STUDIES ON THE GREEN MUSCARDINE FUNGUS: INFLUENCE OF METEOROLOGICAL FACTORS AND INOCULUM POTENTIAL ON THE INFECTION OF *METARRHIZIUM ANISOPLIAE* ON *ORYCTES RHINOCEROS* L.

ELIZABETH GEORGE AND CHANDY KURIAN*

THE coconut black beetle, *Oryctes rhinoceros* L., is subject to a number of diseases due to bacteria, fungi and viruses. Of the fungus infections *Metarrhizium anisopliae* affects all stages of the pest except eggs. Among various factors, environmental conditions and the inoculum potential influence the initiation and development of disease outbreaks. Johnpulle (1938) recorded that exposure to temperature 50° C and above for 5 min inhibited germination of the spores of *Metarrhizium*. He pointed out that the spores had a low thermal death point, but 42° C was definitely not lethal to them and some germinated after an hour's exposure to 48° C. Again, he recorded that the fungus inoculated larvae and pupae at a temperature 48°C in compost pits were controlled successfully within a period of 12 weeks. Pospelov (1940) observed in his experiments with the fungus *Metarrhizium*, on beet weevil *Cleonus punctiventris*, that optimum conditions for the development of the fungus was temperature range between 22 and 28°C and 100 per cent relative humidity. Steinhaus (1949) stated that the growth and fructification of this fungus are retarded by the rays of sun. Nirula (1958) reported that natural incidence of infection due to this fungus on *Oryctes* increased when relative humidity was high and temperature low. The present paper deals with the influence of relative humidity and temperature as well as inoculum potential, on the rate of infection of *rhinoceros* beetle.

MATERIAL AND METHODS

The fungus used for the test was locally isolated from a dead larva of *Oryctes rhinoceros* collected from the areas adjoining Central Coconut Research Station, Kayangulam. Pure culture of the fungus was prepared by single spore isolation and multiplied

* Central Coconut Research Station, Kayangulam, Ochira, Kerala State, South India.

by cultivating in sterilized potato slices contained in flakes for 25 days. The fungus growth on the surface of potato was scraped out by means of sterilized scalpel or spatula.

Experiment 1.—The inoculum was mixed with air-dried cowdung contained in mud pots (Size 18" × 18" at top) at the rate of 1 g of fungus inoculum to 5,000 g of cowdung. Fifteen third instar larvae of the same size collected from identical condition in the field were introduced into the inoculum in each pot. The pots were kept under a thatched roof in the field. 250 ml of water was added to the inoculum in the beginning of the experiment to keep it moist. Control pots had all these conditions except the fungus inoculum. There were three replications at each of the seven trials carried out during the different periods of the year with varying climatic conditions. Daily observations on normalcy and mortality of grubs were made and dead larvae were removed from the pots each day soon after observation. Observations were recorded till all the grubs were dead in the treatments. Temperature and relative humidity were noted daily. Temperature of the cowdung was also recorded. Death due to fungus infection alone was calculated by adjusting natural mortality by Abbotts formula.

Experiment. 2.—Three trails were conducted as mentioned above with the different doses of inoculum. *viz.*, 1 g, 0·5 g, 0·25 g, 0·125 g, 0·0625 g and 0·03125 g to 500 g of dried cowdung. Weather data during the period of experiment were also noted.

Results and Discussion

Experiment 1.—In this experiment, considering the rate of mortality on 10th day in the different trails it is seen that the percentage mortality is positively correlated with the average humidity and negatively with temperature (Fig. 1). Temperature of the cowdung was found to be more or less the same as the atmospheric temperature and the moisture level in the pots at the beginning of the experiment was the same in all the trials. The meteorological factors were seen to influence the mortality which sets in 3·6 days after inoculation, with increasing trend from 7th or 8th day. From 7th to 11th days after inoculation the rate of mortality was comparatively high, and about ninety per cent death occurred by the thirteenth day. The data indicate that seven to ten days are necessary to establish high rate of infection, although death may occur even from 3rd day onwards depending on favourable factors promoting infectivity of the pathogen (Table I).

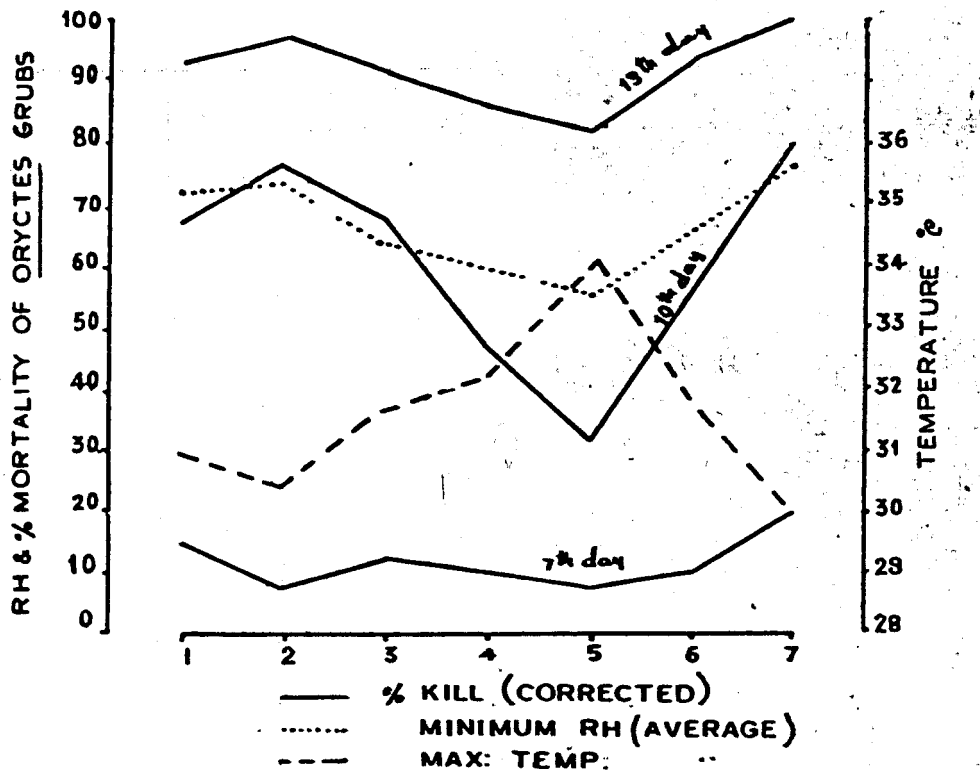

FIG. 1. Correlation of meteorological factors to mortality of *Oryctes* grubs treated with *M. Anisopliae*.

In the seventh trial, 4·6 per cent mortality was observed on 3rd day and all the grubs were found dead by the thirteenth day, in which case high humidity and low temperature acted together to raise the mortality rates. But in fourth and fifth trials, the rate of mortality is comparatively low, *i.e.*, mortality begins only on 5th day, less than 50·0 per cent even on 10th day and maximum mortality was obtained after only 16 and 17 days respectively. Low humidity and high temperature are attributed to this. 100 per cent mortality was not attained even on 17th day in fifth trial because of the high temperature and low humidity during that period. There were not many differences in mortality rates between the trials one and three where the temperature and humidity were also more or less the same. The mortality rate of the second trial was steady upto eighth day and then suddenly increased. It may perhaps be due to the prevailing high humidity and low temperature during the latter half of the experimental period. Table II indicate that the percentage of infection is directly proportional to average maximum relative humidity and inversely proportional to average maximum temperature. The percentage

TABLE I

Mateorological data (Temperature and Relative humidity) and perce
anisopliae *at 1 g spor*

Days after treatment	Replication I			Replication II			Replication III	
	Mc	RH	T	Mc	RH	T	Mc	RH
1	0·0	97	31·1	0·0	99	31·8	0·0	100
2	0·0	96	31·4	0·0	95	31·9	0·0	96
3	0·0	97	31·9	0·0	95	32·0	2·2	97
4	0·0	100	28·9	2·2	91	31·8	4·4	93
5	2·4	100	29·0	2·2	89	32·2	6·9	99
6	9·5	97	31·2	2·4	97	31·7	7·0	97
7	14·6	95	30·5	7·3	99	31·3	11·9	98
8	24·4	95	29·5	10·0	100	29·4	14·6	97
9	28·3	91	30·7	38·5	97	30·4	35·0	98
10	67·5	94	31·2	75·7	98	28·6	68·4	88
11	81·0	92	31·2	83·8	97	29·3	78·9	94
12	91·6	94	31·6	94·4	97	29·4	89·2	94
13	94·2	93	31·6	97·3	100	26·3	91·6	99
14	100·0	97	32·0	100·0	100	29·5	94·4	97
15	..	..	..	..	..	..	100·0	86
16	..	..	..	..	..	..	..	..
17	..	..	..	..	..	..	..	..

Mc = Percentage mortality of *Oryctes* (Corrected); RH = Relative Humidity (Maximu

tage mortality of Oryctes *grubs due to the fungus* Metarrhizium *to* 5,000 *g of cowdung*

Replication IV			Replication V			Replication VI			Replication VII		
Mc	RH	T	Mc	RH	T	Mc	RH	T	Mc	RH	T
0·0	83	29·4	0·0	99	33·3	0·0	99	31·1	0·0	98	30·6
0·0	87	31·1	0·0	98	34·6	0·0	100	33·1	0·0	100	30·5
0·0	89	32·5	0·0	83	33·6	0·0	97	32·2	4·5	99	29·4
2·2	96	32·3	0·0	86	32·6	4·6	97	31·0	4·8	97	31·6
4·6	97	31·5	4·6	89	34·0	4·7	97	29·4	7·6	98	32·9
4·7	86	32·4	7·0	93	32·3	4·7	87	32·0	11·5	97	30·0
9·8	99	33·0	7·4	92	33·4	9·5	97	30·4	19·6	95	30·2
12·5	98	29·9	10·1	96	33·6	14·6	87	32·1	28·5	96	30·9
23·7	95	32·3	18·0	94	33·9	25·0	100	31·9	43·4	97	30·5
47·3	82	33·5	31·5	95	34·1	56·2	97	31·7	80·5	100	26·6
53·9	96	33·5	55·2	95	33·1	71·0	94	32·8	87·8	97	28·0
82·9	91	33·5	80·5	93	33·7	86·1	98	31·9	93·5	100	29·6
85·7	100	33·8	82·4	90	36·4	94·2	99	31·4	100·0	97	29·9
88·2	91	33·1	85·3	84	35·4	100·0	87	31·7	..	..	..
94·0	97	30·9	90·9	88	34·6	..	..	..	..	..	..
00·0	100	32·4	93·7	90	35·6	..	..	..	..	..	..
..	..	..	96·8	91	34·3	..	..	..	..	..	..

T = Temperature °C (Maximum).

of infection (56·2 per cent) remains slightly above the medium level in sixth trial since the temperature and humidity are not too low and high respectively from the mean.

TABLE II

Correlation of meteorological factors to mortality of Oryctes *grubs*

Replication	Temperature °C. (Average maximum)	Relative humidity (Average maximum)	Percentage mortality on 10th day (corrected)
1	30·9 L	95·5 H	67·5 H
2	30·4 L	96·7 H	75·7 H
3	31·7 L	95·9 H	68·4 H
4	32·3 H	92·6 L	47·3 L
5	34·0 H	91·5 L	31·5 L
6	31·6 L	95·4 H	56·2 M/H
7	30·0 L	97·5 H	80·5 H
Mean	32·0	94·5	56·0

H = High M = Medium L = Low.

Experiment 2.—Besides the environmental factors, the quantity of inoculum appears to be equally important in determining the severity of disease. Even when the climatic conditions are favourable for disease incidence the rate of mortality is to a large measure dependant upon the inoculum potential. 50 to 78 per cent mortality was obtained on 10th day in treatment with higher doses of inoculum whereas only five to eleven per cent was obtained in lower inoculum. Mortality reached only upto 35 to 50 per cent even on 50th day where the quantity of inoculum was low. Even when the humidity and temperature are favourable for the fungus infection in first and second replications of the second experiment, mortality reached only about 50 per cent even on 15th day in the treatment with lower inoculum, whereas, 100 per cent mortality

was obtained in the higher inoculum treated on the same day (Table III). Complete mortality could be obtained only on 19th to 21st day in the treatment of lower doses of inoculum. Due to adverse effect of temperature and humidity to fungus infection in third replication of the second experiment the duration of time to obtain 100 per cent mortality also extends.

TABLE III

Percentage mortality (corrected) of Oryctes *grubs at different doses of inoculum.* Metarrhizium anisopliae *and weather data during the period of observation*

Replications	Days after treatment	Doses inoculum						Weather data	
		1 g	0·5 g	0·25 g	0·125 g	0·0625 g	0·03125 g	Temperature °C. average maximum	Humidity average maximum
1	Day							31·3	94
	3rd	10·0	10·0	0·0	0·0	0·0	0·0		
	7th	21·0	21·0	15·8	10·5	5·3	5·3		
	10th	72·2	61·1	44·4	27·8	11·1	5·5		
	13th	94·1	88·2	70·6	53·7	35·3	23·5		
	15th	100·0	93·8	81·3	62·8	50·0	43·8		
	17th	..	100·0	93·3	86·7	60·0	53·3		
	18th	..	..	100·0	100·0	86·7	80·0		
	19th	..	..	..	..	100·0	93·0		
	20th	..	..	..	..	..	100·0		
2	3rd	10·0	5·0	5·0	0·0	0·0	0·0	30·2	96
	7th	26·3	21·0	10·5	10·5	5·3	5·3		
	10th	77·8	66·7	38·9	27·8	16·7	11·1		
	13th	88·2	82·4	64·7	41·2	23·5	17·6		
	15th	100·0	100·0	93·8	68·7	56·2	50·0		
	17th	..	..	100·0	93·8	81·2	68·7		
	18th	..	..	..	100·0	93·3	86·7		
	19th	..	..	..	..	100·0	100·0		
3	3rd	5·0	5·0	0·0	0·3	0·0	0·0	32·5	92
	7th	15·8	15·8	10·5	5·3	5·3	1·3		
	10th	50·0	44·4	33·3	16·7	11·1	5·3		
	13th	83·3	72·5	55·5	38·9	22·2	16·7		
	15th	94·1	88·2	76·5	58·8	47·0	35·3		
	17th	100·0	100·0	87·5	75·0	68·5	56·3		
	18th	..	..	93·8	87·5	81·3	75·0		
	19th	..	..	100·0	93·3	86·7	84·7		
	20th	..	..	..	100·0	100·0	93·0		
	21st	..	..	..	..	..	100·0		

From the various replications of the first experiment, it is seen that humidity and temperature have great influence on the rate of mortality of the grubs. This is in accordance with the observation made by Nirula (1958) in the field that the disease is more prevalent during south-west monsoon period. 15 to 20 per cent mortality occurs in the control series on 10th day in the various trials. Thus, it is to be interpreted that the influence of weather alone is not the determining factor on the mortality of grub population. On the other hand, inoculum potential also appears to play an important role. The influence of weather factors appears to have direct effect in increasing the activity of the pathogen rather than predisposition of the grubs to the infection. In other words, the favourable weather conditions directly boost up the pathogenicity of *M. anisopliae*. The fact that the inoculum potential is the determining factor in the rate of spread of disease is indicated by the data. The data is suggestive of the fact that under favourable weather conditions, *M. anisopliae* can build up its potentiality and take the role of a virulent pathogen and cause epidemics particularly in the larval population. The results of these trials indicate the possibility of building up the population of this fungus in *Oryctes*-infested coconut gardens as a measure of biological control in the immature stages of the pest in its breeding places which would break a connecting link in the life-cycle of the pest.

SUMMARY

Favourable weather conditions (low temperature and high humidity) and higher quantity of inoculum potential have great influence in increasing the activity of the pathogen, *Metarrhizium anisopliae*, rather than predisposition of the grub of *Oryctes rhinoceros* to the infection.

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