

To the Graduate Council:

I am submitting herewith a thesis written by Wayne Malcolm Millington entitled "Population Survey and Bacterial Control of the Japanese Beetle in Happy Valley, Blount Co., Tennessee." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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POPULATION SURVEY AND BACTERIAL CONTROL
OF THE JAPANESE BEETLE IN
HAPPY VALLEY, BLOUNT CO.,
TENNESSEE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Wayne Malcolm Millington
August 1980

3044902

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. L. E. Klostermeyer for his patience, encouragement, and able guidance through the course of this study. Thanks go also to Dr. R. R. Gerhardt and Dr. J. H. Reynolds for their advice and suggestions. The author also wishes to thank Mr. W. J. VanLandingham for the use of his 1978 adult trapping data for comparisons. Also, thanks go to Mr. J. R. White, Mr. J. B. Bogard, and Mr. R. B. Quillin of the Tennessee Department of Agriculture for their moral and financial support of this project. Finally, thanks go to Dr. Carroll J. Southards and to the rest of the staff of the Department of Entomology and Plant Pathology for being there when I needed them.

ABSTRACT

The Japanese Beetle, *Popillia japonica* Newman (Coleoptera:Scarabaeidae) was first detected in the United States in 1916 and entered Tennessee in about 1936. The adult and larval stages damage plants in most of Eastern Tennessee yearly. Adult population surveys were carried out during the summer of 1978 and 1979 in Happy Valley, Blount County, Tennessee. Adults began emerging on 2 June in 1979. A peak of 5,934 beetles per trap was reached on 26 July 1978 and 5,095 per trap on 19 July 1979. Larval surveys in 1978 and 1979 detected densities of 5.2 larvae per 0.09m^2 and 2.0 larvae per 0.09m^2 respectively. In a mark, release, and recapture of adults, 98% of recaptured beetles were within 200 meters of the release site with a recapture range of 5.1m to 2.24km. Milky Spore, a commercial mixture of spores of *Bacillus popilliae* Dutky, *B. lentimorbus* Dutky, and talc, was applied to the study area as a biological control agent. One infected grub was detected in October, 1979. Bioassays were made of all Milky Spore used. Levels of infectivity ranged from 0 to 12%.

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CHAPTER I

INTRODUCTION

The Japanese beetle, *Popillia japonica* Newman (Coleoptera:Scarabaeidae) is a major defoliating pest in the Eastern United States. It was introduced into the United States prior to 1916 at a nursery in Riverton, New Jersey (Davis 1920). It was first detected in Tennessee in 1936 in Sullivan County and by 1968 was found in over 33 counties of eastern Tennessee (Wallace 1968).

Control techniques used against the Japanese beetle are limited. Chemicals have shown some success when used for controlling small infestations, but not for large infestations. Biological control, using bacterial milky disease, appears to be the most effective control or suppression alternative. Milky disease has been proven to be successful in the suppression of Japanese beetle population density in the northeastern states of the United States (Fleming 1968) and thus was chosen as the agent of control for these experiments.

Research on the Japanese beetle in Tennessee has been limited. Ladd et al. (1972) carried out sterile-male releases and a mark, release, and recapture experiment in

Monroe County in 1972. Intensive surveys for the detection of Japanese beetles have been done by the state of Tennessee in cooperation with the Plant Pest Control Division of the Animal and Plant Health Inspection service, the United States Department of Agriculture (APHIS-USDA). On 10 September 1978, the USDA dropped its survey in Tennessee (Bogard 1980). Because of the termination and the increased concerns of the citizens of eastern Tennessee, the Tennessee Department of Agriculture (TDA) initiated its own Japanese Beetle Suppression Program in 1978-1979. The program consisted of state and counties matching funds which were then used to purchase milky disease powder (Milky Spore®, Reuters Laboratory Inc., Haymarket, VA 22069). The material was then spread on permanent turf at public schools, parks, etc. as well as private lawns.

The purpose of this research project, under the leadership of The University of Tennessee, was to evaluate the effects of milky disease on the population density of Japanese beetles in Happy Valley. The purpose was also to examine and record adult dispersal ability and adult emergence dates of the Japanese beetle in eastern Tennessee.

CHAPTER II

LITERATURE REVIEW

I. Anatomy

The adult Japanese beetle is a shining, metallic-green beetle about 15 cm long and 7.5 cm wide. It is broadly oval and characteristically scarablike (Davis 1920). The elytra are coppery brown and cover most of the abdomen. Two small tufts of white hair behind the wing covers and five patches of hair along each side are characters that distinguish this beetle from other similar beetles (Hadley and Hawley 1934). The female and male can be easily distinguished from each other by the shape of the tibial spurs. The spurs of the male are more pointed than those of the female (Fleming 1972). In addition, the first tarsal segment of the female is as long as the other three tarsal segments combined; whereas the male has four small tarsal segments of equal size (Hadley and Hawley 1934).

The eggs of the Japanese beetle are elliptical and translucent. When deposited they are about 1.6 mm in diameter but double in size because of the uptake of moisture and development of the larva (Davis 1920, Fleming 1972).

The 1st instar larvae are approximately 1.5 mm long. At first they are white but turn yellowish after one to three hours. After feeding, an accumulation of fecal or soil material in the hindgut gives the posterior of the abdomen a characteristic black color (Fleming 1972). On the ventral side of the last abdominal segment two conspicuous rows of six or seven short straight spines are arranged in a "v" pattern (Fig. 1). This "v" is a character that distinguishes the Japanese beetle grub from other white grubs (Fleming 1972, Sim 1934, Ritcher 1966).

The pupa is coarctate and greatly resembles the mature beetle, except the legs, antennae, and wings are closely folded to the body. They are pale cream, soon becoming tan. They are about 15 cm long and 7.5 cm wide (Hadley and Hawley 1934, Fleming 1972).

II. Biology

The life span of the adult beetle is 30 to 45 days (Hadley and Hawley 1934). The beetle emerges in late May and early June in the southern USA (Morrill and Dobson 1978, Fleming 1972). After emergence, the beetle seeks out a nearby food source. The adult may attack over 300 species of plants, causing serious damage to many (Whittington 1942). Plants of the family Rosaceae such as roses, blackberries, apples, peaches, cherries, and raspberries are particularly

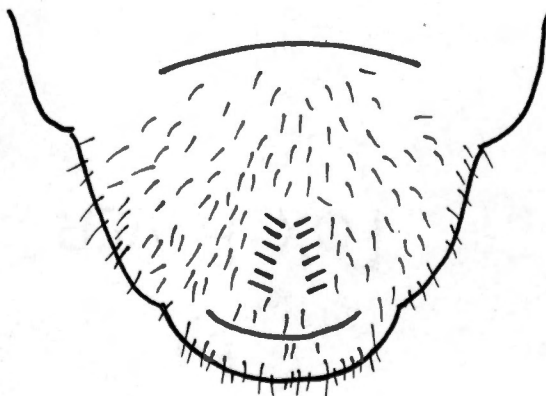


Fig. 1. Raster pattern of the Japanese Beetle larva.
Ventral view of the last abdominal segment.

attacked by the adult. Other plants attacked or damaged are beans, grapes, sassafras, and smartweed (Hadley and Hawley 1934, Fleming 1972). The attractancy of certain preferred plants such as sassafras and hemlock results from the presence of certain plant constituents such as acetic and valeric acids. When these constituents are removed the plants are not nearly so favored (Langford et al. 1943).

Mating may occur at any time during the day. It usually occurs on plants, but may also occur on the ground. The act of mating may be brief or last for several hours. Each female will usually mate between each ovipositional period (Fleming 1972).

After the adult female has mated she returns to the sod to lay her eggs. She burrows 7 to 9 cm into the soil and lays one to three eggs. She then returns to the surface to feed and mate again. She will return to the sod 15 to 25 times during her life to oviposit. A total of 40 to 60 eggs can be deposited during her life (Hadley and Hawley 1934, Fleming 1972). For survival of the eggs to occur, the soil must remain moist to prevent desiccation (Fox 1939), and the soil temperature must remain between 7° and 34°C for normal growth and development (Ludwig 1928, Hawley 1949, Hall et al. 1968). Under drought conditions the

female will oviposit in the lower spots of a field, where moisture is still present (Fox 1939). Eggs normally hatch about two weeks after they are deposited (Fleming 1972).

After hatching, the larvae feed on grass roots. During normal feeding, the larvae remain in the upper 5 to 10 cm of soil. As the soil temperature begins to decrease in the fall, feeding ceases and the larvae prepare for the overwintering process (Fleming and Metzger 1936). When the soil temperature reaches 15°C , the larvae begin moving down in the soil. They cease this movement when the soil temperature reaches 10°C and overwinter where they are. For most larvae this will be 10 to 20 cm below the sod (Hadley and Hawley 1934). Although mature larvae are relatively cold hardy (Fleming 1972), at air temperatures below -9°C the larvae will live only a few days (Ludwig 1928). Also, under conditions where ice forms in the soil, larvae can be crushed and killed (Hawley and Dobbins 1941). As the soil temperature increases above 10°C the following spring, the surviving larvae move up to the sod and resume feeding. After feeding for a short time, the larvae changes into an inactive prepupa. After spending about 10 days in the inactive prepupal condition, they change to the quiescent pupal stage. This stage lasts from

8 to 20 days, depending upon the temperature and other factors. Final transformation to the mature form then takes place, and the adult beetles, emerging from their pupal skins, make their way to the surface of the soil (Hadley and Hawley 1934).

III. Larval Survey Methods

Hadley and Hawley (1934) found that soil surveys for Japanese beetle larvae were time consuming and difficult. Furthermore, they found that after sampling, data often failed to give a valid estimate of the population sampled. With this in mind, Fleming and Baker (1936) developed guidelines for population surveys of immature Japanese beetles. They found that from a consideration of the standard deviations, the standard error of the means, and the concentration of the larvae about the median, that a 30 by 30 cm (1 sq ft) unit is the most dependable for estimating larval populations in the field. They also found that to find valid estimates of a population being sampled, 10% of the area must be sampled. To eliminate the possibility of biased sampling, each unit of the sample was taken at definite intervals over the whole area. For samples of 225 square meters or more the interval should be 3 meters (Fleming and Baker 1936). Unfortunately, these techniques still require large amounts of time and labor.

Regniere et al. (1979) felt that a computer model could estimate total oviposition of field populations from data on the seasonal variation of the egg content of females caught in traps. Such information could constitute the basis for predictions of population density changes without involving time-consuming and costly sampling of larvae in soils. This model has not been tested in the field to date.

IV. Adult Survey Methods

Since the discovery and identification of the Japanese beetle in New Jersey, many different styles and shapes of traps have been used for adult population surveys. The earliest traps were simple funnels placed over collection jars (Richmond and Metzger 1929). Later, traps were designed with lighter materials and mass production in mind. Metzger (1934) designed a trap with a four-wing baffle system set above the funnel to act as an interceptor for the passing beetles. After making a few modifications, Metzger received a US Patent for this trap in 1935 (Armstrong and Metzger 1935). Although Metzger's 1935 design for the trap is still being used today, many different studies have been conducted in an attempt to increase the efficiency of trapping. Studies were made to find the lightest and least expensive construction materials (Langford et al. 1940a). Work was also carried out to streamline the

baitwell to prevent the clinging of the beetles after attraction (Klein et al. 1973a). Efforts were also made to streamline the remaining trap sections (Klein et al. 1973b). Streamlining aided by increasing capture numbers and preventing the escape of any beetles. The color of traps was also studied to decide what color was most attractive to the beetle, yellow (Fleming et al. 1940) or green (Metzger 1932). Today green is considered to be the most attractive for Japanese beetles (Klein et al. 1973a). The distance of the traps above the ground was also studied (Whittington and Bickley 1941, Ladd and Jurimas 1972). The optimum height for an attractant trap was found to be between 55 and 110 cm above the ground. Traps at these heights were significantly more attractive than those at ground level (Ladd and Jurimas 1972).

Another factor that was considered was the location of the traps. Whittington et al. (1942) found that traps in the vicinity of favored host plants were often significantly more attractive to the beetles than more distant traps. Langford et al. (1943) felt that the attractiveness of those favored plants was due to chemicals produced in the plants that attracted the beetles. He felt that by removing those chemicals, those plants could be rendered less desirable.

Studies were also conducted concerning the effectiveness of the attractants used with the traps. Early attractants were molasses and bran mixtures, but these proved somewhat ineffective (Richmond and Metzger 1929). Many attractants have been used and evaluated since then: geraniol (Smith 1924), geraniol plus eugenol (Fleming et al. 1940), phenylethyl butyrate plus eugenol (Schwartz 1968), and the most recent attractant phenylethyl propionate plus eugenol (Ladd et al. 1974).

The value of attractant traps in controlling the Japanese beetle has been evaluated in several ways. The actual cost of trapping versus the benefits received is one method for evaluation. Another method of evaluation is the monitoring of damage levels before and after trapping to determine significance (Langford et al. 1943). Others found that evaluation was difficult because of the extremely high population levels that existed in areas of infestation and the mobility of those individuals (Langford et al. 1940b).

V. Bacterial Control

The existence of diseases among larvae of the Japanese beetle and their usefulness in the reduction of the populations of these larvae has been known for many years (Hadley and Hawley 1934). There are protozoan, fungal, rickettsial,

and bacterial diseases of Japanese beetles (Fleming 1968). The most important diseases are the milky diseases caused by the bacteria *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky. The milky diseases represent the most significant natural control agents available in the US for the Japanese beetle (Fleming 1968).

The milky disease organisms were first discovered in central New Jersey in 1933. Hawley and White (1935) noticed that in areas where heavy infestations had been present for several years, many normally clear Japanese beetle larvae were milky and clouded. Dutky (1940) isolated, separated, and named two organisms that caused these milky symptoms. The first, Type A, *B. popilliae*, was found to be a gram-positive rod with a developmental range of 16° to 36°C. The second, Type B, *B. lentimorbus*, was also a gram-positive rod with a developmental range of 16° to 30°C. Both organisms were apparently indigenous. They are soil borne, and the spores remain viable for extended periods in the soil. The organisms are most resistant in the spore stage (Fleming 1968). Sharpe and Rhodes (1973) found that sporulation will occur on the surface of an artificial media, with growth only on the periphery. They also found that the formation of heat resistant spores would occur on artificial media. St. Julian et al. (1975) found that the strain of organism

being used and the particular host involved are very important. The reaction of each host was unique and thus unpredictable. Even within seemingly identical strains of *B. popilliae* the level of infectivity can be very different (Pridham et al. 1964).

B. popilliae and *B. lentimorbus* invade the hemolymph of the Japanese beetle larvae. Spores are ingested with other materials during feeding. These spores form vegetative rods in the gut which enter into the hemolymph through the malpighian tubules (Beard 1945). These rods will sporulate in the hemolymph and in the later stages of infection produce a milky appearance of the hemolymph. This milky or clouded appearance is caused by a massive buildup of bacterial spores (Dutky 1940, Beard 1945, Fleming 1968, St. Julian et al. 1970). Temperatures must be between 16° and 36°C for sporulation and infection to occur. St. Julian et al. (1970) divided the growth pattern of *B. popilliae* and *B. lentimorbus* into four distinct phases. Phase one was the initial incubation phase of about two days during which there is no evidence of infection macroscopically or microscopically in the hemolymph. The second phase was characterized by a proliferation of vegetative growth in the hemolymph. This phase lasted until day five when limited spore growth occurred. In phase three, days five to ten, concomitant vegetative

growth, prespore formation, and increased sporulation occurred at greatly increased rates. During phase four, the final phase, sporulation occurred and macroscopic determination could be made by the milky appearance of the larvae. Death occurred sometime between days fourteen and twenty-one. Less than 30% of the infected larvae reached this stage (St. Julian et al. 1972). After the death of the larvae, the body decomposes and releases 2 to 5 billion spores into the soil (Fleming 1968). Although the exact mechanism of infection is not known, a major point of entry of the bacterial spores is through the mouth during feeding (Dutky 1941, Beard 1945). Beard (1945) noted that milky disease did not necessarily kill the larvae. He proposed that the larvae eventually died because of an inability to proceed into the next metamorphic phase. Sharpe and Detroy (1979a) proposed that depletion of fat body reserves during development of milky disease was the reason why the next life stage could not be reached. They believed that milky disease developed slowly and caused a depletion of much of the stored reserves, particularly lipids. Even in the later stages of the disease, the larvae continue feeding in an attempt to replenish fat body reserves.

Susceptibility of larvae to *B. popilliae* is not totally understood, but several factors do influence it.

Beard (1944) found that under controlled conditions the incidence of milky disease among third instar larvae was directly related to the concentration of spores present in the inoculum. He also found that larvae failing to contract milky disease after an initial artificial inoculation of spores, were less susceptible to a second inoculation, indicating a kind of resistance. Beard (1944) also found that under ideal conditions, the virulence of *B. popilliae* in causing milky disease of Japanese beetle larvae was much less than some other organisms, for example *B. larvae* in causing foul brood of honeybees. Susceptibility in laboratory experiments was related possibly to the spore material being used. St. Julian et al. (1978) mentioned that although *B. popilliae* spores will store in a dried state for 10 or more years, the level of virulence drops drastically after three or four years.

In the field, the level of infectivity of spores did not have to be extremely high to be useful. With this in mind, the two *Bacillus* organisms were put into use as control agents in the 1930's. White (1940) found that the introduction of Japanese beetle larvae infected with *B. popilliae* into field plots resulted in the establishment of the organism. Adams and Wheeler (1946) found that applications of milky spore dust, given enough time, can bring about a lasting reduction in the larval population.

However, where larval populations are building up to destructive numbers milky disease alone should not be expected to prevent temporary high beetle populations. White (1941) found that the milky diseases were important natural control agents in areas supporting heavy populations of larvae. With the heavy populations, a rapid build-up of disease can be expected with a corresponding decrease in population density. Ladd and McCabe (1967) warned that the effects of milky disease organisms should not be judged by the mortality of third instar larvae alone. They found that in some tests, 50 to 90% of the milky disease infection occurred in the first and second instars. The adults are seldom, if ever, infected by the milky disease bacteria. However, Langford et al. (1942) showed that the adults were viable hosts for the organisms. Also, they were useful in the spread of the organism in the field.

In order for the milky diseases to be viable control agents, a method had to be developed for mass production of the material. In the absence of a practical method for the propagation of *B. popilliae* and *B. lentimorbus* on artificial media, a process was devised and patented in 1939 for producing the spores in the hemolymph of the larvae and incorporating them into talc for storage and distribution to the infested areas (Dutky 1942a). Methods

for application of the spore dust in the field were studied by Beard (1946), Adams and Wheeler (1946), White (1948), and Fleming (1968).

Because of the widespread interest in the use of the disease organisms for Japanese beetle control, and in order to accelerate the natural spread of the bacteria, an extensive program of distribution was undertaken in 1939, 1940, and 1941 by the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture and a number of cooperating states and federal agencies. Eleven eastern US states were involved in this program, with a total of 8371.2 hectares (20,928 acres) treated. The program was very successful with a noticeable decline in population density and with an increase in spore levels in the soil (White and Dutky 1942).

Recent studies have been made in some of the states to determine presence of disease in the field. Hutton and Burbutis (1974) found that milky disease organisms were still present in Delaware in areas treated in 1940. In most of the earlier treated sites, population levels of Japanese beetles have remained low. However, recent population increases have occurred in Connecticut and scientists believe it is due to a loss of virulence by the milky disease organisms (Dunbar and Beard 1975).

VI. Dispersal

The Japanese beetle depends heavily on its ability to fly. It is through flight that the beetle is able to seek out food sources and shelter when necessary. It is also through flight that the beetle is able to expand its range and disperse. Many factors determine the effectiveness of dispersal by flight. The wind is extremely important in determining the direction of beetle flight (Fox 1927), the distance of flight (Hadley and Hawley 1934, Fleming 1972), the actual range expansion of the beetles (Smith and Hadley 1926) and in turn the effectiveness of control. The adult will not fly on cloudy or stormy days (Hawley and Metzger 1940).

The passive dispersal of the Japanese beetle by trains, trucks, buses, automobiles, and even people is also an important dispersal agent (Smith and Hadley 1926, Fleming 1972). Also, the topography of an area greatly affects the effectiveness of the beetle for movement and dispersal (Fox 1927). Areas of level pastures and farms offer no resistance, whereas steep, rolling, and poorly vegetated areas slow the dispersal process considerably.

The potential dispersal of the Japanese beetle has been tested in the past using capture, mark, release, and recapture experiments. Past experiments have found that

the majority of the beetles will be recaptured within 100 meters of the release site (Polivka 1949, Schwartz 1968, Ladd et al. 1972). Recapture percentages are normally low, 0.1 to 58%, in most mark and recapture experiments (Polivka 1949).

Methods for marking the Japanese beetle for release include fast drying enamel paint sprayed on the elytra (Schwartz 1968, Ladd 1972), tape fastened to the elytra (Schwartz 1968), and fluorescent pigments and dusts applied to the dorsum or elytra (Polivka 1949, Staniland 1958, Ladd 1972). Most marking techniques require hand application, but self-marking devices can be built to quicken the process (Sheppard et al. 1973).

CHAPTER III

METHODS AND MATERIALS

I. Study Area

Field work for this experiment was performed in Happy Valley in Blount County, Tennessee (Fig. 2). Because of its isolated location, abundant natural food sources, the cooperation of the residents, the convenience of the roads for trapping, and the large population of Japanese beetles in the area, the Tennessee Department of Agriculture selected this area for study in the summer of 1978.

Happy Valley is located in the foothills of the Blue Ridge Mountains in eastern Tennessee. It is bordered on the northwest by the steep forested sides of Chilhowee Mountain, 630 m above the valley floor, on the southeast by the drainage slopes of the Great Smoky Mountains National Park (GSMNP), on the southwest by the Little Tennessee River, and on the east by Abrams Creek and the main range of the GSMNP. The elevation of Happy Valley ranges from 264 m at the Little Tennessee River to 390 m at the northeastern corner. The GSMNP averages 125 cm of precipitation a year, with the greatest occurring in April and May, and abundant precipitation the remainder of the year (Murlless and Stallings 1973). This availability

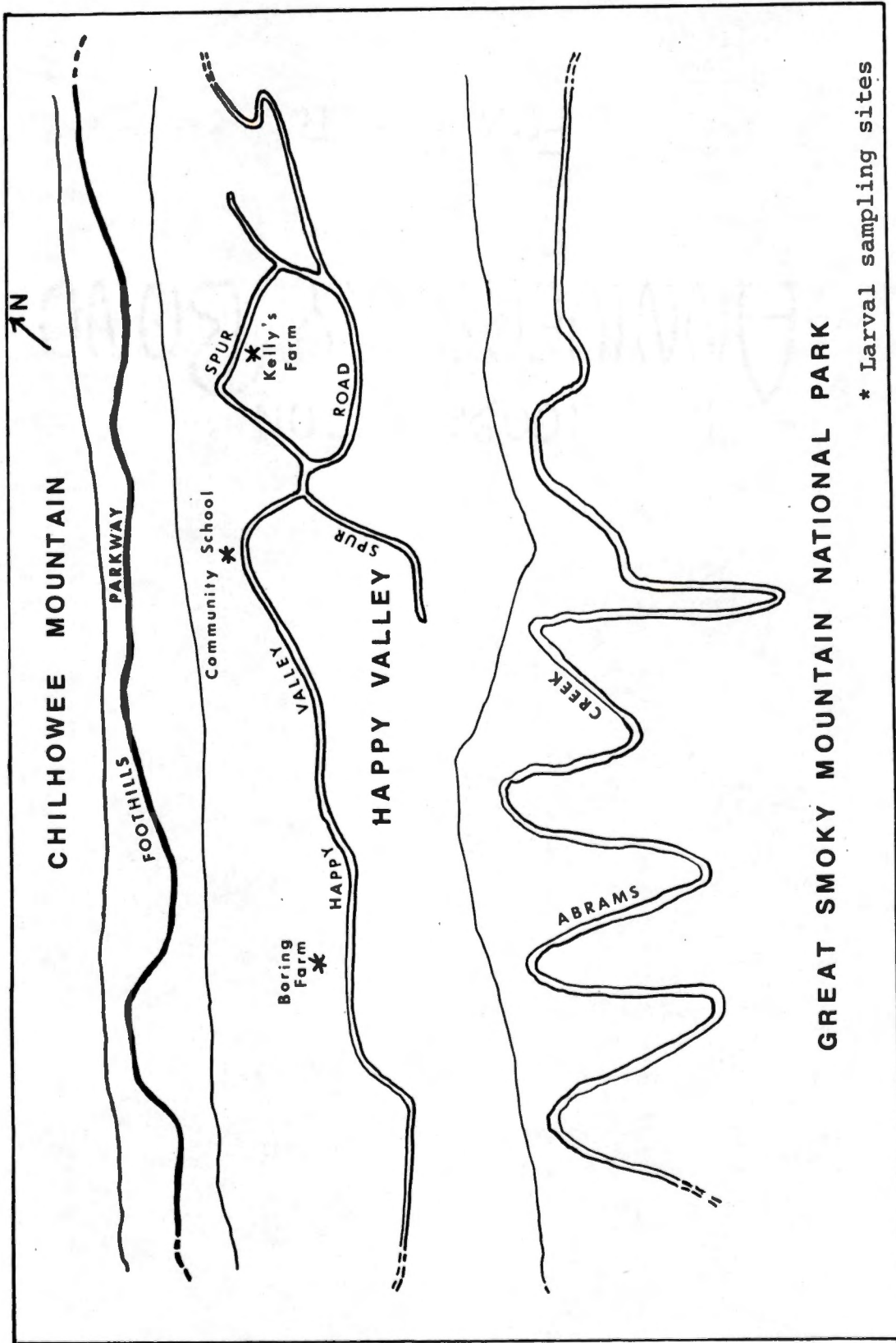


Fig. 2. Diagram of Japanese Beetle study area in Happy Valley, Blount County, Tennessee.

of moisture throughout the year is very important to the life cycle of the Japanese beetle, and under these conditions, rapid and vigorous population growth can take place (Fleming 1972). The area is also rich in available food sources for the beetles. Over 1,300 species of flowering plants are known to occur in the GSMNP which is at the northern and eastern boundary of Happy Valley (Murlless and Stallings 1973). Among these are wild roses, apple, smartweed, wild grapes, plus many other preferred host plants of the Japanese beetle.

The actual basin of Happy Valley consists of rolling pastures and forest; with approximately 175 hectares in pasture. One major road, 12 km long, bisects the valley from northeast to southwest.

II. Larval Survey

Survey periods to be used for Japanese beetle larvae were determined by compiling 10 year averages (National Oceanic and Atmospheric Administration 1969-1979) for the soil temperature 10 cm below the sod (Fig. 3). These soil temperatures were recorded at a site approximately 35 km from Happy Valley. Fall surveys started in September, and continued until average soil temperature at 10 cm was consistently, historically, below 15°C (Fig. 3). Spring

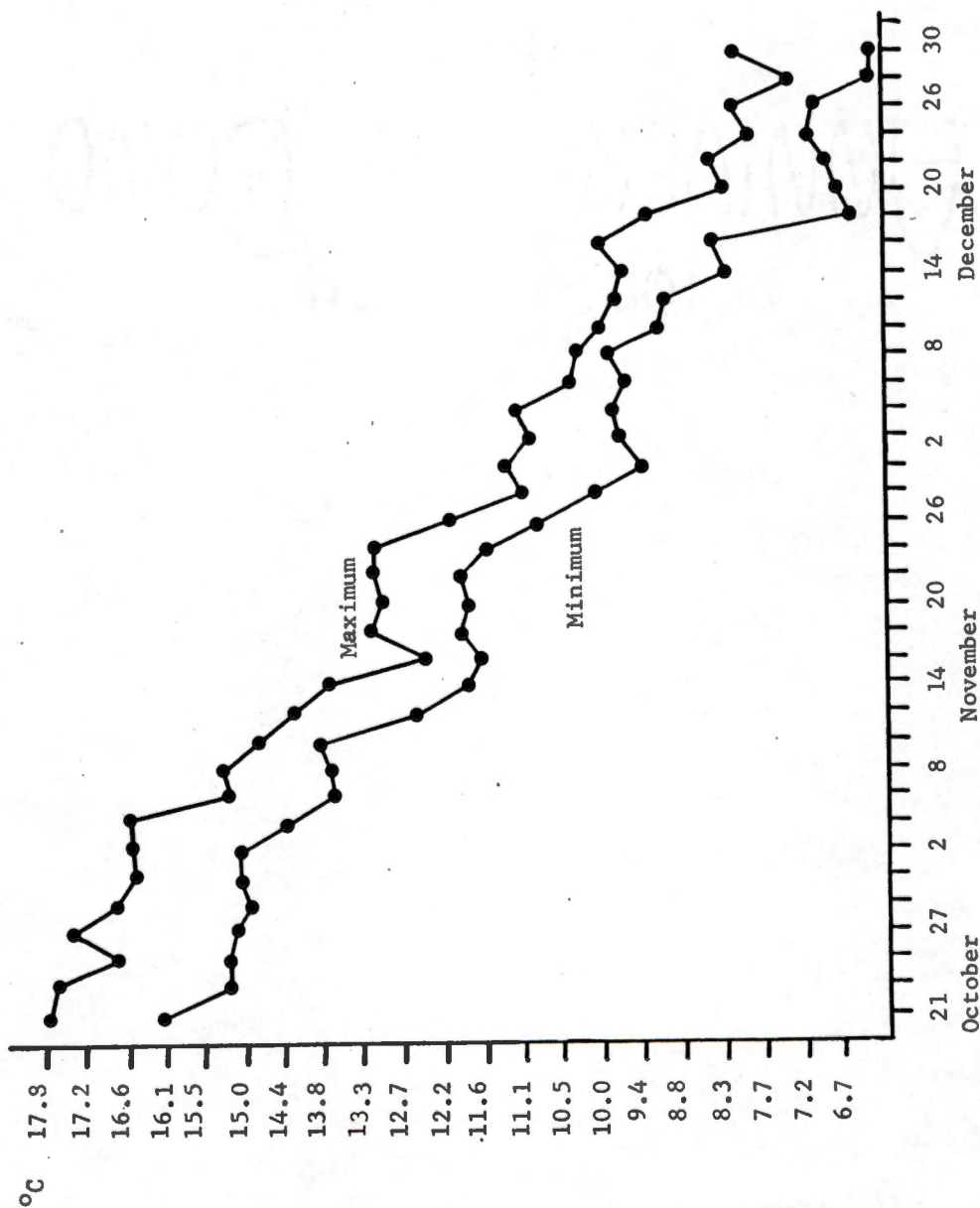


Fig. 3. Maximum and minimum 10-year-mean soil temperatures ($^{\circ}\text{C}$ at 10 cm depth) for October, November, and December 1969-1979 at the Knoxville Experiment Station, Plant Science Farm, The University of Tennessee, Knox County, Knoxville, Tennessee (National Oceanic and Atmospheric Administration 1969-1979).

surveys began when average soil temperature at 10 cm was consistently above 15°C (Fig. 4).

All locations sampled in Happy Valley were treated with Milky Spore (lot number unknown) during the summer of 1978 by the TDA. Areas sampled in 1978 and 1979 were the Boring farm (Fig. 2), in the southwestern region of Happy Valley, and the schoolground in the central region (Fig. 2). Both areas were left untreated in 1979. Each area consisted of homes or lawns with adjacent pastures.

An area added for sampling in the fall and spring of 1979 was the Kelly farm (Figs. 2, 5). It was retreated with a Milky Spore (Lot #411) mixture during August and September of 1979. This area consisted of maintained home lawns with adjacent pasture.

At each location, three parallel sampling lines, 30 m apart, were taken. At the Kelly farm, the first line was 15 m into the treated area, second line at the edge of the treated area, and the third, 15 m outside the treated area. This design was to determine the rate, if any, of the passive dispersal of the *Bacillus* spores. Sampling at the Boring farm (pastures) and at the schoolgrounds (lawn), yielded base line data on larval population levels for use in subsequent sampling.

Each sampling line consisted of ten 0.09 m² samples taken at 3 m intervals. Metal frames, 30.5 cm on

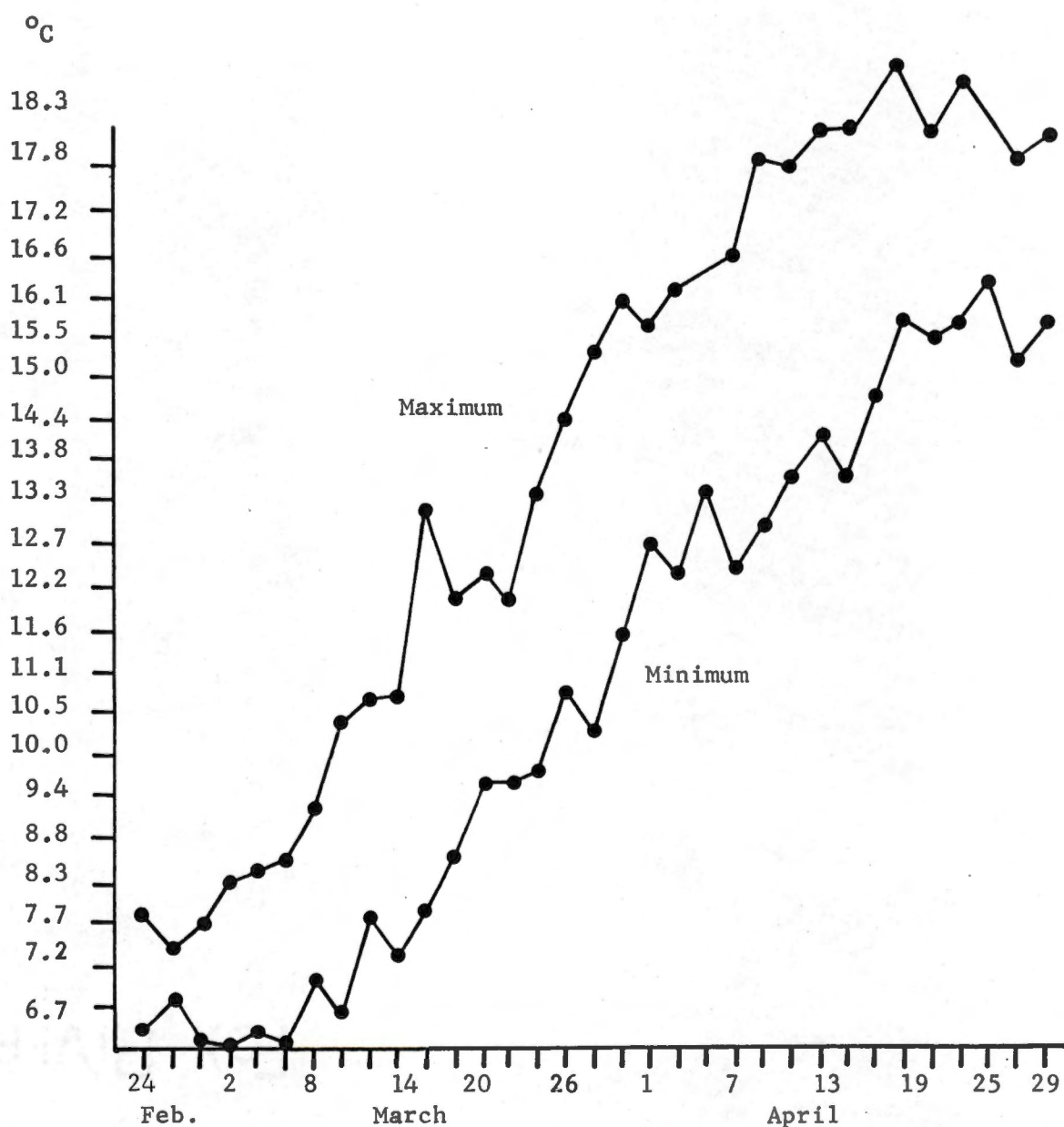


Fig. 4. Maximum and minimum 10-year-mean soil temperatures ($^{\circ}\text{C}$ at 10 cm depth) for February, March, and April 1969-1979 at the Knoxville Experiment Station, Plant Science Farm, The University of Tennessee, Knox County, Knoxville, Tennessee (National Oceanic and Atmospheric 1969-1979).

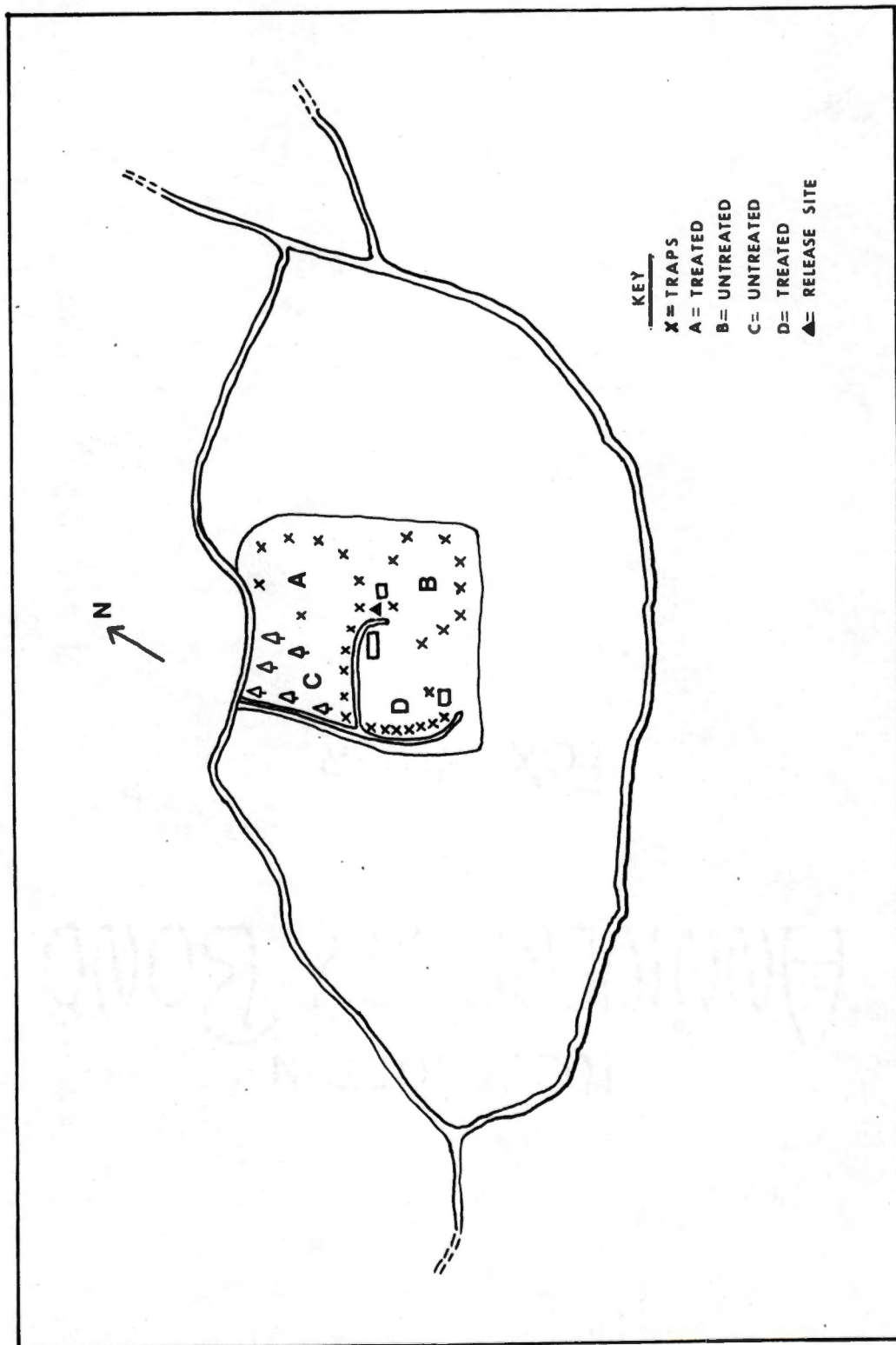


Fig. 5. Diagram of Kelly farm, Northeast section of Happy Valley, Blount County, Tennessee, showing Japanese beetle trap locations, Milky Spore treated areas, and release site for mark-and-release experiment.

a side, were used to standardize sample size. At each sample, the sod was cut and removed, the roots and soil of the sod were examined to a depth of 10 cm for any white grubs. All white grubs found were collected, identified using a hand lens, recorded, and taken to the laboratory. Verification of species was made by using Ritcher's (1966) keys. Final determination of suspected milky diseased larvae was done in the laboratory through microscopic examination of the hemolymph for the characteristic footprint shaped spores (Dutky 1942b).

III. Adult Trapping

Adult population surveys were conducted using the Type A USDA Japanese beetle attractant traps (Metzger 1934) modified with 3.8 l milk jugs as collectors. The traps were set as shown in Fig. 6. The bait used was a 7:3 mixture of phenylethyl propionate and eugenol that was acquired from the USDA by the TDA.

In 1978 adult trapping was conducted by W. VanLandingham and R. Southards of the TDA. Eighty-four traps were set at 0.16 km distances along the main road and spur roads of the valley. These traps were checked from 12 July to early September. On 25 May 1979, 63 traps were set at 0.16 km distances along the same roads. An additional 29 traps

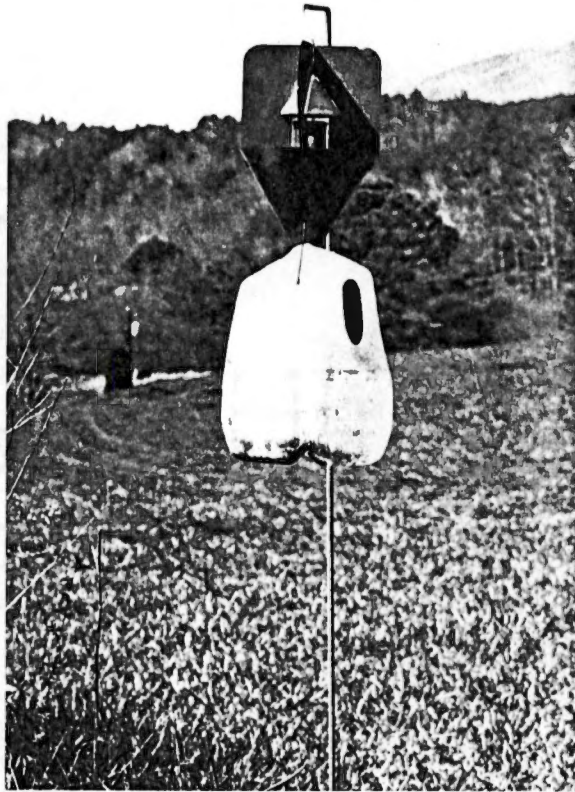


Fig. 6. Modified USDA Type A Japanese Beetle attractant trap, used in the adult surveys in Happy Valley, Blount County, Tennessee.

were set on 5 June and 3 July at the Kelly farm (Fig. 5). Trapping was carried out from 25 May to 12 September in 1979. Green traps were used along roads of the valley, and yellow traps on the Kelly farm. Although previous work had shown differences in the attractability of different colored traps (Fleming et al. 1940), differential attractiveness was not examined in this study.

Traps were examined twice weekly during the summers of 1978 and 1979. At each examination, catches were placed in plastic bags, and returned to the laboratory for counting. In cases of large trap catches, trap contents were weighed and numbers estimated by weighing a subsample of 100 beetles. Average weight was then used to estimate numbers of beetles trapped.

The disappearance of traps was a serious problem during the trapping periods in both 1978 and 1979. In 1979, replacement was attempted early in the summer, but the continued disappearance in some areas made this impractical. Traps disappearing most frequently were those isolated from houses and structures.

IV. Adult Mark and Release

Two experiments were conducted in Happy Valley in 1979 to determine the dispersal of the adults. Live adults

were collected from the USDA attractant traps along the roads of the valley and the additional traps placed at the Kelly farm, and were returned to the laboratory for marking.

Captured beetles were marked on the pronotum with Testors enamel paint using a small brush. No toxicity was noted in preliminary laboratory tests. For each release date a different color was used. The central capture and release area for these experiments was the Kelly farm.

The first adults were captured and marked on 3 July 1979, and released on 4 July. One thousand Japanese beetles were released with several females immediately digging into the soil. Approximately 1,000 left the site of release. The second set of adults were captured and marked on 20 August. On 21 August 1,000 adults were released.

Catches of traps at the Kelly farm and along the roads were examined for marked beetles. Location and date of recapture were recorded. Percent recapture and mean distance of recapture were then determined.

V. Bioassay of Milky Spore Material

Bioassays were conducted to determine the percent infectivity of lots of Milky Spore received by TDA for use

in the Japanese Beetle Suppression Program. Techniques used for the bioassay were similar to those described by Dutky (1942).

Third instar Japanese beetle larvae used in these bioassays were obtained in field sampling in the fall. Larvae were returned to the laboratory and maintained at 5°C in layers of moist soil in plastic dish pans (Dutky 1942b, Fleming 1972). Larvae were obtained from outside Blount County. This was done to avoid any grubs which may have milky disease that might have been previously introduced into the environment.

One hundred grams of milky disease powder or talc was added to 1,250 grams of sterile soil (loam), and 5 grams of red top grass seed (*Agrostis alba* L.) and white clover seed (*Trifolium repens* L.) 1:1 were added. A 1000:1 aqueous-formaldehyde solution was added to the mixture, bringing it to a moisture level of 60% of ballpoint. Ballpoint was determined by adding water to soil until the soil formed balls easily (Dutky 1942b). The soil was then divided among twenty-five 90 ml tins. One healthy larva was placed in each tin. The tins were incubated at 30°C and checked three times a week. Additional seed and formaldehyde solution were added as needed. The presence of milky disease was determined macroscopically, by the

external milky appearance of the legs, or microscopically, through hemolymph examination for the presence of spores.

Each treatment was replicated 25 times. A check was set up using talc instead of milky disease powder. The concentration of milky disease powder used in these bioassays was 8×10^9 spores per kg soil. A previously tested standard (Japidemic®, Fairfax Laboratory, Clinton Corners, New York) was obtained from the USDA Japanese beetle laboratory. This was originally obtained by them from the University of Maryland in May 1978. At a concentration of 8×10^9 spores per kg of soil it had an infectivity of 67% (Klein 1979).

CHAPTER IV

RESULTS AND DISCUSSION

I. Larval Survey

During the fall of 1978, 74 turf samples at the Boring farm were examined. A mean of 7.2 larvae per 0.09m^2 was found. During the same period in 1979, 31 turf samples were examined with a density of 2.2 larvae per 0.09m^2 . This was a significant (t-test, $P \leq 0.05$) difference in the population density (Table 1).

During the fall of 1978, ten turf samples were examined at the old community school. An average of 3.3 larvae per 0.09m^2 was found. During the same period in 1979, thirty turf samples were examined with a population density of 2.2 larvae per sample (0.09m^2). Using the t-test to analyze the data, there was a significant ($P \leq 0.05$) difference in population density (Table 2).

Sampling at the Kelly farm took place during the year 1979 only, with results taken from treated, border, and nontreated areas. Larval densities of 2.0 (treated), 1.9 (border), and 5.5 (nontreated) per 0.09m^2 were obtained in spring 1979. Corresponding larval densities of 2.2, 2.1, and 1.3 per 0.09m^2 were found in the fall of 1979 (Table 3). One milky diseased larva was recovered on the

TABLE 1. Results of fall 1978 and fall 1979 Japanese Beetle surveys from the Boring farm, Happy Valley, Blount County, Tennessee. Area was not treated with milky spore.

	No. of Samples	No. of Larvae	Mean Density/ 0.09m ²
Fall 1978 Survey (Sept. - Dec.)	74	532	7.2
Fall 1979 Survey (Sept. - Dec.)	31	69	2.2*

* Significantly different as determined by t-test,
P ≤ .05.

TABLE 2. Results of fall 1978 and fall 1979 Japanese Beetle larval surveys from the Community School, Happy Valley, Blount County, Tennessee. Area was treated with Milky Spore July 1978.

	No. of Samples	No. of Larvae	Mean Density/ 0.09m ²
Fall 1978 Survey (Sept. - Dec.)	10	33	3.3
Fall 1979 Survey (Sept. - Dec.)	30	66	2.2*

* Significantly different as determined by t-test,
P ≤ .05.

TABLE 3. Results of 1979 Japanese Beetle larval surveys from treated, border, and nontreated areas of the Kelly farm, Happy Valley, Blount County, Tennessee. Area treated with Milky Spore in July 1978 and September 1979.

	Treated		Border			
	Spring-Fall	Spring-Fall	Spring-Fall	Spring-Fall	Spring-Fall	Spring-Fall
Number of Sample units (0.09m ²)	10	30	10	20	10	20
Number of <i>P. japonica</i> larvae	20	65	19	41	55	25
Mean larvae/0.09m ²	2.0	2.2	1.9	2.1	5.5	1.3
Number of Milky Diseased larvae	0	1	0	0	0	0

Kelly farm on 3 October 1979. This larva verified the establishment of milky disease in Happy Valley. The larva was located at the site of a recent retreatment of Milky Spore (Lot #411), and this provides probable evidence that Lot #411 contained some infective spores.

It is important to note, that during the 1978 survey, few species of white grubs other than *P. japonica* were found. However, during 1979, the ratio of Japanese beetle larvae to other white grubs was approximately 1:1. Other species of larvae found were: *Cotinus nitida* (L.) (11 grubs) and *Phyllophaga hirticula* (Knoch) (39 grubs). None of these other larvae were infected with milky disease.

Larval surveys made in 1978 and 1979 were effective in detecting Japanese beetle larvae in Happy Valley. (Tables 1, 2 and 3) However, due to several factors, the surveys did not give an accurate estimation of the population density. Previous studies have shown that when sampling an area, accurate population estimates can be obtained by sampling 10% of the area (Fleming and Baker 1936). Because of the time and manpower available, areas sampled for these experiments ranged from 10 turf samples to 74 turf samples each of 0.09m^2 . In these experiments, 10% of the turf area sampled would have required extensive damage to people's lawns and it was not attempted. Another

factor which might have affected density was the presence of another disease in certain sections of the study area. "Blue Disease," probably caused by the rickettsial organism, *Rickettsiella popilliae* (Dutky and Gorden), appeared occasionally within the study areas in 1978 and 1979. Over 50% of the larvae recovered in December 1978 had "Blue Disease." This disease is extremely pathogenic, and can decrease the larval density in the areas where present (Fleming 1968). No further data on blue disease was collected. A final factor was the inconsistency of larval densities, populations were simply not uniform throughout the sample area. Factors such as moisture, plant cover, and distance from food plants may affect where eggs are to be laid, and in turn where the larvae will be.

II. Adult Trapping

Trapping of adult Japanese beetles for the purpose of a population survey took place in 1978 by TDA and in 1979 by The University of Tennessee.

Trapping by the Tennessee Department of Agriculture began 7 July 1978 (VanLandingham 1978). A peak population of 5,934 beetles per trap was reached during the week of 24 July to 28 July (Fig. 7). A period of extended rainfall occurred from 31 July to 7 August, greatly suppressing the

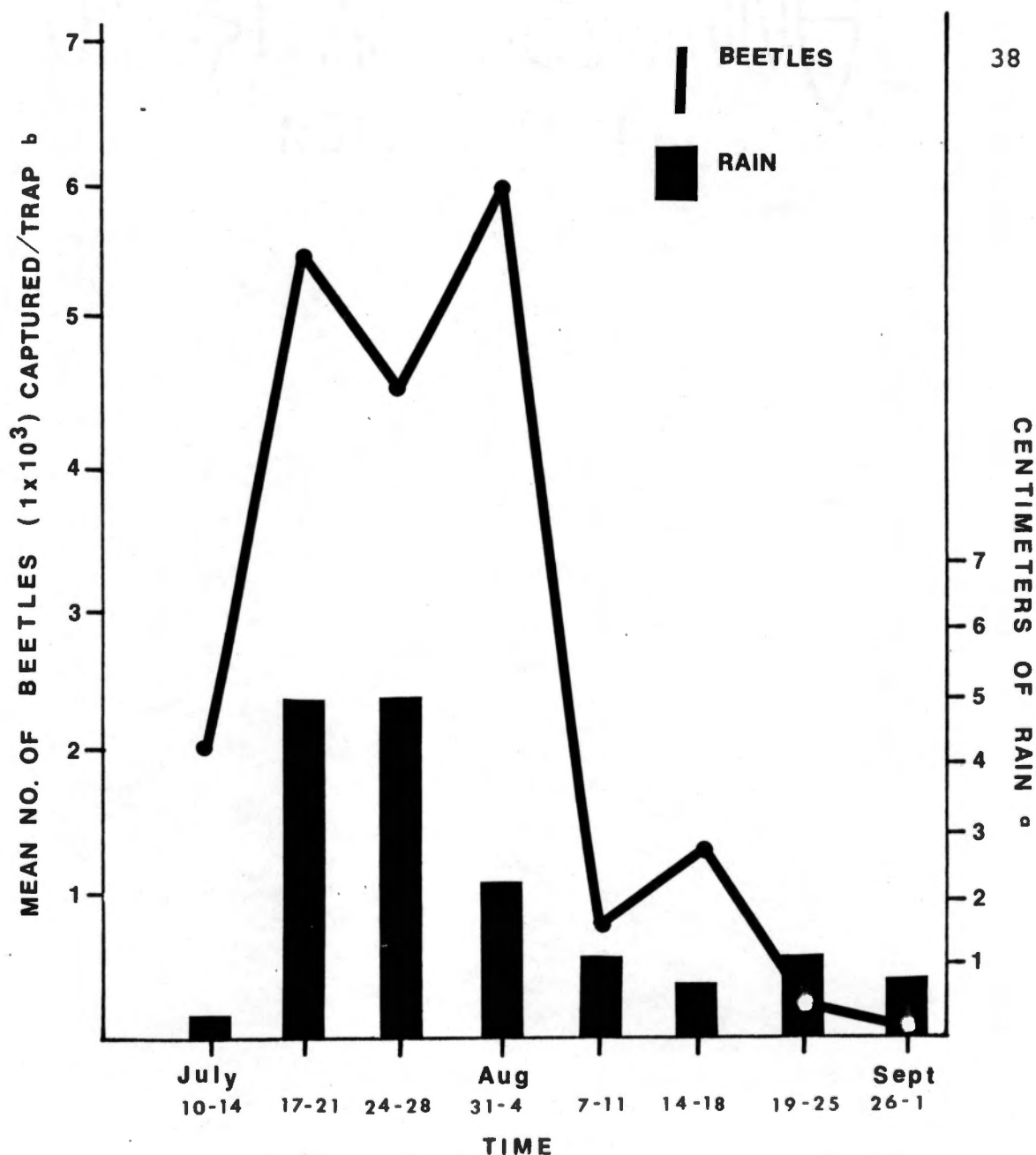


Fig. 7. Adult trapping results from Happy Valley, Blount County, Tennessee, 1978. Rainfall data is plotted to compare affects of rain on mean beetles captured per trap.

^aRainfall data, 1978, for Knoxville Experiment Station, Plant Science Farm, The University of Tennessee, Knox County, Knoxville, Tennessee (approximately 35 km from Happy Valley) (National Oceanic and Atmospheric Administration 1978).

^bBeetle data was obtained from the Tennessee Department of Agriculture, Nashville, Tennessee. (Mr. W. J. Vanlandingham 1978).

trap catches. Trap catches were never above 1,460 per trap after this point (Fig. 7).

Adult trapping for the 1979 season began on 25 May. The first beetles were caught on 2 June with a rapid increase in catch numbers occurring by 14 June. The peak catch of 5,095 beetles per trap occurred on 19 July (Fig. 8). Heavy rainfall throughout July and August greatly affected trap collections. Approximately 13.5 cm of precipitation occurred during 19 July to 22 July (Fig. 8), suppressing trap collections to 431 beetles per trap on 23 July. Collection numbers continued to fluctuate up and down, with a final decline beginning on 9 August. By 17 September, trap collections averaged 8.7 beetles per trap. Trapping was discontinued at this time.

The results obtained in both years of trapping were very similar with peak catch numbers occurring within the same two calendar weeks (Figs. 7 and 8). Weather greatly affected the size of collections in the traps. During periods of precipitation, beetle flight decreased drastically, thus trap catches decreased (Fleming 1972). Another factor was the disappearance of traps during the experiment.

III. Adult Mark and Release

Two adult capture, mark, release, and recapture experiments were conducted during the summer of 1979

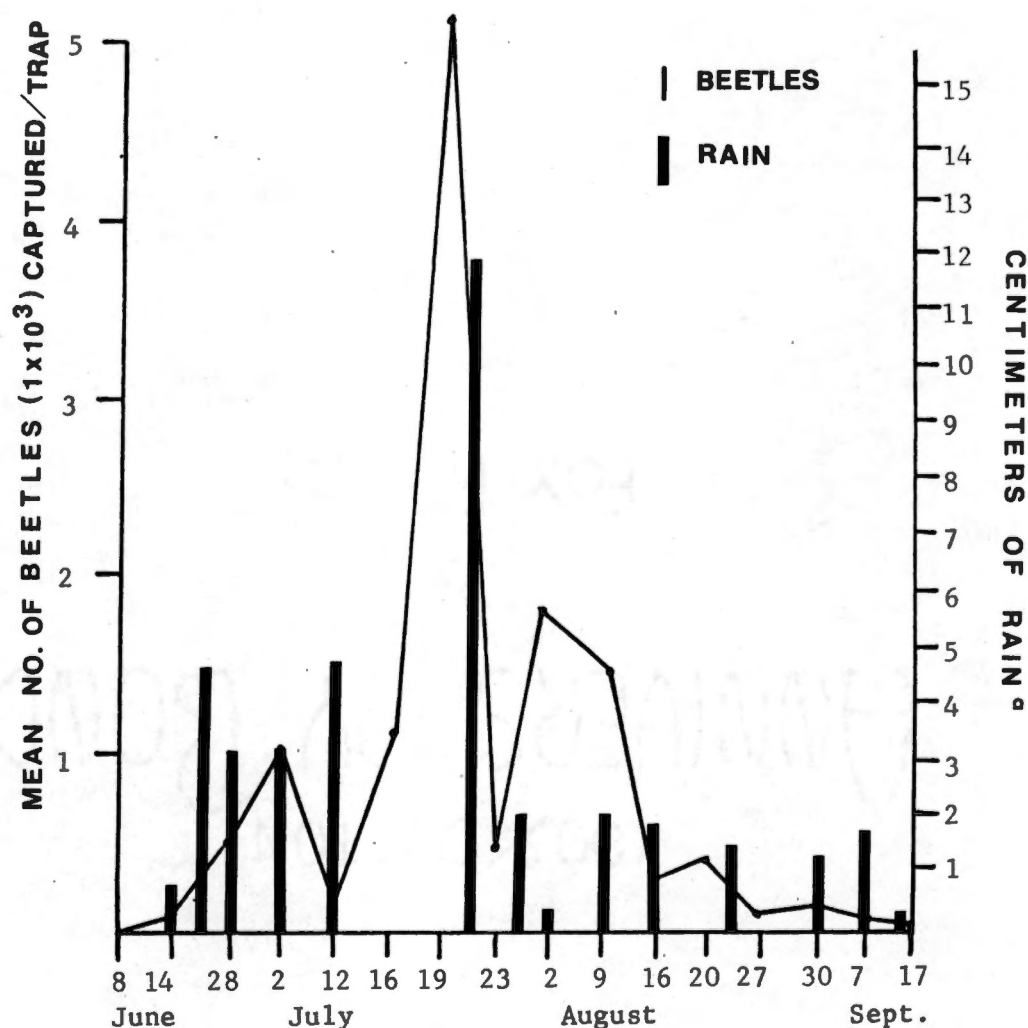


Fig. 8. Adult trapping results from Happy Valley, Blount County, Tennessee, 1979. Rainfall data is plotted to compare affects of rain on mean beetles captured per trap.

^aRainfall data, 1979, for Knoxville Experiment Station, Plant Science Farm, The University of Tennessee, Knox County, Knoxville, Tennessee (approximately 35 km from Happy Valley) (National Oceanic and Atmospheric Administration 1979).

(Tables 4 and 5). The first mark and release took place on 4 July. Two beetles were recaptured, one at 45.7 m (12 July), and one at 5.2 m (16 July) from the release site. After continuously poor weather conditions, the experiment was terminated on 22 July (Table 4).

The second mark and release took place on 21 August. A total of 95 beetles were recaptured with 95.8% being trapped on 23 August. The mean distance from release to recapture was 65.7 m. One beetle was recaptured 2.24 km from the release site. Fifty-three percent of the beetles were recaptured within 50 m of the release area, 88% within 100 m, 99% within 200 m. The mean time from release to recapture was 2.3 days (Table 4).

Results obtained were similar to those obtained in previous experiments (Polivka 1949, Schwartz 1968, Ladd et al. 1972). Environmental conditions were poor for the dispersal of the released beetles. Most Japanese beetles will remain close to the point of release. However, some beetles are capable of dispersing several km.

IV. Bioassay of Milky Spore Material

Five bioassay experiments were conducted with ten different production lots of Milky Spore tested (Table 6). Material applied in Happy Valley in 1978 (Happy Valley Lot)

TABLE 4. Results of 1979 mark, release, and recapture experiments with Japanese Beetles conducted in Happy Valley, Blount County, Tennessee.

Release Date	No. Released	No. Recaptured	Percent Recaptured	Mean Recovery Distance	Maximum Recovery Distance	Mean Recovery Time (days)
4 July	1,000	2	0.2	25.45 m	45.7 m	11.0
21 August	1,000	95	9.5	65.70 m	2.24 km	2.3

TABLE 5. Recapture distances of 1979 mark, release, and recapture experiments with Japanese Beetles conducted in Happy Valley, Blount County, Tennessee.

Release Date	No. of Beetles			
	Recapture Distances (meters)			
	≤50	≤100	≤200	>200
4 July	2	0	0	0
21 August	53	31	10	1
Total	55	31	10	1
Percent	56.7	31.96	10.3	1.04

TABLE 6. Results of laboratory bioassays of Milky Spore lots received by the Tennessee Department of Agriculture for its Japanese Beetle Suppression Program. Twenty-five third stage Japanese Beetle larvae used per lot at a spore concentration of 8×10^9 spores/kg soil.

Lot No. ^a	Exp. No.	Percentage of Larvae		
		Pupated	Dead	Milky Diseased
325	2	16	52	0
329	3	80	84	0
365	1	20	76	0
372	4	64	32	0
	5	32	20	0
373	3	68	84	0
380	2	60	60	0
	3	72	80	0
Happy Valley	1	12	84	0
	2	8	72	0
	4	60	48	0
411	4	20	72	0
	5	16	20	4
421	5	8	16	4
427	5	8	28	12
Japidemic ^b	1	0	84	12
	2	8	72	8
	4	36	40	4
Check	1	4	88	0
	2	40	76	0
	3	60	28	0
	4	12	52	0
	5	16	12	0

^aProduction lot numbers of Milky Spore received by TDA from Reuters Laboratory

^bTest sample received from USDA Japanese Beetle Laboratory: Fairfax Laboratory, origin - University of Maryland, May 1978. Infectivity: 67% (8×10^9 spores/kg soil)

had 0% infectivity in all three bioassays in which it was tested. Production lot 411, used in selected areas of Happy Valley in 1979, had 0 and 4% infectivity. No milky disease was noted in the talc checks. Infectivity in the other 8 Milky Spore lots was low, ranging from 0% in the 300 series to 4 and 12% for lots 421 and 427, respectively. Japidemic, another milky disease product used as a standard, had levels of infectivity of 12, 8, and 4%.

Bioassays of similar lots were conducted by the USDA Japanese Beetle laboratory in 1978 and 1979 (Klein 1979). Low levels of infectivity were obtained in the early 300 lots, ranging from 0% (lots 323, 325) to 12% (lots 326). The Japidemic standard was found to be 67% infective by the USDA personnel. Although one of the lots tested by the USDA had only 12% infectivity in the laboratory, it did work well in the field (Klein 1979). Therefore, low infectivity in laboratory bioassays is not necessarily a measure of field infectivity. When the material shows 0% infectivity in the laboratory and fails to become established in the field there is reason for concern to administrators of milky disease colonization programs.

There are several reasons why the levels of infectivity obtained in these experiments were low. Some of the early 300 series production lots of material used showed no

infectivity (Klein 1979). It was because of this result that the USDA personnel became concerned and started evaluating the production practices. It was discovered that there was a malady with the drying process. This allowed the spores to bake. This malady was corrected by changing the process from heat drying to a lyophilization process (Klein 1979). Since this change in technique, infectivity of production lots, in the 400 series, has increased (Klein 1980).

Another reason for the low infectivity may have been the incubator used in these experiments. For maximum sporulation to occur, the temperature and humidity must be maintained at approximately 30° and 80%, respectively (Beard 1945). However, with the incubator used in these experiments, it was difficult to keep temperatures from rising up to 35°C or near the upper developmental threshold level for the larvae. This created less than optimum conditions for larval development. Also, under these stressed conditions, mortality resulting from other factors may have increased.

Probably the most important factor for obtaining low levels of infectivity in these experiments was the physiological condition of the larvae. All of the larvae used were overwintering third stage, usually collected in

November and December. These larvae often fed for only a short time, if at all, after being removed from cold storage. This period of feeding might have been too short to have allowed ingestion of sufficient spores by the larvae or for sufficient incubation time for the milky disease organisms to develop. Milky disease became evident in 7 days at 30°C (Beard 1945). This was a very big problem in bioassay No. 3, in which pupation by 75% of the larvae by day 10 prompted an early termination of the experiment (Table 6). In most of these experiments, external signs of milky disease in the larvae occurred between day 10 and 14. Although all larval stages of the Japanese beetle are susceptible to milky disease pathogens, late second and early third stage actively feeding larvae would probably be better suited for laboratory bioassays. "Blue Disease," probably caused by *Rickettsiella popilliae* (Dutky and Gooden 1952), was present in many of the feral larvae collected for use in bioassay experiment 4. Care was taken to separate these diseased larvae from the healthy ones at the laboratory, but it is possible that some infected larvae were not detected. If that occurred, some of the nonsymptomatic deaths might be attributed to this.

If these experiments are to continue, better laboratory equipment and techniques must be used. Younger, more active

feeding larvae must be used. This will help remove the problem of pupation during testing. A range of spore concentrations must be tested as well as more larvae used per spore concentration to better determine infectivity. This will help insure that spore powder of sufficient infectivity is being used in the Japanese Beetle Suppression Program. This will increase the prospect of a successful biological control program for the Japanese beetle.

CHAPTER V

SUMMARY AND CONCLUSIONS

The Japanese beetle is a major pest in the Eastern United States. It was first recorded in Tennessee in the northeastern county, Sullivan, in 1936. It has since spread south and west into the eastern third of Tennessee. The Japanese beetle causes damage in both larval "root feeding," and adult "defoliation" stages. The adult feeds on over 300 plant species.

Adult trapping peaks as determined by trapping were 5,934 beetles per trap between 24 and 28 July 1978 and 5,095 on 19 July 1979. During both 1978 and 1979, trap catches were reduced, after heavy rainfall. Larval density changed significantly ($P \leq .05$) from fall of 1978 to fall of 1979. The larval density was 5.2 per 0.09m^2 in fall of 1978 and declined to 2.0 per 0.09m^2 by fall of 1979. This decline could be due to many factors, including sampling. Often larvae were not uniformly dispersed throughout the areas sampled.

Adult mark and release experiments conducted in 1979 determined that the Japanese beetle can disperse effectively. Beetles were recaptured as far as 2.24 km from the area of

release. Flight, aided by wind, represents the most effective dispersal mechanism of the beetle (Fleming 1972).

Bioassays of milky disease powder, Milky Spore, were performed in 1978 and 1979 to determine infectivity of the material being used in the Japanese Beetle Suppression Program. Biological control of the Japanese beetle using Milky Spore was attempted in 1978 by TDA. Retreatment with Milky Spore was necessary in the valley as the result of poor bioassay results on material applied in 1978. Establishment of the new material, lot 411, in the field was verified on 3 October 1979 when one diseased larvae was detected in the valley. Further verification of the effectiveness of the Milky Spore should be detected during subsequent summers.

Considering the terrain of Tennessee, the availability of host plants, the mild weather, and the reproductive capabilities of the beetle, the spread of the Japanese beetle throughout Tennessee is probable. However, through the use of attractant traps for monitoring the movement, widespread distribution and use of bacterial milky disease, and the supportive use of appropriate chemicals, the Japanese Beetle Suppression Program can greatly slow the buildup and expansion of the beetle in the state of Tennessee.

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