

United States Department of Agriculture
Agricultural Research Administration
Bureau of Entomology and Plant Quarantine

EFFECT OF THE SUN'S RAYS UPON THE VIABILITY OF SPORES OF
BACILLUS POPILLIAE DUTKY, THE ORGANISM CAUSING
MILKY DISEASE OF JAPANESE BEETLE LARVAE

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The method of distribution of milky disease spore dust used by the Bureau of Entomology and Plant Quarantine, and by certain agencies cooperating with the Bureau, for the control of the Japanese beetle (Popillia japonica Newm.) usually consists in placing spore dust in spots at intervals on the surface of turf or soil and depending upon heavy dews or rains to wash the material into the soil. Such spots have remained visible during drought periods for as long as 10 days. Although the dust layer has an appreciable thickness, and a portion sifts through the grass, a large portion remains exposed for a time to the direct rays of the sun. Another method of distribution given consideration consists in applying the material over an area as a fine dust. By this method the material would be spread out as a very thin film, and practically all of it would be exposed to the rays of the sun until washed in by rain or covered in some other manner.

In August 1943 tests were started to determine the effect of direct sunlight on the spores of Bacillus popilliae Dutky in the spore-dust mixture. Preliminary tests were carried on in that year and more extensive ones in 1944. Since the results were in general agreement, only the more extensive tests will be reported.

The spore mixture was dusted very thinly over shallow trays and exposed out of doors to full sunlight for various periods from 1 to 80 hours. These trays had removable covers of perforated cellophane to prevent the blowing of the fine spore dust. Dust to be exposed to the sun for more than 6 hours was brought indoors at the end of each 6-hour period, remixed, redusted over the trays, and returned to the sunlight until the required number of hours' exposure had accumulated. Periods during which the sun was even partially hidden by clouds were deducted from the time of exposure.

A similar series of spore-dust samples, not exposed to sunlight, was used to test the viability of the spores in the original material.

A parallel series, in which equal samples of the original spore dust were placed in an oven at 140° F. for the same periods as those exposed in the sunlight, was also run to test the effect of heat on the viability of the spores.

At the close of the various exposure periods during July and August 1944, the samples were placed in new 2-ounce tins, labeled, and put aside until November 27, 1944, when enough healthy third-instar larvae from one source were available for running biological tests on all series simultaneously.

In each series 40 grams of the dust was incorporated into 2 kilograms of soil together with 20 grams of equal parts of white clover and red top grass seed. This soil was then divided into 50 equal parts and placed in 2-ounce tins. One healthy larva was placed in each tin and allowed to feed for 37 days, or until it became milky or died. There were 14 exposure periods requiring 50 larvae each, or a total of 700 larvae for each of the 3 treatments. The same amount of pure chalk and talc without spores was used with a series of 700 larvae to determine the normal disease incidence that might be expected from the larvae and soil used in the tests.

The results of the tests are summarized in table 1.

Table 1.--Incidence of milky disease among Japanese beetle larvae in soil containing milky disease spores that had been given various exposures to sunlight and heat; 50 larvae in each lot.

Hours of exposure	Number of infected larvae in soil containing spore dust			
	Check	Exposed	Not exposed	Heated at
	(chalk plus talc, no spores)	to sunlight	to sunlight	140° F.
1	4	38	37	41
2	2	43	37	42
4	2	36	38	36
6	2	37	42	38
8	3	39	36	42
16	3	28	37	41
24	4	28	39	40
32	5	31	42	43
40	2	29	39	39
48	2	22	44	40
56	3	24	41	38
64	4	22	44	38
72	3	21	44	38
80	<u>3</u>	<u>20</u>	<u>36</u>	<u>37</u>
Totals	42	418	556	553
Percentages	6		79.4	

The data indicate that there is no reduction in viability when milky disease spores are exposed to the sun for 8 hours or less, that there is a significant reduction in viability when they are exposed from 16 to 40 hours, and that there is a further marked reduction in viability when they are exposed for 48 hours or more.

The infection rate in the series exposed to 140° F. for as much as 80 hours was similar to that obtained from impregnated spore dust not exposed to the sun. This indicates that the effect of heat alone on the potency of Bacillus popilliae is of minor importance under field conditions. However, the effect of temperature in combination with exposure to direct sunlight on milky disease spore dust was not included in this study. Neither was any attempt made to determine the particular factor or factors responsible for the effect of the sun on viability of the spores. The disease incidence of 6 percent occurring in the check series compares very favorably with that in numerous other check series run during the same period in other work.

Another series of tests was run to determine the effect of the sun on the viability of the spores in deposits of dust applied by the accepted method of spore-dust distribution used by the Bureau and cooperating agencies. Deposits of spore-dust material 1/32, 1/16, and 1/8 inches in thickness were exposed to direct sunlight as previously described, for 56, 64, and 72 hours, respectively. This material was left undisturbed for the entire exposure period. The exposed spore dust was then mixed with soil as in the other tests, 50 larvae being used for each series. These results are given in table 2, and may be compared with those of the checks used in similar exposure periods with a thin film of dust (56, 64, and 72 hours, table 1).

Table 2.--Incidence of milky disease among Japanese beetle larvae in soil containing milky disease spores that had been exposed to the sun's rays in layers varying in thickness;
50 larvae in each lot

Thickness of spore-dust layer (inch)	Hours of exposure to sun	Number of infected larvae recovered
1/32	56	35
	64	38
	72	32
1/16	56	37
	64	30
	72	32
1/8	56	36
	64	25
	72	40



These results indicate that sunlight does not materially reduce the viability of the spores of Bacillus popilliae in the standard milky disease spore-dust mixture applied for the control of grubs of the Japanese beetle in accordance with the spot method followed by the Bureau and cooperating agencies. Most of the dust is deposited on the turf or soil in layers of sufficient thickness to prevent damage to any except the thin top layer of spores. On the other hand, a considerable reduction in the viability of the spores may result when the dust mixture is broadcast in a very thin film, if several sunny days pass before the material can be washed in by rains or otherwise worked into the soil or turf.