32	72.4	60	35.4
1.25	98	17.3	34	77.3	64	31.7
0.625	99	10.6	36	23.3	64	18.7

1/ All grubs were 3rd-instar.
2/ Corrected for check mortality by Abbott's formula.

Table 3. Toxicity of chlordane applied topically to Japanese beetle grubs from eight localities in Connecticut.[1]

Location	Dosage and % mortality[2] 0.87%	0.29%	0.09%
Patton Brook Country Club, Southington	100	100	83.2
Goodwin Park, Hartford	100	88.0	38.1
Wallingford Country Club, Wallingford	100	88.3	35.3
Mt. St. Benedict, Hartford	100	64.4	35.8
Watertown Country Club, Watertown	79.6	71.5	28.5
Madison Country Club, Madison	76.1	86.3	61.7
Colt Park, Hartford	69.5	65.2	4.3
Yale Golf Course, New Haven	18.2	0	6.9

1/ 30 3rd-instar grubs/treatment.
2/ Corrected for check mortality by Abbott's formula.

especially Colt Park, may indicate that resistance is beginning to develop in those populations.

Field Evaluation of Insecticides

In the event that chlordane resistance spreads to other areas in the state, alternative insecticides will be required to protect turf from damage. A field test was conducted during August and September 1974, to evaluate seven insecticides against grubs at three different golf courses. Test plots 10 x 10 ft. were set out in fairway turf at Yale, Patton Brook Country Club, Southington, and at the Wallingford Country Club. Each treatment was replicated six times in a randomized complete block design at each course.

Premeasured amounts of insecticides were applied to each plot in both directions to insure proper coverage. Liquid and wettable powder formulations were mixed in 5 gals. water and applied by sprinkling can. Granular formulations were mixed with sifted sand and applied with a lawn fertilizer spreader. Each plot was thoroughly irrigated after application of the insecticides. Treatments applied on August 20-22 were evaluated on September 20-23. The method was the same as that used in the summer field test at Yale.

Soil from Patton Brook was classified as Manchester loamy sand with 2.8% organic matter. Soils from both Yale and Wallingford had considerably heavier textures and contained more organic matter than that from Patton Brook. The Yale soil is Charlton sandy loam with 7.0% organic matter and the Wallingford soil is Cheshire sandy loam with 6.0% organic matter.

The density of grubs was greatest at Patton Brook and lowest at Yale (Table 4). Approximately 95% of the grubs identified from the three test areas were those of the Japanese beetle. The other grubs were mainly those of the Oriental beetle and the Asiatic garden beetle, *Maladera castanea* (Arrow). With the exception of chlordane at Yale, all of the insecticides reduced the numbers of grubs in the treated plots to levels significantly lower than those in the untreated plots.

While Dasanit provided the highest level of control of all the insecticides tested, Dursban, diazinon and Primicid also were highly effective under all three soil conditions. Mocap was effective at Patton Brook and Yale, but was not effective at Wallingford. This is especially puzzling because the soil type at Wallingford is nearly the same as at Yale where Mocap was effective. The lowered effectiveness of Dylox at Patton Brook may be associated with rapid leaching since it is highly soluble in water and the Patton Brook soil is high in sand and low in organic matter.

Should resistance to chlordane spread, effective insecticides are available for use.

Table 4. Effectiveness of different insecticides in controlling Japanese beetle grubs, fall, 1974.

Insecticide and formulation	lbs. A.I./acre	Mean no. grubs/six 0.23 ft.[2] samples/plot[1] Walling-ford	Patton Brook	Yale
Dasanit (10% G)	4.0	0.0 a	0.0 a	0.3 a
Diazinon (AG 500)	5.0	1.3 a	1.2 a	5.2 ab
Dursban (2E)	4.0	3.3 a	3.2 a	0.2 a
Dylox (80% SP)	8.0	3.7 a	13.5 b	0.8 a
Chlordane (74% EC)	5.0	3.8 a	10.8 a	12.2 bc
Primicid (EC -500g/1)	2.0	4.8 a	1.8 a	1.6 a
Mocap (10% G)	5.0	14.5 b	1.7 a	3.5 ab
Untreated	—	36.8 c	52.0 c	18.5 c

1/ Data were transformed to $\sqrt{x} + 0.5$ for analysis. Means within a column followed by the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

SUMMARY

A strain of Japanese beetle highly resistant to chlordane was discovered in 1974 at Yale Golf Course in New Haven. Chlordane resistance does not appear to be widely distributed in the state, but with widespread use of chlordane, development of resistance could accelerate.

In a field test conducted during August and September 1974, several insecticides including Dasanit, Dursban, Primicid and diazinon were found highly effective in controlling Japanese beetle grubs in infested turf. Although Dylox, Mocap and chlordane were less effective, they reduced the numbers of grubs in treated plots to levels significantly lower than in untreated plots.

Diazinon, Dursban, Dylox and chlordane are currently registered for control of grubs in turf.

Acknowledgments

We thank Gene Hubeny for his assistance in these studies. We also thank Dr. David Hill of this Station for classifying soil from the different test areas and the Superintendents of the Yale Golf Course and the Patton Brook and Wallingford Country Clubs for use of their turf for our field tests.

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ADDENDUM

(Additional tests to evaluate insecticides for control of Japanese beetle grubs were conducted by the senior author after this manuscript was submitted.)

FIELD EVALUATION OF INSECTICIDES SPRING, 1975

Infestations of Japanese beetle grubs in turf are often not discovered until early spring. Field tests were conducted at the Madison Country Club to determine if the same insecticides effective for fall grub control were also effective in the spring. Two additional insecticides (Ciba-Geigy A 12223 and Dyfonate), not included in the fall tests, were also included.

Plot size and methods of application and evaluation were the same as those described above. Treatments were applied on May 2 and evaluated on June 3 and 6. Each treatment was replicated five times in a randomized complete block design. Soil from the test area was classified as Hinckley gravelly sandy loam with approximately 3% organic matter.

All of the insecticides reduced the grub population in the treated plots to levels significantly lower than in the untreated plots (Table 5). As in the fall tests, Dasanit and diazinon provided high levels of control. Ciba-Geigy A 12223 was also highly effective and shows promise as a new insecticide for grub control because of its low application rate. Dyfonate, Dursban and chlordane were less effective, but still reduced the grub population 71-78% below the level in the untreated plots. Mocap reduced the grub population by only 47.2%. This poor control was similar to that achieved at Wallingford in the fall.

Acknowledgement

The assistance and cooperation of the Superintendent of the Madison Country Club is appreciated.

Table 5. Effectiveness of different insecticides in controlling Japanese beetle grubs at the Madison Country Club, spring, 1975.

Insecticides and formulation	lbs. A.I./acre	Mean no. grubs/six 0.23 ft.[2] samples/plot[1]
Dasanit (10% G)	4.0	0.0 a
Ciba-Geigy A 12223 (2E)	2.0	0.2 ab
Diazinon (AG 500)	5.0	0.6 ab
Dyfonate (10% G)	5.0	7.2 abc
Dursban (2E)	4.0	8.8 bcd
Chlordane (74% EC)	5.0	9.4 cd
Mocap (10% G)	5.0	17.0 d
Untreated	—	32.2 e

1/ Data were transformed to $\sqrt{x + 0.5}$ for analysis. Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).