TEMPERATURES LETHAL TO THE GREEN MUSCARDINE FUNGUS, METARRHIZIUM ANISOPLIAE (METCH.) SOROK.

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THE fact that a compost pit provides an ideal breeding ground for the larvae of the coconut beetle, *Oryctes rhinoceros* L., constitutes a serious objection to laying compost pits on coconut land. The possibility of controlling the larvae in these pits by the use of the entomogenous fungus *Metarrhizium anisopliae* (Metch.) Sorok., has been frequently suggested. As temperatures as high as 70°C. are of common occurrence in compost heaps and as there is the likelihood of these temperatures being lethal to both the mycelium and the spores of the fungus, information regarding the temperature relations of this fungus is an essential preliminary to attempts at biological control of rhinoceros beetle larvae. The investigation recorded below represents an attempt at securing this information.

EXPERIMENTAL METHODS

The experimental material consisted of a strain of *M. anisopliae* isolated by Mr. L. S. Bertus from diseased rhinoceros beetle larvae. Stock cultures of the fungus were maintained in Erlenmeyer flasks on boiled rice, a medium on which *Metarrhizium* sporulates freely. In one series of experiments single spore cultures were used, and single hyphal tip cultures in another. Single spore isolations were made by the method of Rawlins (1933) and single hyphal tip cultures by Brown's method (1924).

The technique of determining lethal temperatures consisted in performing viability tests in Van Tieghem cells on spores subjected to various heat treatments. Preliminary experiments on the suitability of various concentrations of glucose (0·2 per cent. – 0·025 per cent.) and of dung extract for spore germination were carried out. Germination in glucose solutions was

poor. A comparatively high percentage germination was obtained with dung extract prepared by steaming 200 gms. dried cow dung for one hour, filtering through cotton wool and making up to a litre with distilled water. The extract was autoclaved at 20 lb. pressure for twenty minutes. Germination in 75 per cent., 50 per cent., and 25 per cent. dung extract was poorer than in the 100 per cent. solution.

Spore suspensions were made by dispersing 5 loopfuls of spores in 10 cc. of sterile 100 per cent. dung extract in a test tube. A thermometer was inserted in the test tube which was maintained at the requisite temperature in a thermostat. The variation in temperature was ± 1°C. The following temperatures were investigated: 60°C., 55°C., 50°C., 48°C., and 42°C. The following were the durations of exposure to the various temperatures: 0 min., 5 min., 15 min., 30 min., 1 hour, 2 hours, 3 hours, 24 hours, and 48 hours. Hanging drop cultures of spores subjected to the above-mentioned treatments were made by inverting a cover-slip carrying a loopful of the treated spore suspension over a Van Tieghem cell. A drop of sterile dung extract was placed on the floor of the cell in order to maintain high air humidity within the cell and to reduce evaporation from the surface of the hanging drop. Soft paraffin wax was used for sealing joints in the cell.

Hanging drop cultures were carried out in triplicate for each treatment.

Daily examinations of the hanging drops were made under the microscope up to usually the third day and occasionally the fifth day.

RESULTS

The results of the experiments are given in table I. Germination is recorded as being present (+) or absent (—). In some cases records have been made of percentage germination.

TABLE I.—Killing of Metarrhizium Spores by Heat

Temp.	Duration o exposure	Germination at end of				
		1st day Per cent.	2nd day Per cent.	3rd day Per cent.	4th day	5th day
42°C.	0 min.	2·0	2·1	+	+	+
	5 „	—	1·4	+	+	+
	15 „	—	1·8	+	+	+
	30 „	—	1·7	+	+	+
	60 „	—	+	+	+	+
	24 hours		—	+	+	+
	48 „			—	—	+
48°C.	0 min.	1·6	2·2	+		
	5 „	—	—	+		
	15 „	—	—	+		
	30 „	—	—	+		
	60 „	—	—	+		

Temp.	Duration of exposure	Germination at end of 1st day Per cent.	2nd day Per cent.	3rd day Per cent.	4th day	5th day
50°C.	0 min.	3·9	4·1	+		
	5 ,,	—	1·0			
	15 ,,	—	0·7			
	30 ,,	—	—	—		
	60 ,,	—	—	—		
	2 hours	—	—	—		
	3 ,,	—	—	—		
	30 ,,	—	—	—		
55°C.	0 min.	1·2	1·8	2·2		
	5 ,,	—	—	—		
	15 ,,	—	—	—		
	30 ,,	—	—	—		
	60 ,,	—	—	—		
60°C.	0 min.	1·5	4·4	+		
	5 ,,	–	—	—		
	15 ,,	—	—	—		
	30 ,,	—	—	—		
	60 ,,	—	—	—		

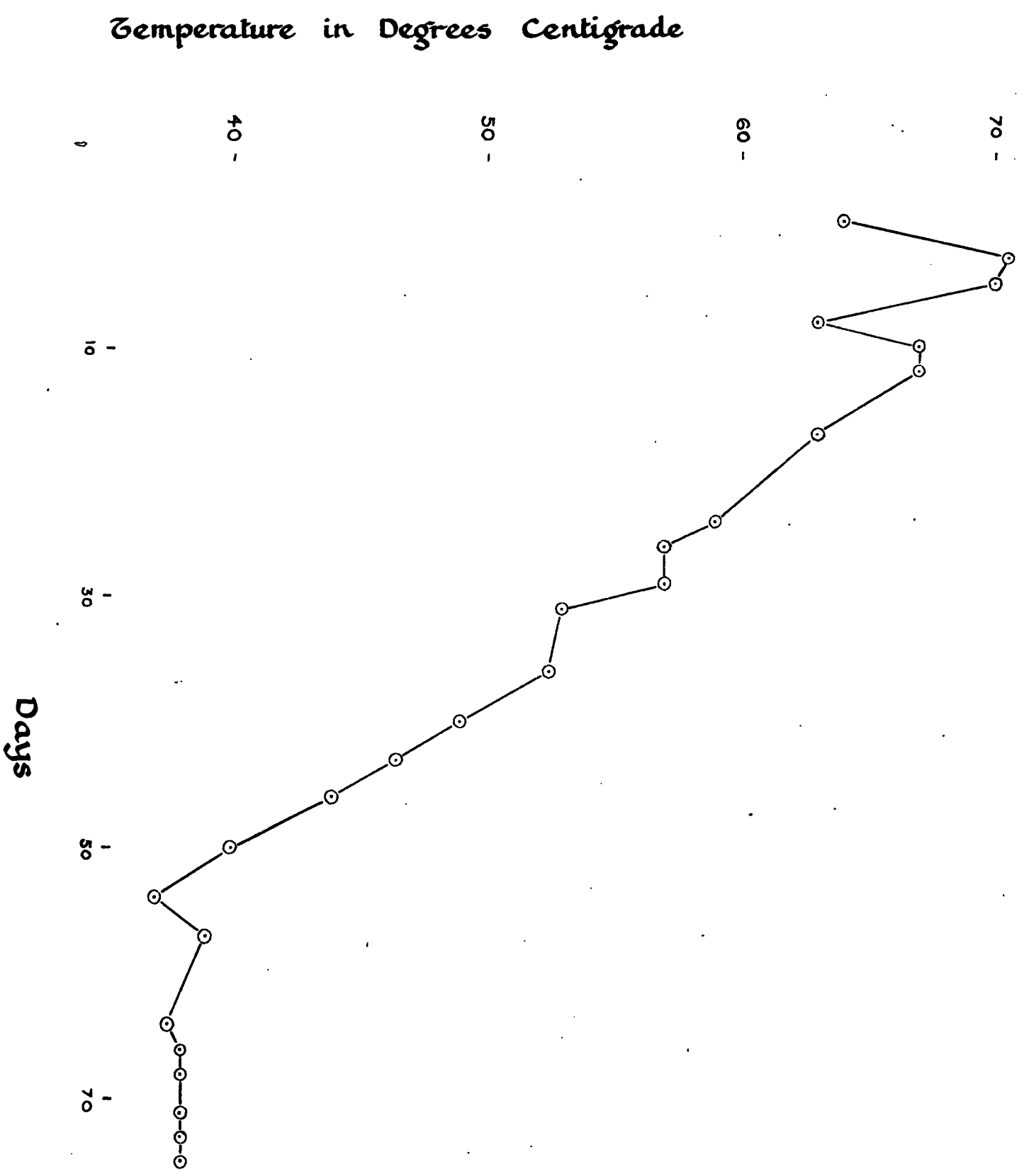
DISCUSSION

The results exhibit a considerable degree of uniformity. Exposures to temperatures of 50°C. and above for 5 minutes inhibited germination in every case. The spores appear to have a comparatively low thermal death point and it is only when the temperature falls below 48°C. that conditions favourable for spore germination are obtained. A temperature of 42°C. is definitely non-lethal to *Metarrhizium* spores.

Fig. 1 illustrates the temperature drift in a compost pit of the type generally laid down in coconut land. A temperature in the neighbourhood of 70°C. is soon reached and is maintained at that level for nearly two weeks. A gradual decline in temperature follows, but the temperature does not fall well below 48°C. till the pits are nearly six weeks old. Inoculation of the pits may then be undertaken. The susceptible larval and pupal stages of the beetle cover a period of nearly eighteen weeks. The inoculation of six-week old pits gives the fungus at least twelve weeks within which to effect destruction of the larvae or the pupae. As killing of the larvae by the fungus is effected under laboratory conditions in a much shorter time, successful control in compost pits appears possible.

SUMMARY

Temperatures lethal to spores of *Metarrhizium anisopliae* have been studied with a view to testing the possibility of biological control of the coconut beetle in compost pits.



TEMPERATURE DRIFT IN A COMPOST PIT.

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The thermal death point of the spore has been shown to be well below the temperatures normally occurring in compost pits during the first six weeks.

The possibility of successful inoculation of the pits after the temperature has fallen below 48°C. is suggested.

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