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EFFECTS OF SOIL TYPE, TEMPERATURE, AND MOISTURE ON PINK BOLLWORM LARVAE
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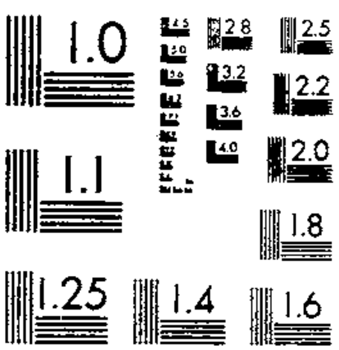

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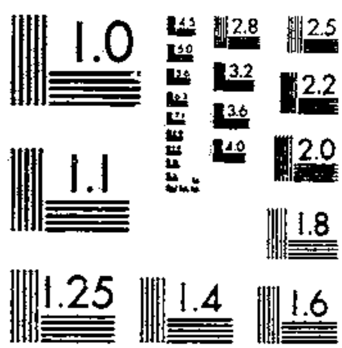

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EFFECTS OF SOIL TYPE, TEMPERATURE, AND MOISTURE ON Pink Bollworm Larvae and Pupae Buried Under Laboratory Conditions

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CONTENTS

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EFFECTS OF SOIL TYPE, TEMPERATURE, AND MOISTURE ON PINK BOLLWORM LARVAE AND PUPAE BURIED UNDER LABORATORY CONDITIONS

By CLYDE A. RICHMOND and EDGAR W. CLARK,[1] *Entomology Research Division, Agricultural Research Service*

Soil moisture and burial of larvae were recognized many years ago as beneficial for control of the pink bollworm (*Pectinophora gossypiella* (Saunders)). Willcocks (*16*)[2] and Williams (*17*) in Egypt found that diapause larvae in bolls buried 20 to 30 cm. seldom survived after April of the following year, even under dry conditions.

Soil moisture detrimental to larval survival has been noted by many authors. Loftin et al. (*9*) in Mexico observed that mortality was greater when larvae were buried in irrigated than in dry soil. Williams and Bishara (*18*) in Egypt found that the mortality rate increased with increased soil moisture and no moths emerged to infest the following year's crop in excessively irrigated fields. Ohlendorf (*11*) in Mexico observed that pink bollworm mortality in soil increased as the moisture increased. Chapman and Cavitt (*3*) in Texas found that soil moisture above 16 percent resulted in mortality of diapause larvae and that survival was greater in sandy than in adobe soil. Squire (*13*) stated that in the West Indies postharvest crop residues subjected to about a 9-inch rainfall per month decomposed in 1 month and larvae disappeared in a shorter period. In India, Nangpal (*10*) observed that larvae in buried infested bolls disappeared at the break of the monsoon season about mid-October, and low survival was probably due to excessive moisture. Recently more detailed studies by Chapman et al. (*4*) and Richmond and Clark (*12*) demonstrated that soil type, soil moisture, and depth of burial affect the activities and survival of both nondiapause and diapause larvae, pupae, and adults.

Moisture and depth of burial have been widely used for the cultural control of overwintering pink bollworm populations. At present the major fall and winter cultural practices in the United States consist of shredding cotton stalks after harvest, burying or plowing under debris, and, in arid regions, increasing soil moisture by fall and winter irrigation. These practices are carried out so as to take advantage of weather conditions adverse to the insects (Fenton and Owen *8*, Adkisson et al. *1*, Chapman et al. *4*). Mortality of the pink bollworm caused by impact with stalk shredders is fairly well understood.

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[1] Now with Southeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture.

[2] Italic numbers in parentheses refer to Literature Cited, p. 29.

Larval mortality resulting from burial and subsequent processes is not well understood nor has it been intensively studied. Present knowledge is based primarily on field observations.

Insofar as could be determined, few field or laboratory studies have been made of mortality of nondiapause larvae (Richmond and Clark *12*). Therefore, laboratory studies were conducted at Brownsville, Tex., to determine the effects of soil type, soil temperature, and soil moisture on diapause and nondiapause larvae buried in the soil. An additional purpose was to determine the possible deleterious effect of cultural practices now used during the growing season on nondiapause pink bollworm larvae that leave cotton blooms or bolls to pupate in the soil.

PROCEDURE

Nondiapause fourth-instar larvae were obtained from firm, green cotton bolls by collecting only those larvae that voluntarily emerged from bolls. Diapause fourth-instar larvae were obtained by means of a Berlese funnel technique (Clark et al. *7*) or by removal by hand from infested seed cotton or dried okra seed pods that had been stored at approximately 15° C. The collected larvae were stored at 15° for periods up to 1 week in petri dishes filled with tissue paper. Pupae were obtained from nondiapause larvae held at 29° under moist conditions.

Ten larvae were buried 1 to 2½ inches deep in each 8-dr. screwcap vial. Care was taken to separate them with soil to avoid injury. Ventilation was provided by drilling a ¼-inch hole in the cap and inserting a wire-gage disk. All glassware was sterilized before use.

Larvae were subjected to combinations of three soil types, three soil temperatures, and three soil-moisture levels for periods ranging from 0.42 to 36 days. Four replicates, or vials, were used for each soil type, temperature, moisture, and period of time. Previous work (Richmond and Clark *12*) prompted the use of Nueces fine sand (pH 6.1), Amarillo fine sandy loam (pH 7.4), and Cameron heavy clay (pH 7.6), which represented the three classes of general surface soil texture. The temperatures employed were 8.5°, 29°, and 38° C. The soil-moisture levels adopted were dry (dried at room temperature with a relative humidity of 60 percent), one-half field capacity, and field capacity. Distilled water was used to maintain these moisture conditions.

The percent moisture in the selected soil types was as follows:

	Percent moisture content in—		
Soil-moisture levels	*Sand*	*Sandy loam*	*Heavy clay*
Dry	0.2	1.1	4.2
One-half field capacity	5.0	10.0	15.0
Field capacity	9.0	19.0	31.0

In general, the total duration of each test varied according to the time needed to reach 100-percent mortality and was as follows:

Temperature (° C.)	Duration of test (days) for— Nondiapause larvae	Diapause larvae
8.5	21	36
29.0	10	10
38.0	7	4

The period of time or interval at which a set of replicates was examined varied with the temperature as follows:

Temperature (° C.)	Days
8.5	6.
29.0	1.
38.0	.42 (10 hours).
38.0	1.

In addition to overall mortality recorded for each exposure, mortalities were recorded for free larvae, for those in cocoons, and for three categories of final larval position indicating larval movement; i.e., above the soil, on the soil, and in the soil. The above-soil category consisted of larvae that crawled above the soil surface and either clung or attached themselves by means of a few threads or a loosely spun cocoon to the inner surface of the vial. Larvae in the soil were removed by carefully washing the soil through a sieve. Recovered larvae from all three positions were placed in petri dishes containing pieces of paper toweling for concealment and separation. They were examined for mortality immediately after transference to dishes and again in 24 hours. Those held at 8.5° C. were also observed at 72 hours. Since the surviving larvae appeared normal, only occasional samples were set aside to determine whether their more gross life processes had been affected.

Median lethal times (LT–50) and b values (slope) were obtained from time-mortality data using regression techniques. Percent mortality was transformed to an angle (arc sine $\sqrt{\text{percentage}}$). To obtain the large number of diapause larvae required for this study, collections were made and each group of experiments was conducted over a period of 4 months. Thus a larval age factor was introduced, which was minimized by taking each of the four replicates from a different age group within the 4-month period. Certain tests in the 29° C. series were repeated 1 year later fo · confirmation of results.

Attempts were made to correlate these factors with certain characters within the population such as size and sex, and to determine whether mortality was affected by certain other factors such as micro-organisms.

RESULTS

Effects of Soil Type, Temperature, and Moisture

The combined mortality (LT–50) of larvae above, on, and in the soil and the mortality of the larvae in the soil alone are given in figure 1 and table 1. Typical results are illustrated in figures 2–4.

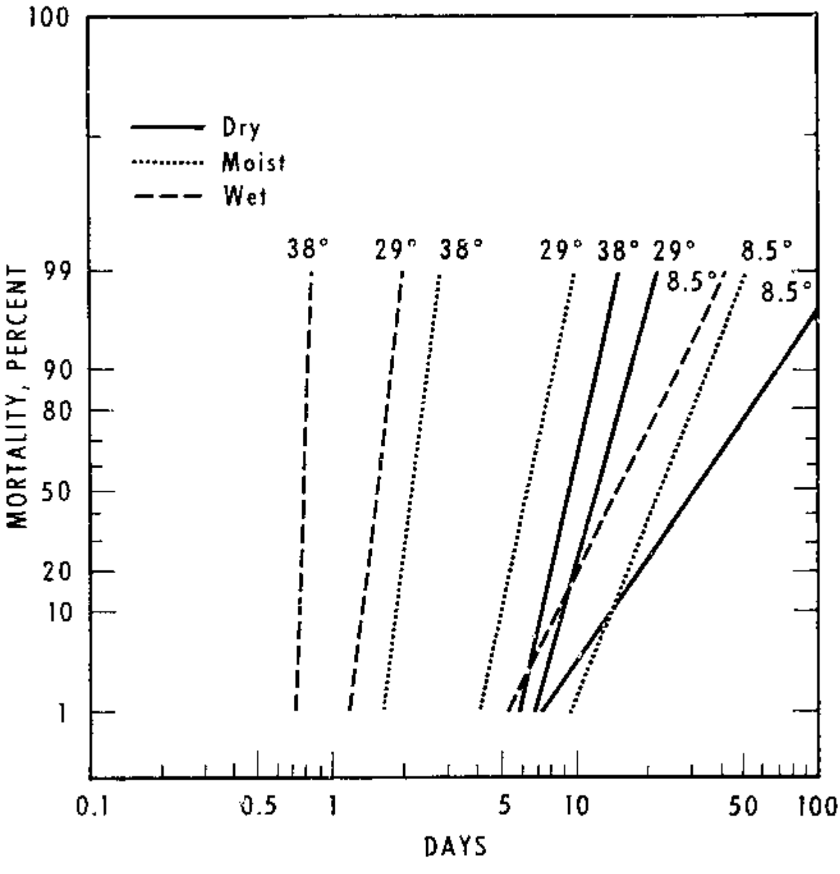

FIGURE 1.—Comparison of probit mortality of diapause larvae buried in sand at three soil moistures and three soil temperatures (° C.) (moist and wet refer to one-half field capacity and field capacity, respectively).

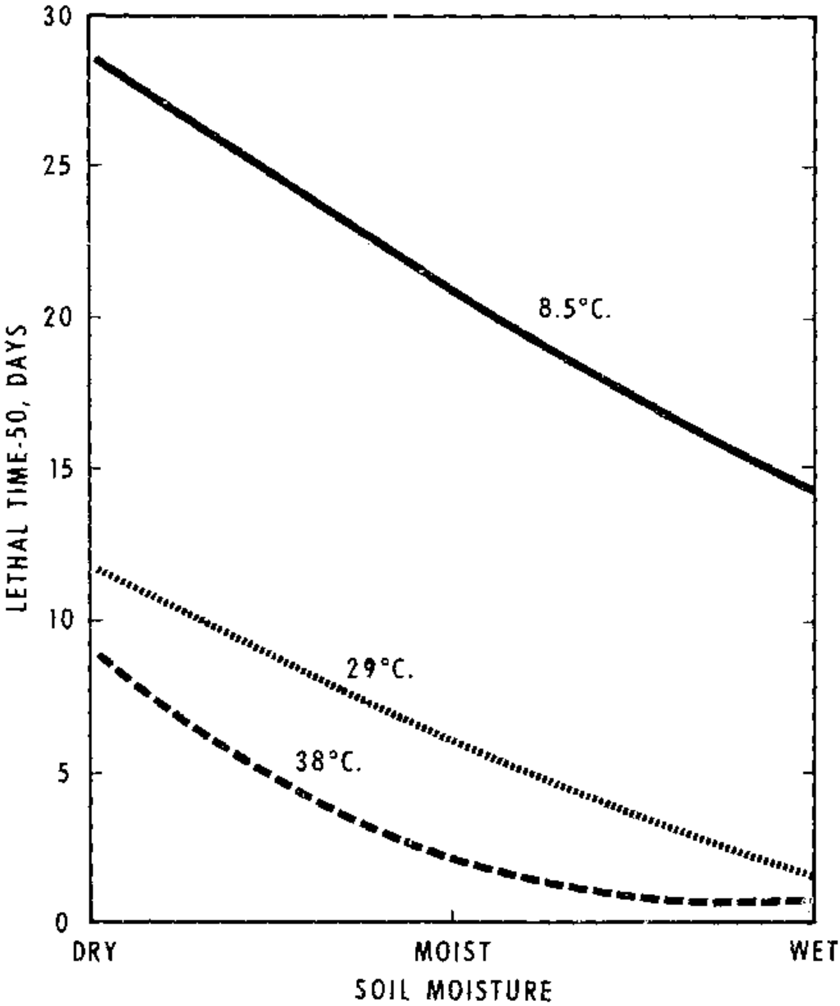

FIGURE 2.—Typical lethal effect of soil moisture and temperature on diapause larvae buried in soil (moist and wet refer to one-half field capacity and field capacity, respectively).

TABLE 1.—*Median lethal time (LT–50) and b values (slope of line) for nondiapause and diapause larvae under various conditions after burial in soil*

Soil moisture and soil temperature (° C.)	Lethal time and slope of line for nondiapause larvae buried in—						Lethal time and slope of line for diapause larvae buried in—					
	Sand		Sandy loam		Clay		Sand		Sandy loam		Clay	
	LT–50	b	LT–50	b	LT–50	b	LT–50	b	LT–50	b	LT–50	b
COMBINED MORTALITY [1]												
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Dry:												
8.5	16.13	3.11	16.87	2.49	23.47	0.65	29.04	1.47	22.70	1.84	23.40	1.60
29.0	18.60	1.62	25.24	1.11	95.85	.32	14.80	1.79	11.21	2.44	11.81	3.05
38.0	3.92	11.69	2.20	12.74	4.15	12.12	8.64	4.48	7.83	5.65	7.08	7.41
One-half field capacity:												
8.5	12.26	3.97	17.18	2.01	14.23	4.48	20.94	2.52	17.27	2.58	15.55	2.70
29.0	11.71	2.53	15.61	2.01	14.05	2.51	6.77	4.08	11.12	3.90	15.66	2.26
38.0	1.57	10.25	2.56	9.10	2.23	9.23	2.13	9.83	3.16	3.22	4.65	7.92
Field capacity:												
8.5	10.65	4.48	18.84	.58	7.76	4.13	14.14	2.06	20.53	2.37	10.65	3.86
29.0	1.47	5.99	5.88	3.90	2.12	5.29	2.40	3.89	5.65	4.56	1.42	5.66
38.0	.01	24.46	1.08	39.44	.89	39.95	.79	43.64	1.94	6.30	1.37	14.32

Dry:												
8.5	15.61	2.85	15.26	2.27	22.30	0.64	28.81	1.46	22.55	1.86	23.42	1.58
29.0	7.70	4.49	7.54	3.12	22.74	1.71	11.89	3.73	7.16	3.98	10.23	3.40
38.0	3.51	13.69	3.62	12.98	3.49	8.89	9.03	4.54	8.60	4.48	8.60	6.03
One-half field capacity:												
8.5	12.26	3.97	17.15	1.97	14.23	4.48	20.94	2.52	17.27	2.58	15.55	2.70
29.0	13.70	2.05	15.54	2.01	13.99	2.52	6.09	4.78	10.02	4.72	13.24	2.77
38.0	1.56	10.19	2.45	9.62	1.61	9.13	2.04	8.42	3.30	3.39	4.18	9.45
Field capacity:												
8.5	10.65	4.48	18.84	.58	7.76	4.13	14.14	2.06	20.09	2.29	10.65	3.86
29.0	2.11	11.40	5.24	4.31	1.82	6.28	1.49	9.00	4.79	5.00	-9.88	1.73
38.0	-.01	24.43	1.07	38.52	.44	27.48	.74	42.08	1.91	6.30	1.35	14.16

[1] Above, on, and in soil.

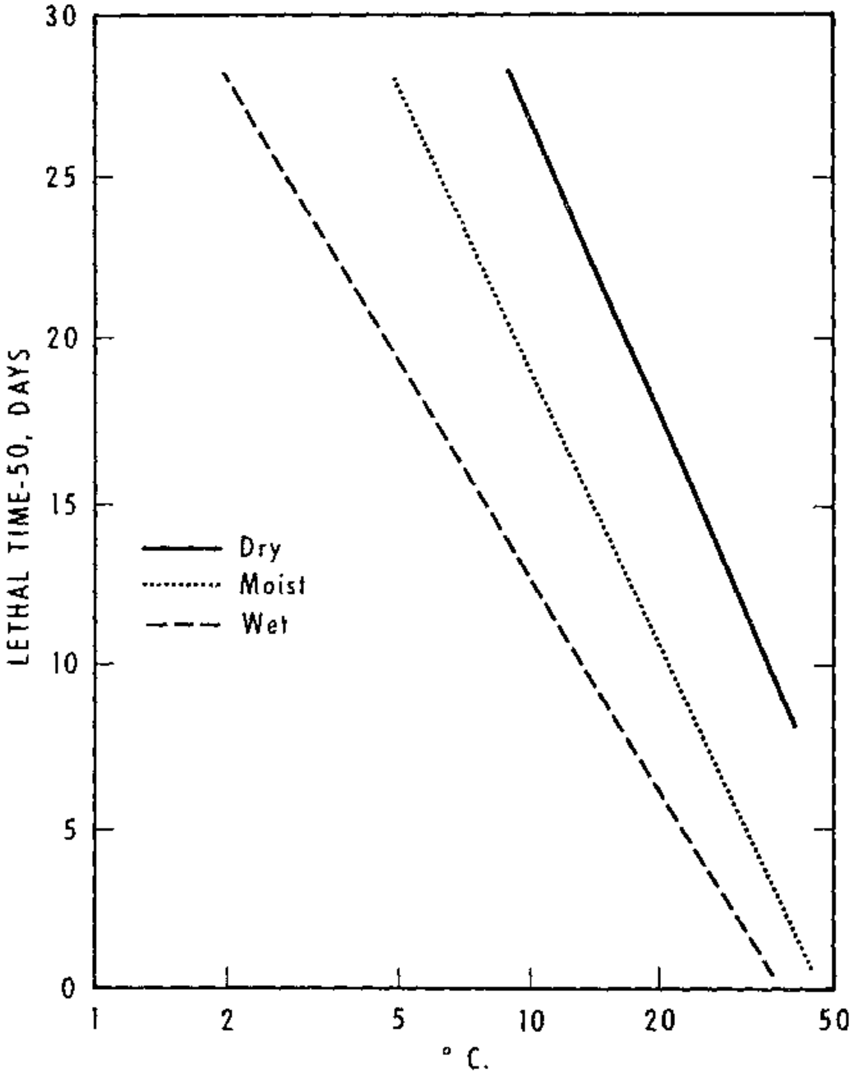

FIGURE 3.—Typical lethal effect of temperature on diapause larvae buried in sand at three soil moistures (moist and wet refer to one-half field capacity and field capacity, respectively).

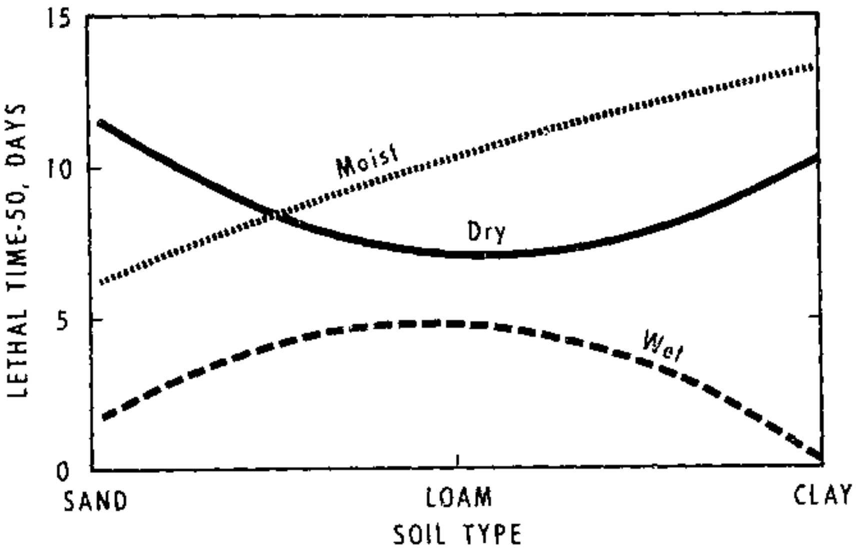

FIGURE 4.—Typical lethal effect of decreasing sand content in soils in which diapause larvae were buried and subjected to 29° C. and three soil moistures (moist and wet refer to one-half field capacity and field capacity, respectively).

Usually any differences in comparative LT–50 values were due to the greater survival of the larvae on the surface. Figures 5 and 6 and table 2 show the mean percent movement of the buried larvae, as indicated by their location at the end of the experimental period, and their subsequent mortality. Mean percentages for the entire experimental period are presented, because only the larvae in the soil had an increased mortality with time. Movement to and above the surface and subsequent mortality in these locations were at random and did not correlate with time. The effects of these terrestrial conditions on the spinning of cocoons and subsequent mortality of larvae with and without cocoons are given in figure 7 and tables 3 and 4.

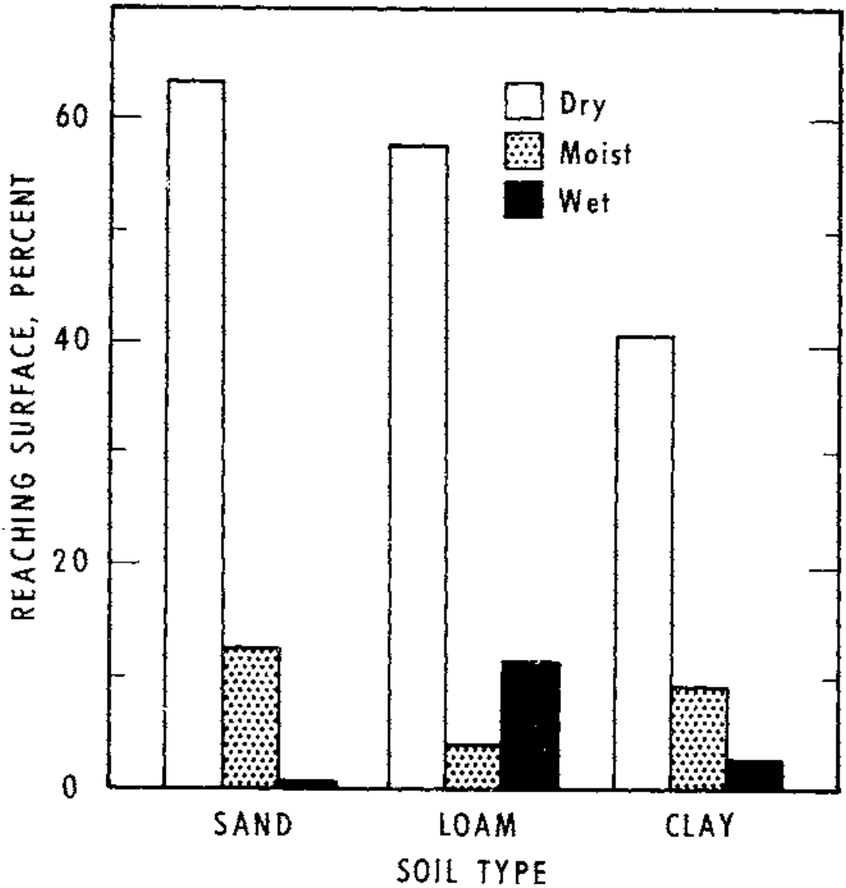

FIGURE 5.—Percent diapause larvae reaching surface after burial in three soil types and three soil moistures and held at 29° C. (moist and wet refer to one-half field capacity and field capacity, respectively).

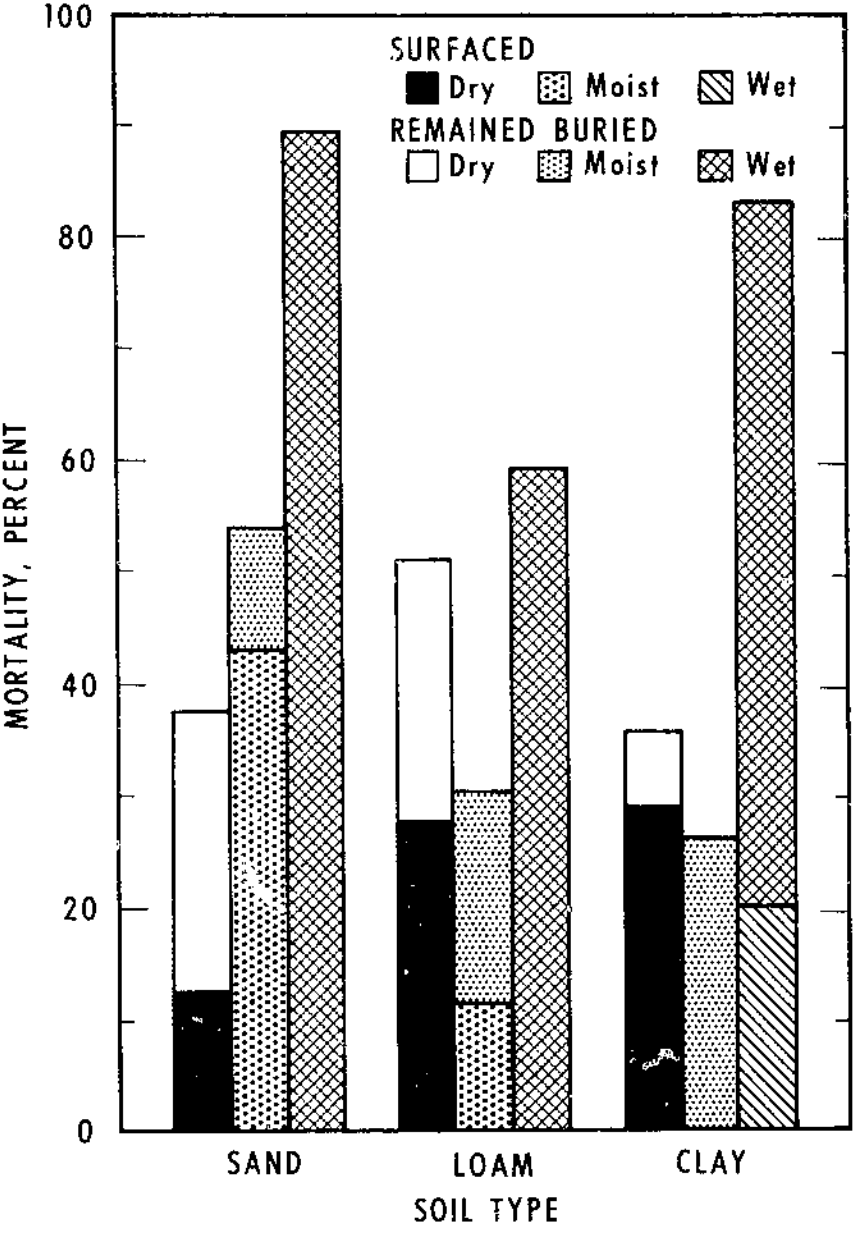

FIGURE 6.—Comparison of mortalities of diapause larvae that surfaced and larvae that remained buried in three soil types and three soil moistures and held at 29° C. (moist and wet refer to one-half field capacity and field capacity, respectively).

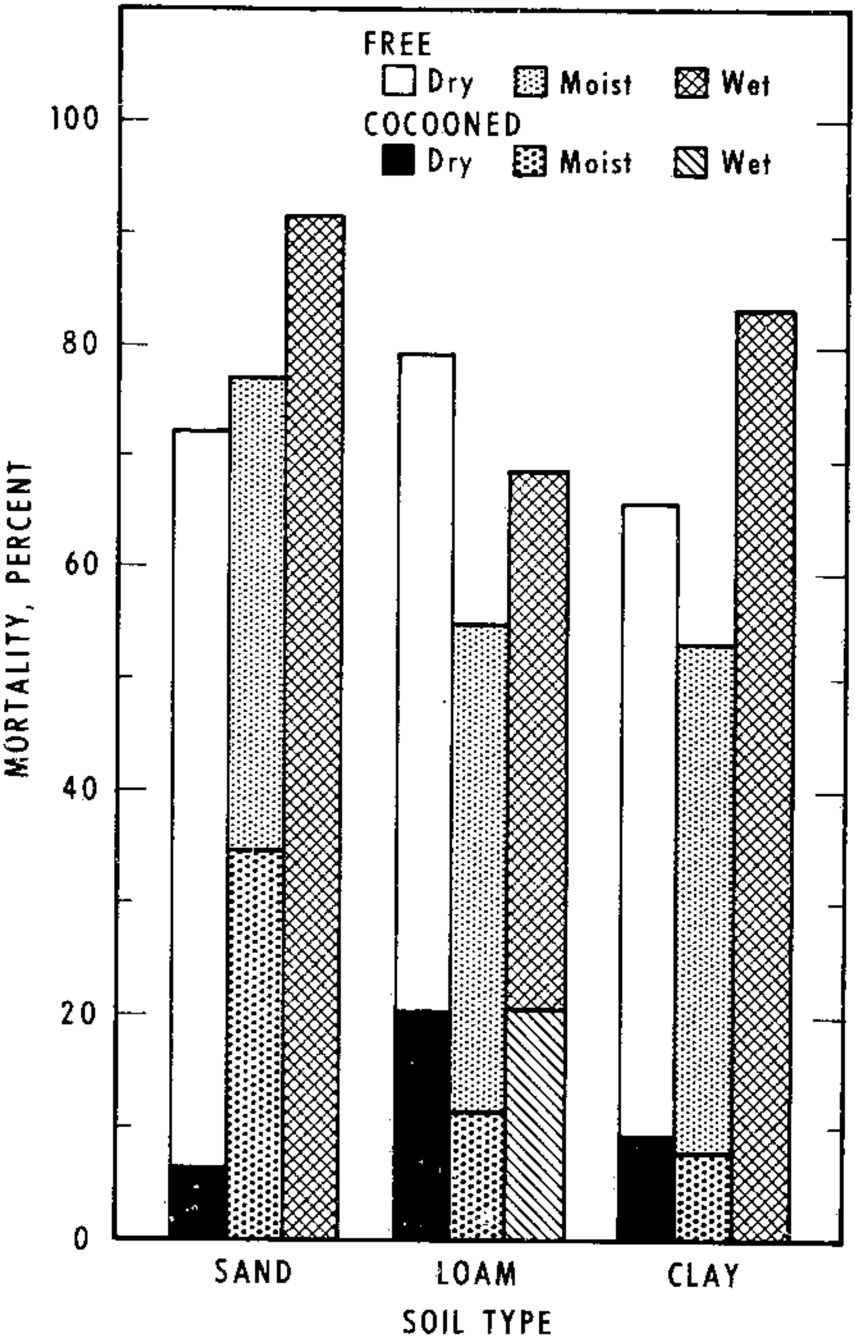

FIGURE 7.—Differences in mortality due to protection by cocoons when larvae were buried in three soil types and three soil moistures and held at 29° C. (moist and wet refer to one-half field capacity and field capacity, respectively).

TABLE 2.—*Nondiapause and diapause larvae found in 3 locations and resulting larval mortality under various conditions after burial in soil*

Soil moisture and soil temperature (° C.)	Nondiapause larvae and mortality when buried in—						Diapause larvae and mortality when buried in—					
	Sand		Sandy loam		Clay		Sand		Sandy loam		Clay	
	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality
ABOVE SOIL												
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry:												
8.5	1.1	0	4.4	0	0	-------	0.4	0	0.4	0	0.8	0
29.0	3.2	23.1	5.2	4.8	1.2	0	8.3	35.0	9.2	36.4	1.7	0
38.0	1.9	0	5.0	25.0	.6	0	1.0	0	5.3	0	.5	0
One-half field capacity:												
8.5	0	-------	0	-------	0	-------	0	-------	0	-------	0	-------
29.0	0	-------	0	-------	0	-------	3.3	87.5	1.7	0	.4	0
38.0	0	-------	5.0	100	0	-------	0	-------	0	-------	1.5	0
Field capacity:												
8.5	0	-------	0	-------	0	-------	0	-------	0	-------	0	-------
29.0	1.8	0	8	21.8	2.2	0	0	-------	2.5	0	.5	0
38.0	.5	0	1.5	0	0	-------	.4	0	1.0	25.0	2.5	0

TABLE 2.—*Nondiapause and diapause larvae found in 3 locations and resulting larval mortality under various conditions after burial in soil*—Continued

Soil moisture and soil temperature (° C.)	Nondiapause larvae and mortality when buried in—						Diapause larvae and mortality when buried in—					
	Sand		Sandy loam		Clay		Sand		Sandy loam		Clay	
	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality	Larvae	Mortality
ON SOIL												
Dry:												
8.5	11.1	0	15.6	7.2	2.2	0	5.0	0	1.2	33.3	0.8	50.0
29.0	58.7	11.9	72.0	11.1	62.0	8.1	55.0	9.1	48.4	25.8	38.8	30.1
38.0	50.6	23.4	40.0	23.4	30.0	18.7	19.0	31.6	22.5	38.9	13.8	48.1
One-half field capacity:												
8.5	0	------	1.1	0	0	------	0	------	0	------	0	------
29.0	.8	100	1.0	0	.5	0	9.2	27.2	2.1	20.0	8.8	0
38.0	1.5	66.6	2.5	0	.5	0	.5	0	.5	0	3.5	42.8
Field capacity:												
8.5	0	------	0	------	0	------	0	------	0	------	0	------
29.0	4.0	43.7	22.5	18.9	5.8	26.1	.5	0	9.0	0	2.0	25.0
38.0	.5	100	2.0	75.0	.5	0	.4	0	2.0	0	0	------

IN SOIL[1]

Dry:												
8.5	87.8	44.3	80.0	50.0	97.8	40.9	94.6	38.8	98.4	47.4	98.4	42.7
29.0	38.1	38.8	22.8	56.1	36.8	19.7	36.7	37.5	42.4	50.9	59.5	35.6
38.0	47.5	52.6	55.0	54.6	69.4	42.3	80.0	16.9	72.2	22.9	85.7	14.5
One-half field capacity:												
8.5	100	29.0	98.9	22.7	100	22.6	100	51.3	100	61.7	100	65.4
29.0	99.3	20.3	99.0	49.2	99.5	42.6	87.5	53.8	96.2	30.3	90.8	26.1
38.0	98.5	70.0	92.5	52.9	99.5	66.8	99.5	60.0	99.5	42.7	95.0	47.8
Field capacity:												
8.5	100	66.6	100	45.6	100	76.7	100	67.5	100	51.7	100	80.0
29.0	94.2	67.4	69.5	58.3	92.0	78.8	99.5	89.5	88.5	59.3	97.5	83.1
38.0	99.0	85.3	96.5	66.3	99.5	74.3	99.2	73.9	97.0	65.8	97.5	57.3

[1] Effects on larvae that remained in soil increased with time.

TABLE 3.—*Total number of nondiapause and diapause larvae and percent spinning cocoons under various conditions after burial in soil*

Soil moisture and soil temperature (° C.)	Nondiapause larvae buried in—						Diapause larvae buried in—					
	Sand		Sandy loam		Clay		Sand		Sandy loam		Clay	
	Total	Spinning cocoons	Total	Spinning cocoons	Total	Spinning cocoons	Total	Spinning cocoons	Total	Spinning cocoons	Total	Spinning cocoons
ABOVE SOIL												
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Dry:												
8.5	1	0	4	0	0	------	1	100	1	100	2	50.0
29.0	13	61.5	21	76.3	5	80.0	20	55.0	22	54.6	4	50.0
38.0	3	66.6	8	37.5	1	0	2	100	17	76.5	1	100
One-half field capacity:												
8.5	0	------	0	------	0	------	0	------	0	------	0	------
29.0	0	------	0	------	0	------	8	12.5	4	75.0	1	100
38.0	0	------	10	50.0	0	------	0	------	0	------	3	0
Field capacity:												
8.5	0	------	0	------	0	------	0	------	0	------	0	------
29.0	7	71.4	32	68.7	9	89.0	0	------	5	40.0	1	0
38.0	1	0	3	0	0	------	1	100	4	25.0	6	------

ON SOIL

Dry:												
8.5	10	0	14	14.3	2	50.0	12	41.6	3	0	2	0
29.0	235	79.3	288	75.3	248	80.3	132	70.5	116	45.6	93	53.8
38.0	81	69.2	64	62.5	48	70.8	38	44.7	72	34.8	27	51.8
One-half field capacity:												
8.5	0	------	1	0	0	------	0	------	0	------	0	------
29.0	3	100	4	25.0	2	0	22	63.6	5	40.0	21	47.6
38.0	3	0	5	0	1	0	1	0	1	0	7	0
Field capacity:												
8.5	0	------	0	------	0	------	0	------	0	------	0	------
29.0	16	18.8	90	75.6	23	30.4	1	0	18	77.8	4	0
38.0	1	0	4	0	1	0	1	0	8	87.5	0	------

IN SOIL

Dry:												
8.5	79	24.1	72	27.8	88	21.6	227	10.6	236	3.0	236	5.1
29.0	152	53.9	91	25.2	147	74.8	88	37.5	102	48.0	143	53.2
38.0	76	36.8	88	37.5	111	42.3	160	61.8	231	55.8	172	63.9
One-half field capacity:												
8.5	90	5.6	89	15.7	90	10.0	240	7.5	240	5.4	240	4.6
29.0	397	67.2	396	68.5	398	60.3	210	54.8	231	56.7	218	59.7
38.0	197	60.4	185	56.2	199	37.2	199	5.0	199	13.6	190	18.9
Field capacity:												
8.5	90	1.1	90	14.4	90	0	240	.4	240	12.1	240	2.1
29.0	377	7.7	278	28.1	368	6.0	199	2.0	177	19.2	195	0
38.0	198	0	193	1.0	199	0	238	0	388	3.6	234	0

TABLE 4.—*Mortality of nondiapause and diapause free larvae and larvae in cocoons under various conditions after burial in soil*

Soil moisture and soil temperature (° C.)	Nondiapause larvae in indicated state when buried in—						Diapause larvae in indicated state when buried in—					
	Sand		Sandy loam		Clay		Sand		Sandy loam		Clay	
	Free	In cocoons	Free	In cocoons	Free	In cocoons	Free	In cocoons	Free	In cocoons	Free	In cocoons
ABOVE SOIL [1]												
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry:												
8.5	0	------	0	------	------	------	------	0	------	0	0	0
29.0	60.0	0	20.0	0	0	0	55.6	18.2	80.0	0	0	0
38.0	0	0	40.0	0	0	------	------	0	0	0	------	0
One-half field capacity:												
8.5	------	------	------	------	------	------	------	------	------	------	------	------
29.0	------	------	------	------	------	------	100	0	0	0	------	0
38.0	------	------	100	100	------	------	------	------	------	------	0	------
Field capacity:												
8.5	------	------	------	------	------	------	------	------	------	------	------	------
29.0	0	0	10.0	27.3	0	0	------	------	0	.0	0	------
38.0	0	------	0	------	------	------	------	0	0	100	6	0

ON SOIL [1]

Dry:												
8.5	0	-------	8.3	0	0	0	0	0	33.3	-------	50.0	-------
29.0	20.2	9.7	35.2	3.2	14.3	6.5	23.1	3.2	43.8	3.8	48.8	14.0
38.0	44.0	14.3	54.2	5.0	57.2	2.9	42.9	17.6	59.6	0	92.3	7.1
One-half field capacity:												
8.5	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
29.0	-------	100	0	0	0	-------	12.5	35.8	0	50.0	0	0
38.0	66.6	-------	0	-------	0	-------	0	-------	0	-------	42.8	-------
Field capacity:												
8.5	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
29.0	46.2	33.3	31.8	14.7	37.5	0	0	-------	0	0	25.0	-------
38.0	100	-------	75.0	-------	0	-------	0	-------	0	0	-------	-------

IN SOIL

Dry:												
8.5	53.3	15.8	65.4	10.0	50.7	5.3	42.8	4.2	48.9	0	45.1	0
29.0	71.5	11.0	74.6	4.4	62.2	5.4	73.2	6.4	79.2	20.4	65.7	9.2
38.0	62.5	35.7	72.8	24.2	55.8	26.0	26.2	11.1	48.1	3.1	29.0	6.3
One-half field capacity:												
8.5	62.3	20.0	48.1	14.3	43.2	88.8	47.3	100	59.5	100	63.8	100
29.0	59.3	14.2	49.6	10.3	38.0	12.5	76.8	34.8	55.0	11.4	53.4	7.7
38.0	83.3	61.3	64.2	44.2	80.0	44.6	65.6	30.0	52.9	25.9	40.2	52.7
Field capacity:												
8.5	67.5	0	46.8	38.4	76.7	-------	67.3	100	48.8	72.5	79.6	100
29.0	83.5	51.7	74.5	16.7	81.8	31.8	91.3	0	68.7	20.6	83.0	-------
38.0	85.4	-------	67.0	0	74.4	-------	74.0	-------	66.8	35.7	57.3	-------

[1] These values largely calculated from small total numbers (<10) (see table 3) and must be viewed with caution.

In general, larvae that survived after 48-hour posttreatment time were normal and completed their life cycles with no abnormalities. Surviving larvae held under moist conditions pupated sooner than those under dry conditions.

The effect of soil types and soil moistures on the pupation of larvae held at 29° C. for 10 days is summarized in table 5. Because pupation showed little or no correlation with time under experimental conditions, pupation is presented as the mean percentage of those larvae in the specific set of conditions and of the total larvae used in the 10-day experiment. The latter percentages are given because the very small numbers of larvae found in certain conditions could cause erroneous conclusions to be drawn from the former percentages (e.g., above soil surface). Pupation data at 8.5° and 38° are not given, because pupation did not occur at 8.5° under these conditions and no appreciable pupation occurred at 38° because of the short experimental period.

Observations made during these studies with nondiapause larvae held at 15.6°±0.6° C. in damp tissue paper showed that they began pupating after 6 weeks. Pupation continued for 5 months, with about 70 percent ultimately pupating. Subsequently, a group of these pupae was divided into two parts, one part was held at 29° and the other at 15.6°. At 29°, 86.5 percent of the pupae emerged as moths in 18 days, but at 15.6°, 50 percent emerged in 47 days. The remainder of the pupae, 13.5 and 50 percent, respectively, died.

Influence of Sex or Size

Studies were conducted to determine whether the effects of burial were influenced by sex or size of the larvae. The general procedure was the same as for previous experiments. There were 4 replicates, each replicate consisting of 50 larvae separated into groups of 10 larvae per vial, for each sex and for each of 2 size groups (20 and 30 mg. per larva). The larvae were placed in sand held at a moisture of field capacity and 29° C. for 3 days. There were no significant differences in the movement or mortality of males and females or between sizes. Webs were spun by 2 percent of the males but none by females.

Effect of Physiological Activity

Attempts were made to correlate the respiratory rate of the larvae as a measure of physiological activity and their mortality rate. The general procedure was the same as for previous experiments except that the respiration was first measured using standard Warburg techniques (Umbreit et al. *15*) at 18° C. The larvae, 3 replicates of 20 larvae each of nondiapause and hand- and Berlese-removed diapause larvae, were buried in sand held at field capacity and 29° for 3 days.

The results are given in table 6. There were no significant differences in the respiratory rates of larvae that lived and those that died, between the hand- and Berlese-collected diapause larvae, or between the weight of dead and live larvae. There was a slight difference in weight between the hand-removed and Berlese funnel-collected larvae because of water absorption by the latter during the collection procedure. An additional experiment involving hand-removed and

TABLE 5.—*Pupation and pupal mortality in cocoons of nondiapause and diapause larvae under various conditions after burial in soil at 29° C. for 10 days* [1]

Location, pupation, and pupal mortality [2]	Burial in indicated moisture of—								
	Sand			Sandy loam			Clay		
	Dry	Moist	Wet	Dry	Moist	Wet	Dry	Moist	Wet
NONDIAPAUSE LARVAE									
ABOVE SOIL									
Pupation:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
In location	53.8	0	23.6	33.3	0	40.6	60.0	0	44.5
Of total	1.8	0	1.0	1.8	0	3.2	.8	0	1.0
In cocoons	85.8	0	75.0	100	0	84.7	100	0	100
Pupal mortality:									
Total	14.3	0	0	0	0	23.1	0	0	0
In cocoons	0	0	0	0	0	23.1	0	0	0
ON SOIL									
Pupation:									
In location	80.9	0	18.8	68.1	100	61.2	74.2	50.0	39.1
Of total	47.5	0	.8	49.0	1.0	13.8	46.0	.2	2.2
In cocoons	85.8	0	66.7	78.2	25.0	80.0	88.2	0	44.5
Pupal mortality:									
Total	5.8	0	0	7.2	0	20.0	4.9	0	0
In cocoons	6.8	0	0	3.1	0	18.2	4.9	0	0
IN SOIL									
Pupation:									
In location	34.2	54.4	.5	45.1	65.9	12.2	61.8	62.2	6.8
Of total	13.0	54.0	.5	10.2	65.2	8.5	22.8	61.5	6.2
In cocoons	44.3	91.7	50.0	31.7	89.0	94.2	80.3	94.4	80.0

See footnotes at end of table.

TABLE 5.—*Pupation and pupal mortality in cocoons of nondiapause and diapause larvae under various conditions after burial in soil at 29° C. for 10 days* [1]—Continued

Location, pupation, and pupal mortality [2]	Burial in indicated moisture of—								
	Sand			Sandy loam			Clay		
	Dry	Moist	Wet	Dry	Moist	Wet	Dry	Moist	Wet
NONDIAPAUSE LARVAE									
IN SOIL—continued									
Pupal mortality:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Total	53.8	12.0	50.0	39.0	8.8	0	14.3	12.6	36.0
In cocoons	5.8	11.1	0	2.4	8.0	0	4.4	8.5	20.0
DIAPAUSE LARVAE									
ABOVE SOIL									
Pupation:									
In location	5.9	12.5	0	22.7	100	20.0	33.3	100	100
Of total	.5	.5	0	2.5	.5	.5	.5	.5	.5
In cocoons	100	100	0	80.0	0	100	100	100	0
Pupal mortality:									
Total	0	0	0	0	0	0	0	0	0
In cocoons	0	0	0	0	0	0	0	0	0
ON SOIL									
Pupation:									
In location	36.2	72.2	100	32.6	100	44.5	25.6	88.2	75.0
Of total	21.0	6.5	.5	16.5	1.5	4.0	10.0	7.5	1.5
In cocoons	90.5	92.3	0	81.7	0	87.5	84.9	60.0	0

Pupal mortality:									
Total	9.5	38.4	0	6.1	0	0	10.0	0	0
In cocoons	9.5	29.4	0	6.1	0	0	10.0	0	0
IN SOIL									
Pupation:									
In location	20.9	10.9	0	7.8	29.6	5.6	3.4	32.4	0
Of total	7.0	9.5	0	3.0	29.0	5.0	2.5	29.5	0
In cocoons	35.8	100	0	33.3	98.3	70.0	60.0	89.8	0
Pupal mortality:									
Total	35.8	5.3	0	0	5.2	30.0	40.0	3.4	0
In cocoons	0	5.3	0	0	5.2	0	20.0	3.4	0

[1] Percentages calculated from total larvae in entire experiment as pupation did not correlate with time. Moist=one-half field capacity; wet=field capacity.

[2] In location=larvae that pupated in indicated location; of total=total larvae exposed to soil condition that pupated in indicated location.

Berlese-collected diapause larvae, using 4 replicates of 10 larvae, each buried in sand at field capacity for 4 days, again showed no difference in mortality (85 percent).

TABLE 6.—*Comparison of larval weight, respiratory rate, and mortality of nondiapause larvae and of diapause larvae collected by hand and Berlese funnel*

Weight, respiration, and mortality of larvae	Nondiapause larvae	Diapause larvae collected by—	
		Hand	Berlese funnel
Mean weight (mg.):[1]			
Total	24.6 ±0.6	20.5 ±0.8	23.5 ±0.9
Larvae dead	24.0 ± .8	19.9 ±1.0	22.3 ±1.2
Larvae alive	25.3 ± .7	21.3 ±1.2	25.3 ±1.1
Mean respiratory rate (μl./O_2/hr./mg.):[1]			
Total	.37± .01	.22± .01	.24± .02
Larvae dead	.37± .02	.23± .01	.27± .02
Larvae alive	.37± .01	.20± .02	.20± .02
Mortality (percent)[2]	49.4	61.4	59.1

[1] At 18° C.
[2] In sand for 3 days at 29° C.

Mortality From Terrestrial Micro-Organisms

Terrestrial micro-organisms were possible mortality factors. The general procedure was the same as for other tests. A freshly collected field sample of clay was divided into two parts. One part was sterilized by autoclaving at 15 pounds per square inch for 20 minutes and the other untreated. Ten larvae per vial were placed in unsterilized and sterilized clay and held at a soil moisture of field capacity and 29° C. for 3 days. Four replicates of 100 diapause larvae were used. There was no significant difference in the larval mortality between unsterilized (64.8 percent) and sterilized soils (60.8 percent), in the number of webbed larvae, number that pupated, nor number reaching the surface.

In other studies it was noted that a white fungus developed after 30 to 35 days in the vial containing diapause larvae being held in sandy loam or clay soils at 8.5° C. under moist conditions. Dead larvae were at the center of these fungal growths, which after 35 to 40 days formed comparatively large mycelial mats. However, these growths were probably saprophytic, because fungi having similar characteristics developed when gelatin was placed under the same conditions as larvae.

Effect of Burial on Pupae

Ten replicates of ten pupae each from nondiapause larvae were buried in sand held at field capacity for 4 days at 29° C. with an equal number as controls. This burial gave a 79-percent mortality.

Effect of Arizona Sandy Loam

A related problem that arose during this research was the high, unaccountable reduction in pink bollworm population in the Buckeye area of Arizona during the winter of 1958–59. With the hypothesis that soil was a major factor, diapause larvae were buried in soil from this area. It was a gravelly sandy loam with a wide range of particle sizes and a field capacity of 13-percent moisture. The buried larvae were subjected to soil-moisture conditions of dry, one-half field capacity, and field capacity at 29° C. for 2-day intervals up to 10 days.

The results were similar to those obtained with sand, but the mortality rate was somewhat higher, particularly at lower moisture levels. The LT–50's in days for overall mortality were dry 5.04, one-half field capacity 4.63, and field capacity −3.21. The LT–50's for soil mortality at the respective moistures were 4.22, 3.76, and −3.07. This soil became extremely hard when wetted and then dried, and thus the larvae were virtually imprisoned at the burial site since not one reached the surface. This condition was much worse than a hard crust, which was previously found so unfavorable for survival (Richmond and Clark *12*). Only 12 percent of the total larvae spun cocoons and this inhibition of spinning undoubtedly aided in causing a high mortality, as previous experiments established that nonwebbed larvae had a high death rate (a mean rate of 75 percent in this study).

DISCUSSION

Larval Behavior

Active and diapause larvae had, in general, the same reactions except in length of reaction time. Diapause larvae took longer to reach an observable point, due probably to their quiescent state. The greatest larval movement occurred in the dry soils held at 29° and 38° C. Both nondiapause and diapause larvae buried in wet soil attempted to seek a drier and more desirable location—nondiapause larvae for pupation and diapause larvae for spinning cocoons.

The percentage of the larvae reaching the surface was low in the soils held at field capacity; however, this was not by choice, as in the half-field capacity group, but probably by suppression of larval movement. Suppression is logically indicated because it would not be normal for larvae to remain in such an environment causing high mortality (see figs. 6 and 7 and table 2).

In a previous study Richmond and Clark (*12*), by observing larvae in glass-walled observation boxes, showed that larval activity was least and mortality greatest in soils with a high moisture content. Also, the observation that aqueous submersion caused general paralysis in larvae within a few minutes (Clark and Richmond *5*) implies that low oxygen tensions bring about inactivity. Willcocks (*16*) also noted that high moisture affected larvae adversely, because he found that diapause larvae crawled from buried, wet bolls either to pupate or to seek a drier location.

In addition to larval and pupal mortality due to high moisture, if development were completed in the soil, the moths could not reach the surface (Richmond and Clark *12*). Figure 5, prepared from data on larvae held at 29° C., more graphically illustrates this surfacewise movement in regard to the differences between dry and wet soils. In dry soils 50 percent of the larvae reached the surface in 3 days, whereas in wet soils the 50-percent level was not reached in 10 days at this temperature.

Soil type and depth of burial also affected larval movement. In these experiments and in previous work (Richmond and Clark *12*), it was noted that packed or hardened soil, particularly clays, tended to imprison the larvae, and they consequently perished. Depth of burial as a deterrent to larval survival has been noted by many workers in the field.

Cocoons protected the larvae, as shown in figure 7. The period of protection ranged from 14 days at 29° C. to 3 days at 38°.

Favorable Conditions for Larvae

Of all the combinations of conditions, the one most favorable for the larvae was 29° C. and one-half field moisture. Here, the soil was not a major factor. Under this set of conditions, larvae made no attempt to get above and little attempt to reach the soil surface. Those remaining in the soil totaled more than 87 percent (see table 2). In this connection, Barber and Dicke (*2*) concluded that normal soil moisture probably was not injurious to pupae of the corn earworm (*Heliothis zea* (Boddie)) and may have been advantageous in maintaining water balance.

Pupation

The percent pupation was less for larvae remaining in the soil than above or on the soil surface during the observed periods. In general, more pupation occurred in moist soils, and there was less mortality in cocoon-encased pupae, indicating that cocoons gave some protection.

Soil Moisture

It was difficult to evaluate the effects of any single factor in this study as they were all interdependent. In general, high soil moisture caused the greatest mortality and most adverse conditions for the larvae (see table 2). It deterred cocoon spinning and greatly reduced the period of time the cocoon protected the larvae, particularly in conjunction with high temperature. Larval movement was greatly impaired as pointed out previously. Swailes (*14*) also found that increased soil moisture caused an increased mortality in the buried beet webworm (*Loxostege sticticalis* (L.)) and five species of its parasites.

The effect of soil moisture within a soil type can be compared in terms of percent moisture. However, in comparing the effect between soil types, it is not the percent, or actual amount of, water present per unit of soil weight, but the retentive capacity of the soil that is correlated with soil forces, gravitational forces, atmospheric pressure, and such soil properties as compaction and granulation. Thus, the percent water present in these three soil types at any one of the three moisture levels was quite different, but the action on the larvae was similar within each level (see fig. 2).

Soil Temperature

Consistently high temperature was detrimental, particularly above 38° C. Temperatures of 8.5° and 38° affected the mortality rates more than 29°. Temperature and high soil moisture were interrelated and the mortality rate increased with corresponding increases of these two factors (see fig. 3).

Temperature and time were inversely interdependent; the lower the temperature the longer the time required to cause any adverse effect (see fig. 3). Thus, low temperatures could protect larvae from other adverse conditions if the time in which the larvae were subjected to these conditions was short.

Soil Types

Of the three soil types, sand generally caused the greatest mortality, then clay, and finally sandy loam. The mortality in sand was to some extent due to integumental abrasion by the sharp particle edges (Clark and Richmond *6*). In clay and loam, compaction, particularly when wet, may have contributed to the mortality rate. Swailes (*14*) also speculated that the mortality rate may increase with depth of burial because of soil compaction. Certain of the data obtained with sandy loam were without an apparent pattern and were therefore difficult to interpret. This irregularity of data was similar to that obtained in field hibernation experiments conducted in a sandy loam soil on overwintering pink bollworm larvae.

CONCLUSION

Results of these laboratory experiments in general agree closely with the results and conclusions of the extensive field experiments and observations on overwintering larvae by Chapman et al. (*4*). Their general findings were briefly as follows: Mortality was directly proportional to the amount of irrigation or rainfall, length of burial period, and depth of burial; mortality was greatest in the soil; and sandy soil was least favorable for survival. Also, mortality was greatest among buried larvae in simulated climates studied in bioclimatic cabinets. They concluded that mortality from present cultural practices was due largely to climatic conditions, and carryover of populations in cocoons would appear to be of little economic importance in areas with heavy rainfall.

From a practical standpoint, the research on nondiapause larvae indicated that field studies should be done in the cotton-growing season to determine whether there is significant mortality in the nondiapause larvae that drop to the soil surface and whether any cultural control could be developed. Wet, cultivated soil in hot weather could eliminate an appreciable number of larvae. Such a condition was examined for 1 week using soil thermographs. The soil surface temperature was found to vary from 23.3° C. at night to 37.8° in the day, and there were 7- to 9-hour periods in which the temperature stayed above 32.2°. Although no extensive tests were conducted to determine the effect of diurnal fluctuations of the soil temperature, it is reasonable to assume that these repeated conditions will have a detrimental effect on the larvae or pupae. In a single experiment carried out for a 5-day period in an outdoor insectary in which the temperature varied from 13° to 31°, the mortality was 10 percent in dry sand and 97.5 percent in sand at field capacity.

This extensive investigation demonstrated the complexity and interrelationship of soil factors and how much they affect the behavior and survival of pink bollworm larvae. Undoubtedly, the results of this study could be applied in a general way to many insects subjected to such subterranean conditions. The physiological causes of mortality due to burial were not determined. These specific causes probably were a combination of such adversities as exhaustion, water imbalance, and asphyxiation.

SUMMARY

Among the factors long recognized as detrimental to overwintering populations of the pink bollworm (*Pectinophora gossypiella* (Saunders)) is burial of larvae in soil with excessive moisture. However, these adverse effects on the larvae have never been well understood and this study was conducted to gain a better knowledge of them. Nondiapause and diapause larvae and pupae were subjected to burial under various conditions in the laboratory. The major conditions studied were all combinations of three soil types, three temperatures, and three moisture levels for discrete periods of time until 100-percent mortality was approached. Data presented include the effects of these soil conditions on mortality rate (median lethal time, LT–50), pupation rate, larval movement and final location, and cocoon spinning and subsequent protection.

Three soil factors examined were all interrelated. However, high soil moisture had the most adverse effects and in conjunction with high temperature became very detrimental to the larvae and pupae. Larval activity was least and mortality greatest in high moisture. Mortality and nonlethal adverse effects varied directly with increasing temperature. Of the three soil types, sand was the most detrimental. Soil compaction was very detrimental, particularly in clay types.

Size, sex, respiratory rate of the larvae, and terrestrial microorganisms could not be correlated with the mortality rate.

Of the conditions tested, the most favorable combination for larval survival was a sandy loam soil at 29° C. and one-half field-capacity moisture. Cocoons protected larvae.

Studies on the effects of gravelly sandy loam on diapause larvae from Buckeye, Ariz., an area with an unaccountable reduction in pink bollworm population, gave results similar to those obtained with sand. This soil became extremely hard when wetted and then dried, imprisoning the larvae at the burial site.

Generally, the results of these laboratory studies agree closely with the results of extensive field experiments and observations previously made. In addition, the data help explain the results of these field studies.

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