Biology of *Margarodes vredendalensis* De Klerk (Coccoidea: Margarodiae) in South Africa*

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ABSTRACT

Various aspects of the biology of *Margarodes vredendalensis* De Klerk, a vine infesting species, were studied. Under laboratory conditions adult females emerged during January and February, and only 10–16% of the cysts developed into females annually. Although cysts were detached from their host plant, females emerged during four successive years from the same population. Adult females did not migrate to the soil surface for mating, and reproduced parthenogenetically. The average oviposition period of females was 18 days, and their average lifespan 40 days. An average of 507 eggs per female were oviposited, the highest number being produced during the first 9 days. The effects of temperature, relative humidity and soil moisture on oviposition, as well as on incubation of the eggs, are reported on.

Cysts in the field were found to a depth of 1,2 m in the soil, the highest number occurring at a depth of 46–60 cm. The vertical distribution of cysts was directly related to the vertical distribution of roots. Significant negative correlations were also found between the vertical distribution of cysts and soil moisture, as well as the percentage clay of the soil.

INTRODUCTION

Numerous *Margarodes* species occur almost throughout the world on a wide range of host plants. However, vine infesting species have only been reported from North and South America and from South Africa. *Margarodes vitis* (Phillippi) is one of the three American species found on vines, and severe damage occurs especially in Chile, where approximately 600 ha of vineyards are infested (Faure & Pinto, 1959). According to Brain (1915) *Margarodes capensis* Giard and *Margarodes greeni* Brain were found on vine roots in South Africa. A closely related species, *Sphaeraspis prieskaensis* Jakubski was also found on vine roots in South Africa (Jakubski, 1965).

According to De Klerk (1975), Margarodes is an increasingly serious pest in the Orange River (Northern Cape) and Olifants River (North Western Cape Province) irrigation areas where numerous vineyards are infested, resulting in vines dying in patches. The problem has also become very serious in the Western Cape Province, especially in the Malmesbury area where approximately 86 ha are infested, and several vineyards have been completely destroyed.

Margarodes attacks the vine roots, and the above-ground symptoms of infested vines correspond largely to those of phylloxera infested vines. The first symptom is a gradual decline in vitality which becomes more severe in time, the shoots becoming shorter and thinner, with smaller leaves. Later, one or more arms of the vine die and, finally, the whole vine. The duration of the process varies greatly. Damage usually starts in patches which gradually become larger, probably due to slow migration of larvae and adult females in the soil. In the event of a severe and uniform infestation the whole vineyard shows a decline in vitality. No characteristic galls or other symptoms are found on the roots as is the case with phylloxera.

At present no pesticide is registered for the control of Margarodes in vineyards, and no rootstock resistant to this pest is known. According to De Klerk (1975), Margarodes attacks and destroys three of South Africa's best phylloxera resistant rootstocks, viz. 99 Richter, 101–14 Mgt and Vitis rupestris (var. du Lot). It would, therefore, seem that an infestation is permanent and that infected land may become unsuitable for economic vineyard cultivation. This is particularly serious where practically a whole farm is infested with Margarodes.

Except for a brief description of the life-history of *Sphaeraspis prieskaensis* Jakubski by Du Toit (1975), virtually nothing is known of the biology and ecology of South African Margarodidae. Consequently, an extensive study of the biology of *M. vredendalensis* De Klerk was made under laboratory and field conditions, the results of which are presented in this paper. This economically important vine infesting species occurs in the Olifants River irrigation area in the North Western Cape Province.

MATERIAL AND METHODS

Laboratory Studies

Emergence of adult females from cysts: To obtain cysts for laboratory observations, soil samples were taken at different vines in a heavily infested vineyard on a farm near Vredendal in the Olifants River irrigation area. In the laboratory the soil was washed with water through a sequence of sieves with apertures of 2,8, 2,0, and 1,0 mm respectively. The cysts obtained in each sieve were re-

moved, and those with an emergence orifice (empty cysts) discarded. The remaining cysts were divided into live and dead cysts (live cysts sink; dead cysts float in water). Live cysts of various sizes were placed on moist filter paper in plastic petri dishes (8,5 cm in diameter and 1,5 cm deep) with punctured lids. These dishes were kept in the laboratory at room temperature, unless stated otherwise. Observations were made daily, and each female was removed on emergence.

Oviposition: On emergence during January and February, 29 females were placed individually on moist filter paper in petri dishes and inspected daily. The females were kept in the laboratory at temperatures ranging from 22 °C to 30 °C. After commencement of oviposition each female was removed to another dish daily and the number of eggs counted.

To determine the effect of temperature at a constant relative humidity on oviposition, six females were placed individually in petri dishes in a desiccator. Females were used directly after emergence from cysts, and as their size could possibly have an influence on the number of eggs produced, only females with approximately the same size were selected for all treatments. One desiccator each was kept at 10 °C, 25 °C, 30 °C and 40 °C. The relative humidity in each desiccator was kept constant with a saturated solution of sodium chloride. The relative humidity in the desiccators varied from 75,0 to 76,5%, depending on the specific temperature (Winston & Bates, 1960). After a period of one month the dishes were removed from the desiccators and the total number of eggs per female determined. Because the eggs were covered with wax threads, they were difficult to observe. To facilitate counting, the wax was dissolved with xylene.

Procedures to determine the effect of temperature at a constant soil moisture content on oviposition were as follows. Small glass tubes (2 cm in diameter and 6 cm deep) were each filled with 25 g air dried soil, to which were added 3 mℓ water. Five tubes with one female each were kept at 10 °C, 25 °C, 30 °C and 40 °C respectively. The original mass of each tube was kept constant by replacing evaporated water every second or third day. After one month the females were removed, and the total number of eggs per female counted. The percentage moisture content of the soil, determined on a dry-mass basis as described by Gardner (1965), was 14%.

The effect of relative humidity on oviposition was tested at two different percentages at 25 °C. Relative humidities of 32,5% and 75,5% were obtained by saturated solutions of magnesium chloride and sodium chloride respectively (Winston & Bates, 1960). Other procedures were similar to those applied to determine the effect of temperature on oviposition at a constant relative humidity.

Procedures to determine the effect of soil moisture were similar to those applied to determine the effect of temperature at a constant soil moisture. The soil in four tubes was kept dry (2% soil moisture), while increasing moisture content was obtained by adding 1, 2, 3, 5 and 7 mℓ water respectively to four groups of tubes each. The tubes were kept at a constant temperature of 25 °C. The field capacity and saturation percentage of the soil were 20 and 32% respectively.

Incubation of eggs: To determine the effect of relative humidity on incubation, five embryonic watch glasses containing 50 one day old eggs each were placed in a desiccator. Three desiccators, with constant relative humidities of 32,5, 75,5 and 100% each, were kept at a constant temperature of 25 °C. A relative humidity of 100% was obtained with pure water in the desiccator, while that of the other two humidities were obtained as described under oviposition.

The containers were inspected after certain periods, as shown in Table 6 under the results, and at each inspection date the number of larvae and live eggs were counted. The intervals between inspections were kept relatively long in order to keep disturbance of the eggs and conditions in the desiccators as low as possible. Larvae were removed during each observation.

The effect of temperature on the incubation of eggs was tested at 10 °C, 25 °C, 30 ° C and 40 °C at a constant relative humidity of 100%. The same methods were applied as those described for the determination of the effect of relative humidty.

To determine the effect of soil moisture on incubation, fifteen eggs were placed in an embryonic watch glass and covered with 6 g air-dried soil. The moisture content of the soil was determined on a dry-mass basis as described by Gardner (1965). The soil in four of the containers was kept dry (2% soil moisture), while increasing moisture content was obtained by adding 0,5; 1,0; 1,5 and 2,0 mℓ water respectively to four groups of containers each. The field capacity and saturation percentage of the soil were 20% and 32% respectively. Each container was weighed and kept at a constant temperature of 25 °C. The original mass of each container was kept constant by replacing evaporated water every second or third day. At each inspection the containers were covered with a small glass plate and turned upside down under the microscope. The number of dead as well as live eggs in each container were counted. On hatching from eggs, larvae burrowed into the soil and could not be observed. Their number was determined by subtracting the total number of live and dead eggs from the original number of eggs.

The same methods were applied to determine the influence of different temperatures at a constant percentage soil moisture of 27%. Five containers with 15 eggs each were used.

Field Conditions

Vertical distribution in the soil: To determine the vertical distribution of *M. vredendalensis* in the soil, observations were made in a seven year old infested vineyard on a farm near Vredendal in the Olifants River irrigation area. The vineyard was irrigated every 16 days by flood irrigation, and observations were made 6 days after a normal irrigation. Regardless of vigour, three vines were chosen at random during January 1976. A ditch, 45 cm wide, 1,5 m long and 1,2 m deep, was dug at right angles to the vine rows and 30 cm away from each of the three vines.

Layers of soil, each 15 cm thick, were removed from the end of the ditch closest to the vine to a depth of 1,2 m. Of the total amount of soil from each layer, small quantities were taken at random to obtain a sample of three or four litres. In the laboratory one litre of soil was taken at random from this sample and washed with water through three sieves with apertures of 2,8, 2,0 and 1,0 mm respectively. The cysts in each sieve were removed and those with an emergence orifice (empty cysts) were separated

from the others and counted. The total number of live and dead cysts on each sieve was then counted, and the percentage mortality calculated. The adult females in each sieve were removed and counted.

During excavations in the field, a small quantity of soil from each layer was placed in an airtight container. The percentage moisture content of the soil was determined on a dry mass basis as described by Gardner (1965). At each of the three vines a soil sample was also taken from each layer down to 1,2 m for soil analysis. The soil from each layer was bulked, and particle size analysis done by the Soil Science section of the Oenological and Viticultural Research Institute according to the hydrometer method of Day (1956). The percentage coarse sand (2,0–0,5 mm), medium sand (0,5–0,21 mm), fine sand (0,21–0,02 mm), silt (0,02–0,002 mm) and clay (< 0,002 mm) of each sample were determined.

RESULTS AND DISCUSSION

Laboratory Studies

Emergence of adult females from cysts

Time of emergence: From a total number of 669 cysts collected during November 1974, 72 adult females emerged from 17 January to 12 February 1975. During the third week of January the number of females was very low but increased from the last week of January, reaching a peak during the first week of February. During the second and third week their numbers decreased again, and from the end of February no emergence of females was observed (Fig. 1A).

The remaining cysts were kept in the laboratory, and a total of 106 females emerged from 19 January to 11 February 1976, again reaching a peak during the first week of February (Fig. 1B).

Percentage emergence: During February 1973, live cysts were collected in the vineyard at Vredendal and observed in the laboratory. Of these only 12,5% developed into adult females during 1974. From a number of live cysts collected in the same vineyard during November 1974 and kept in the laboratory 10,8% developed into adult females during 1975. Of the remaining cysts of this group 15,8% developed into females during 1976. These results show that only a small percentage of cysts develop into females annually.

Emergence during successive years: The cysts collected during February 1973 and referred to in the previous paragraph were kept under laboratory conditions for a number of years. During January and February of 1974, 1975, 1976 and 1977 the percentages emergence of adult females from the original number of cysts were 12,5; 25,0; 12,5 and 6,3 respectively. At the end of this period the remaining cysts were opened to determine whether the nymphs were alive or not as indicated by the presence or absence of body fluid. Of the original number of cysts, 31,3% were still alive after four years.

These results clearly indicate that adult females could still emerge four years after collection of cysts from a vineyard, even if the cysts are detached from the roots and received no nourishment. If an infested vineyard is replanted within four years, the new vineyard could thus still become infested from cysts that had developed on the original planting.

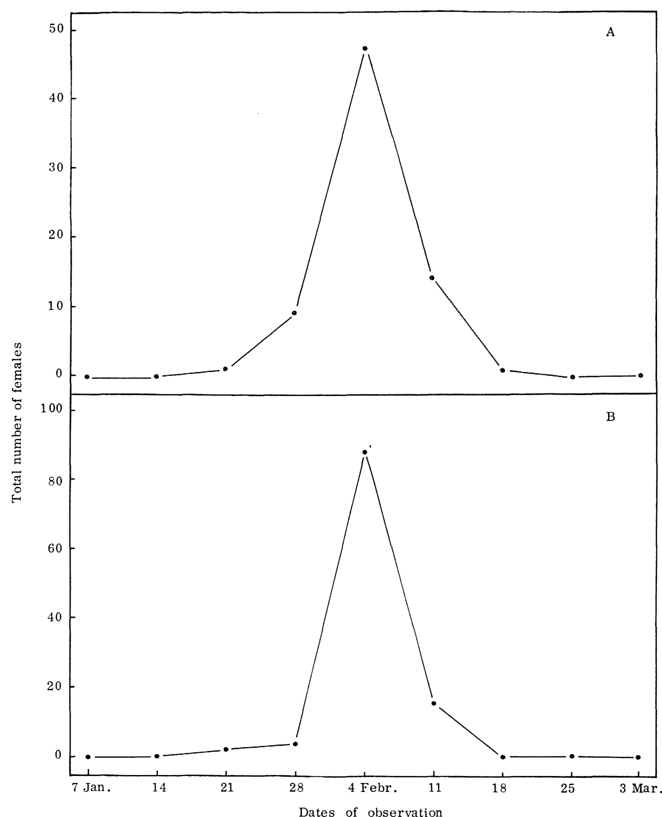


FIG. 1

Total number of adult females emerged from cysts of *M. vredendalensis* on various dates during (A) 1975 and (B) 1976 under laboratory conditions.

Oviposition and fecundity

Pre-oviposition period: Under laboratory conditions the adult females were very active during the first two to four days after emergence from cysts, moving around and trying to burrow through the filter paper placed in the petri dishes. On reaching the edge, most of the females crawled underneath the paper. After this period of activity they became sedentary, and only weak movements of legs and abdomen were noticed. During the inactive period wax threads were excreted, first on the dorsal and ventral sides of the last abdominal segment, but two to three days later on all the abdominal segments. As oviposition proceeded, these wax threads became longer and denser to cover the whole body as well as the eggs.

The average period from emergence to the first appearance of wax threads was 11 days, with a minimum and maximum of 6 and 15 days respectively. The average period of wax production before oviposition started, was 4 days with a minimum of 1 day and a maximum of 7 days. The pre-oviposition period thus lasted 15 days on average, with a minimum and maximum of 10 and 20 days respectively (Table 1).

Since it is known that some species migrate to the soil surface for mating during the pre-oviposition period (Du Toit, 1975) the migration pattern of *M. vredendalensis* after emergence from the cysts, was investigated. Glass jars, 6 cm high and 4 cm in diameter, were filled with moist soil, and one female was placed on the soil surface in each of 30 containers directly after emergence. Thirteen of these containers were kept in the laboratory, and all the females burrowed immediately into the soil, not returning

TABLE 1

Pre-oviposition period, oviposition period, longevity, total number of eggs per female and number of eggs per female per day of *Margarodes vredendalensis* under laboratory conditions

Replicates	Duration from emergence to formation of wax threads (days)	Duration from formation of wax threads to commencement of oviposition (days)	Duration from emergence to commencement of oviposition (days)	Oviposition period (days)	Duration after egg laying until death of female (days)	Longevity of adult female (days)	Total number of eggs per female	Minimum number of eggs per female per day	Average number of eggs per female per day	Maximum number of eggs per female per day
1	13	4	17	17	9	43	814	3	48	134
2	11	4	15	19	4	38	694	1	37	100
3	13	2	15	20	8	43	1 121	3	56	169
4	11	1	12	17	8	37	1 238	5	73	185
5	10	3	13	16	8	37	1 136	1	71	164
6	11	4	15	16	5	46	313	2	20	59
7	11	2	13	16	8	37	599	3	37	100
8	9	4	13	16	7	46	913	2	57	127
9	11	3	14	14	7	35	392	1	28	70
10	10	4	14	14	7	35	406	4	29	88
11	14	2	16	30	3	49	883	1	29	139
12	11	6	17	16	7	40	164	2	10	30
13	6	4	10	13	5	28	368	2	28	74
14	9	4	13	18	7	38	397	2	22	67
15	12	4	16	21	5	42	443	3	21	68
16	14	5	19	23	6	48	403	1	18	63
17	9	4	13	9	5	27	254	5	28	61
18	9	3	12	5	13	30	212	23	42	71
19	9	2	11	12	7	30	326	5	27	52
20	13	4	17	25	6	48	329	2	13	49
21	15	3	18	13	7	38	681	6	52	118
22	14	5	19	23	6	48	248	2	11	50
23	15	5	20	23	5	48	227	1	10	30
24	9	5	14	14	6	34	193	1	14	49
25	11	4	15	24	5	44	450	1	19	84
26	13	5	18	23	6	47	306	2	13	53
27	12	5	17	24	6	47	675	2	28	78
28	12	7	19	24	4	47	200	1	8	34
29	10	4	14	24	6	44	321	1	13	71
Average	11	4	15	18	6	40	507	3	30	84

to the soil surface during the next 7 days. The other 17 jars were buried in a box filled with soil, with their openings level with the soil surface and placed in direct sunlight from 08h00 to 17h00 daily. One of the females died without entering the soil, becoming brown and shrivelled after 8 hours. The rest of the females burrowed immediately into the soil, without returning to the soil surface. After 7 days the soil was removed, and all the females were found at the bottom of the jars. Only 23,5% of them were still alive. Females kept in the laboratory were also found at the bottom of the containers after 7 days, but 100% were still alive.

The results show clearly that females do not stay at or migrate to the soil surface for mating and that normal summer temperatures could cause their death in the upper 6 cm of the soil.

Oviposition period and longevity of females: As shown in Table 1 the average period of oviposition was 18 days, with a minimum and maximum of 5 and 30 days respectively. The period from the end of oviposition to the death of the females was on average 6 days, with a minimum and maximum duration of 3 and 13 days respectively. The average longevity of adult females was 40 days, with a minimum and maximum of 27 and 49 days respectively (Table 1).

Fecundity: When the female oviposits in the soil, eggs are laid in a bundle and covered with wax threads to form a compact egg sack. In an open space such as in a petri dish, however, the eggs are laid attached to one another, forming a string of eggs, covered with wax threads. Up to 139 eggs were counted in a single string. The total number of eggs per female averaged 507, with a minimum of 164 and a maximum of 1 238 (Table 1).

Rate of oviposition: The lowest oviposition per female per day varied between 1 and 23, with an average of 3. The maximum varied between 30 and 184, with an average of 84. The average oviposition per female per day varied between 8 and 73, with an average of 30 (Table 1).

The number of eggs produced per female per day was determined for 29 females, and the average of each successive day during the oviposition period is shown in Figure 2. On the first day of oviposition the average number of eggs per female was relatively high, increasing gradually to reach a peak on the fifth day. Afterwards the production of eggs decreased, and from the 25th day, no eggs were oviposited. Eighty six per cent of the total production were oviposited during the first 9 days.

Number of eggs with regard to the size of the female: The length and width of 29 adult females were measured just before commencement of oviposition, and an index value for size was obtained for each female by multiplying its length by its width. The relationship between body size and fecundity was determined by calculating a linear regression coefficient. A highly significant ($P < 0,01$) linear correlation was found. As shown in Figure 3A the total number of eggs per female is positively correlated with the size of the female.

A highly significant ($P < 0,01$) linear correlation (Fig. 3B) was also found between the body size of females and the average number of eggs per female per day.

Effect of temperature at a constant relative humidity: As shown in Table 2, a temperature of 10 °C was evidently too low for the production of eggs. However, all the females kept at this temperature were still alive after one month. They were then transferred to room temperature, and after 14 days, normal oviposition commenced.

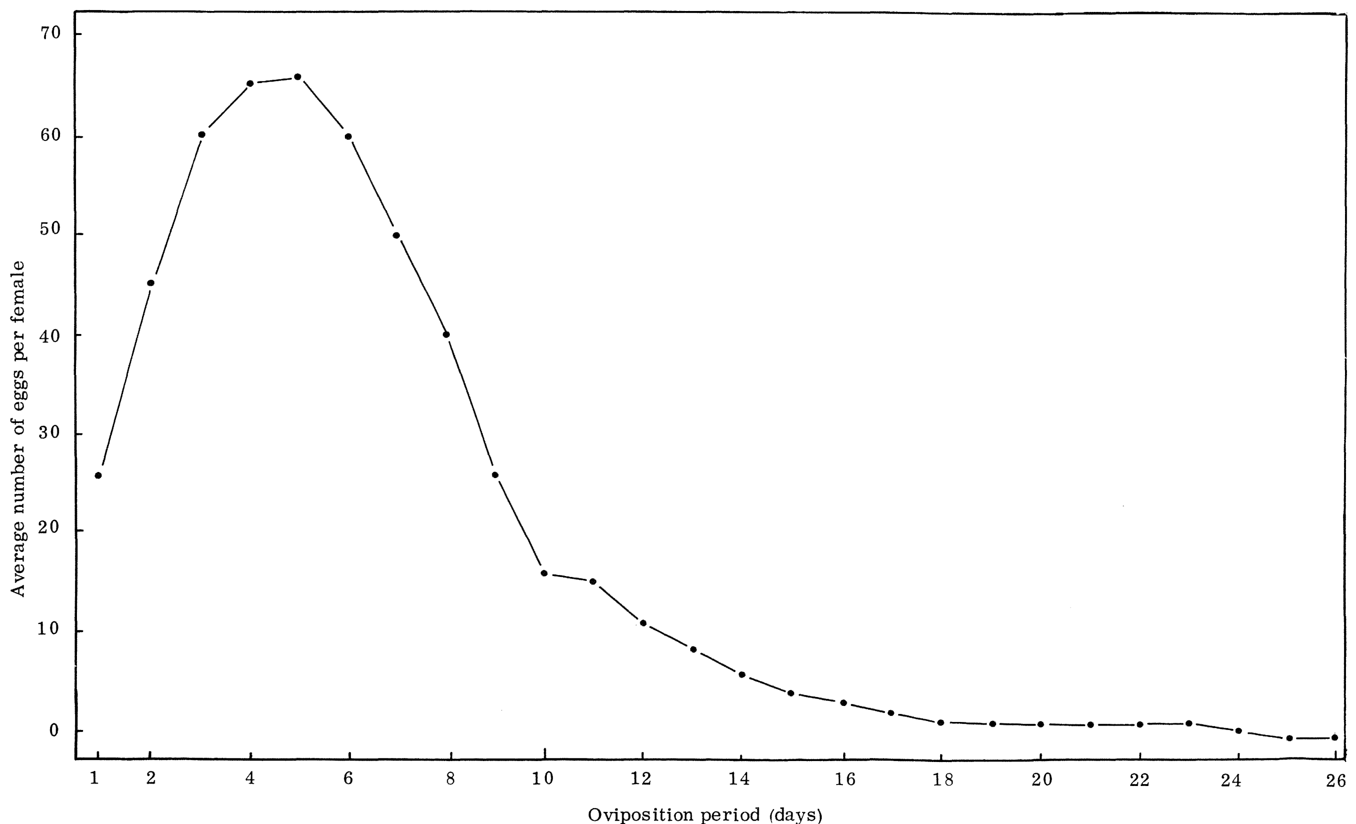


FIG. 2
Average number of eggs oviposited per female per day by *M. vredendalensis* under laboratory conditions.

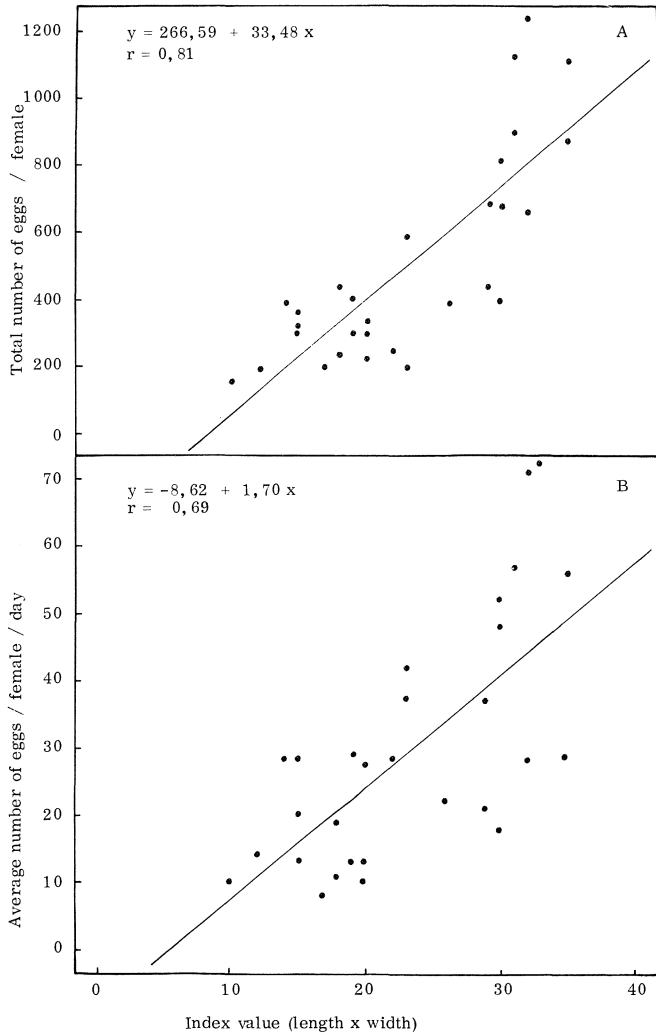


FIG. 3

Correlation between size index of adult females of *M. vredendalensis* and (A) total number of eggs oviposited per female and (B) average number of eggs oviposited per female per day.

Although females were not killed at 10 °C, egg production was inhibited. A temperature of 40 °C was too high for egg laying, and all the females were dead after one month. Eggs were produced normally by females kept at constant temperatures of 25 °C and 30 °C, and an analysis of variance showed no significant differences at the 5% level.

TABLE 2

Total number of eggs oviposited by females of *M. vredendalensis* at different temperatures and 75,5% relative humidity

Replicate	Temperature			
	10 °C	25 °C	30 °C	40 °C
1	0	185	227	0
2	0	521	120	0
3	0	369	456	0
4	0	576	700	0
5	0	580	993	0
6	0	886	1 054	0
Average	0	520	592	0

Effect of temperature at a constant soil moisture: As indicated in Table 3, temperatures of 10 °C and 40 °C were too low and too high respectively for the production of eggs. However, eggs were oviposited normally at temperatures of 25 °C and 30 °C. An analysis of variance showed no difference at the 5% level between these two treatments. These results show a similar pattern to those obtained with the same temperatures at a constant relative humidity of 75,5%.

TABLE 3

Total number of eggs oviposited by females of *M. vredendalensis* at different temperatures and 14% soil moisture content

Replicate	Temperature			
	10 °C	25 °C	30 °C	40 °C
1	0	288	216	0
2	0	—	204	0
3	0	116	—	0
4	0	382	428	0
5	0	305	335	0
Average	0	273	296	0

Influence of relative humidity: The results are shown in Table 4, which clearly indicates that oviposition took place at the different relative humidities tested. An analysis of variance, however, showed a highly significant ($P < 0,01$) difference between the two treatments. A relative humidity of 32,5%, therefore, had a detrimental effect on the production of eggs.

TABLE 4

Total number of eggs oviposited by females of *M. vredendalensis* at different relative humidities and 25 °C

Replicate	Relative humidity	
	32,5%	75,5%
1	101	185
2	164	521
3	106	369
4	149	576
5	107	580
6	315	886
Average	157	520

Effect of soil moisture: The total number of eggs per female at each moisture level is shown in Table 5.

TABLE 5

Total number of eggs oviposited by females of *M. vredendalensis* at different percentages soil moisture and 25 °C

Replicate	Percentage soil moisture					
	2	6	10	14	22	30
1	0	839	539	672	1 114	815
2	0	376	721	322	308	479
3	0	445	464	377	523	325
4	60	426	867	563	396	223
Average	15	522	648	484	585	460

The results show clearly that oviposition occurred over a wide range of soil moisture conditions. Eggs were laid in dry soil with a moisture content as low as 2% as well as in wet soil with a moisture content of 30%. Oviposition was not inhibited even when the percentage soil was very near the saturation percentage.

An analysis of variance of these results showed that significant (P < 0,05) differences occurred between the treatments. Tuckey's test of D-values indicated that the number of eggs produced at 2% soil moisture was lower than those of the other treatments. No other differences could be detected. Oviposition is thus not totally inhibited by low soil moisture but the number of eggs produced at 2% soil moisture could be considerably lower than that at percentages varying from 6% to 30%. However, this finding will have to be verified at a later stage because the technique used for obtaining different soil moisture contents may have been unable to ensure uniform soil moisture conditions in all cases.

Incubation of eggs

Effect of relative humidity: Eggs did not hatch at relative humidities of 32,5% and 75,5%, which were evidently too low (Table 6). At a relative humidity of 100% a number of eggs hatched and the incubation period was longer than 34 but shorter than 48 days.

It can be calculated from the results in Table 6 that only 28% and 8% of the original number of eggs were still alive after 34 days at 32,5% and 75,5% respectively. These relative humidities were possibly too low for the normal development of eggs to larvae. At 100% humidity, 85% of the eggs were alive after 34 days, while 55% were still alive after 41 days. This indicates that the larvae develop normally in the eggs at a relative humidity of 100%.

Effect of temperature at a constant relative humidity: As indicated in Table 7, eggs did not hatch at temperatures of 10 °C and 40 °C, which were evidently too low and too high respectively. A number of eggs, however, hatched at 30 °C and 25 °C. The results indicate that the incubation period is possibly shorter at 30 °C than at 25 °C and was on average more than 20 but shorter than 48 days.

The results also show that all the eggs at 40 °C were dead after 20 days, indicating that this temperature was too high for the normal development of eggs to larvae. At 10 °C, 79% of the original number of eggs were alive after 34 days and 25% lived for 62 days. The eggs were eventually all dead after 76 days. A temperature of 10 °C was therefore not fatal to the eggs, but it was evidently too low for their normal development. As shown in Table 7, the total number of live eggs at 30 °C after 34 days as well as after 41 days was significantly lower than that for the same periods at 25 °C.

Effect of soil moisture: As shown in Table 8, eggs hatched at 10% to 35% soil moisture but not at 2%. An analysis of variance of the results showed that significant differences occurred between treatments at the 5% level. Tuckey's test of D-values indicated that the number of larvae that hatched at 35% soil moisture was significantly higher than at the other percentages of soil moisture tested. No other statistical differences could be detected.

Table 8 further shows that all the eggs were dead after 34 days at a soil moisture content of 2%, indicating that this was too low for the normal development of eggs to larvae. An analysis of variance of the numbers of dead eggs after 41 days at the various soil moistures, showed that significant (P < 0,05) differences occurred between the treatments. Tuckey's test of D-values indicated that the number of dead eggs at 35% moisture content was signi-

TABLE 6

Total number of eggs hatched (A) and total number of live and ecloded eggs (B) of *M. vredendalensis* at certain periods after oviposition at different relative humidities and 25 °C (250 eggs per treatment)

Percentage humidity	Number of eggs											
	After 20 days		After 34 days		After 41 days		After 48 days		After 55 days		After 62 days	
	A	B	A	B	A	B	A	B	A	B	A	B
32,5	0	235	0	70	0	44	0	18	0	0	0	0
75,5	0	181	0	20	0	0	0	0	0	0	0	0
100	0	236	0	213	95	138	2	17	0	9	0	0

TABLE 7

Total number of eggs hatched (A) and total number of live and ecloded eggs (B) of *M. vredendalensis* at certain periods after oviposition at different temperatures and 100% relative humidity (250 eggs per treatment)

Temperature °C	Number of eggs											
	After 20 days		After 34 days		After 41 days		After 48 days		After 55 days		After 62 days	
	A	B	A	B	A	B	A	B	A	B	A	B
40	0	0	0	0	0	0	0	0	0	0	0	0
30	0	222	79	150	2	41	0	22	0	5	0	0
25	0	236	0	213	95	138	2	17	0	9	0	0
10	0	239	0	197	0	174	0	120	0	86	0	62

TABLE 8

Total number of eggs hatched (A) and total number of dead eggs (B) of *M. vredendalensis* at certain periods after oviposition at different percentages soil moisture and 25 °C (60 eggs per treatment)

Percentage soil moisture	Number of eggs									
	After 20 days		After 34 days		After 41 days		After 48 days		After 55 days	
	A	B	A	B	A	B	A	B	A	B
2	0	38	0	60	0	—	0	—	0	—
10	0	33	0	44	2	53	0	58	0	—
19	0	44	0	49	1	57	0	59	0	—
27	0	33	0	47	6	50	0	52	0	54
35	0	15	0	25	19	30	1	40	0	—

TABLE 9

Total number of eggs hatched (A) and total number of dead eggs (B) of *M. vredendalensis* at certain periods after oviposition at different temperatures and 27% soil moisture content (75 eggs per treatment)

Temperature °C	Number of eggs									
	After 20 days		After 34 days		After 41 days		After 48 days		After 55 days	
	A	B	A	B	A	B	A	B	A	B
10	0	36	0	55	0	60	0	63	0	65
25	0	36	2	63	3	68	0	70	0	—
30	0	53	0	72	0	75	0	—	0	—
40	0	75	0	—	0	—	0	—	0	—

ficantly lower than at the other treatments. No other statistical differences could be detected. However, as explained under the effect of soil moisture on oviposition, these results have to be verified at a later stage.

Effect of temperature at a constant soil moisture: As indicated in Table 9, larvae emerged only at 25 °C, and the incubation period was more than 20 but shorter than 48 days. A temperature of 10 °C was possibly too low for the hatching of eggs, while 30 °C and 40 °C were too high.

It can be calculated from the results in Table 9 that 100% of the eggs were dead at 40 °C after 20 days, while 96% were dead at 30 °C after 34 days. These temperatures were evidently too high for the normal development of eggs to larvae. Although 75% of the eggs were dead after 34 days at 10 °C, 100% mortality was reached only at a period longer than 55 days. Eggs were therefore not killed at a temperature of 10 °C but it was evidently too low for their development. Eggs hatched only at a temperature of 25 °C, indicating that at this temperature the eggs can develop normally.

In the previous results concerning egg incubation, the hatching percentage was often very low, even under apparently favourable conditions. This was probably partly due to the fact that the eggs were removed manually and were often disturbed during observations.

As mentioned under Material and Methods, intervals between inspections were kept relatively long in order to keep disturbance of eggs and conditions in the desiccators as low as possible, and the exact incubation period could therefore not be determined. This period was, however, more accurately determined in a succeeding experiment. Four hundred one day old eggs were placed in embryonic watch glasses and kept at 25 °C in desiccators with a constant relative humidity of 100%. After a period of 20 days the eggs were inspected every second or third day, and emerging larvae were counted and removed. Of the total number of eggs 77,0% hatched, the rest being dead after 58 days. The incubation period was between 36 and 45 days. Of the total number of eggs hatched 92,7% hatched between the 37th and 42nd day after oviposition.

FIELD STUDIES

Vertical distribution in the soil

Cysts: The average number of cysts of all sizes per vine at the various depths, is shown in Figure 4. As can be seen from the graph the number of cysts was at a very low level in the upper 15 cm of soil, increasing gradually to reach a peak at a depth of 46–60 cm. In the following layers it decreased again gradually and at a depth of 91–120 cm, cysts occurred only in limited numbers.

As shown in Figure 5A, the distribution in depth of cysts larger than 2,8 mm in diameter was almost the same as that of cysts of all sizes together, with a peak at a depth of 46–60 cm. The total number of cysts of the size range 2,8–2,0 mm was very low in the first 30 cm of soil, increasing sharply, to reach a peak at a depth of 46–60 cm. In the following layers their numbers decreased gradually, and at a depth of 91–120 cm cysts occurred only in limited numbers (Fig. 5B). The vertical distribution of cysts of the size range 2,0–1,0 mm followed almost the same pattern as that of cysts in the size range 2,8–2,0 mm, with a peak at a depth of 61–75 cm (Fig. 5C). Evidently little differences occur in the vertical distribution of cysts of various sizes.

The average root mass per vine at each of the eight layers of soil down to a depth of 1,2 m was determined. The results are shown in Figure 6. In the upper 15 cm of soil the root mass was very low, increasing gradually to reach a peak at a depth of 46–60 cm. In the following

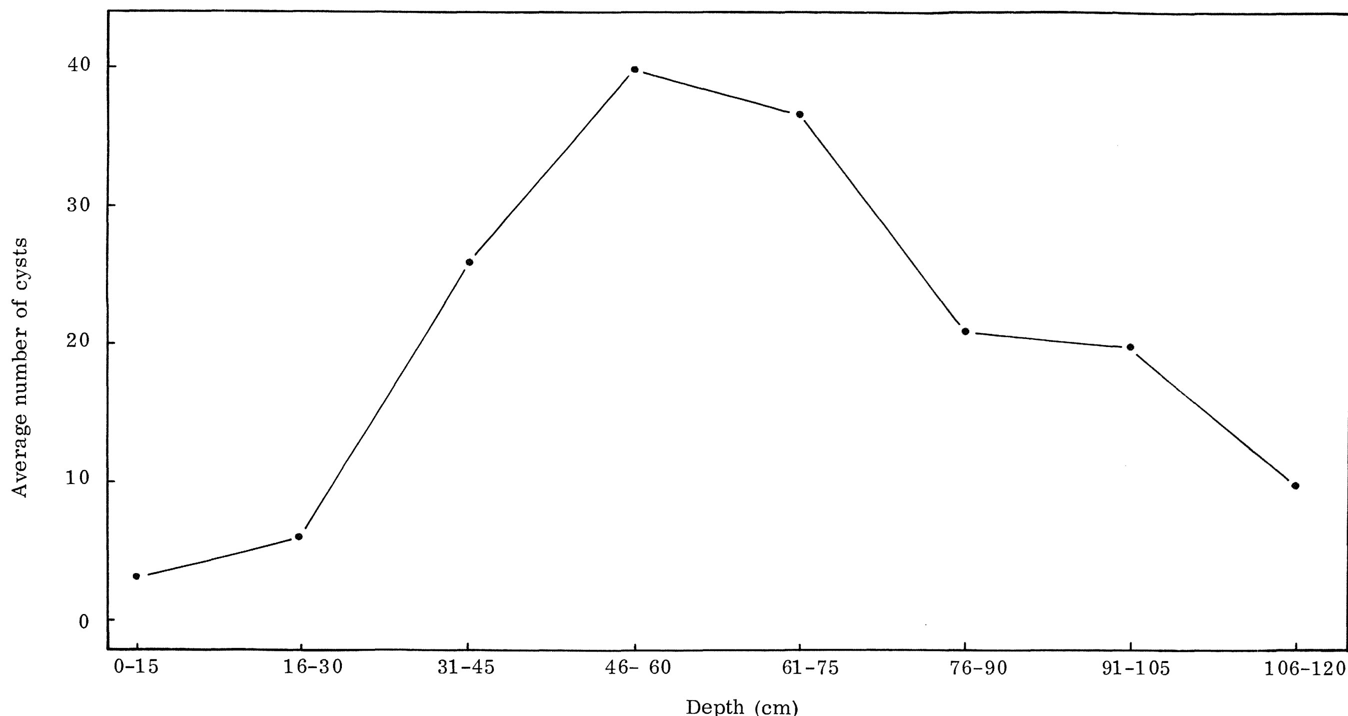


FIG. 4
Average number of cysts (all sizes) per vine of *M. vredendalensis* at various soil depths sampled at Vredendal during January 1976.

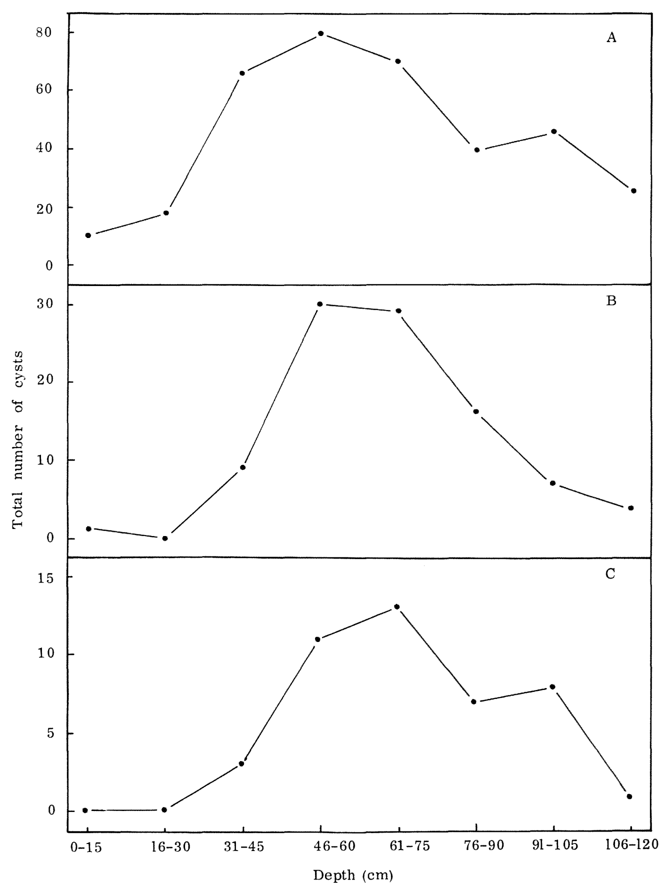


FIG. 5
Total number of cysts of *M. vredendalensis* at various soil depths sampled at Vredendal during January 1976 (A) cysts greater than 2,8 mm (B) cysts 2,8–2,0 mm and (C) cysts 2,0–1,0 mm in diameter.

layers it decreased again gradually, and at a depth of 91–120 cm the root mass was very low. A comparison between Figure 4 and Figure 6 shows that the vertical distribution of cysts followed almost the same pattern as that of root mass with a peak at a depth of 46–60 cm. The relationship between the number of cysts and root mass was highly significant and linear (Fig. 7).

The relationship between the number of cysts and the percentage of each of the various soil fractions were analysed statistically, and the linear regression coefficient determined. No significant correlation at the 5% level was found between the number of cysts and any of the soil fractions, except for the percentage clay which showed a significant ($P < 0,05$) negative correlation ($r = -0,41$; $y = 47,23 - 1,88x$).

A linear regression coefficient was also determined between the number of cysts and the percentage soil moisture for each of the various depths, and a significant ($P < 0,05$) correlation was found ($r = -0,4805$). The negative correlation coefficient indicates that the number of cysts decreases as the percentage soil moisture increases and vice versa ($y = 63,19 - 6,08x$).

Although no causal relationship could be determined, root mass, percentage clay content of the soil and soil moisture could be direct factors affecting the vertical distribution of cysts, or the percentage clay and soil moisture could be indirect factors by affecting the vertical distribution of roots.

The percentage dead cysts of each of the three size ranges and at each of the various depths was determined. As the figures of the different size ranges were almost similar, cysts of all sizes were taken together, and their mortality at different depths compared. An analysis of variance showed no differences at the 5% level, indicating that the normal mortality of cysts is at the same level from 0–1,2 m in the soil. The average mortality of cysts was 18,81%.

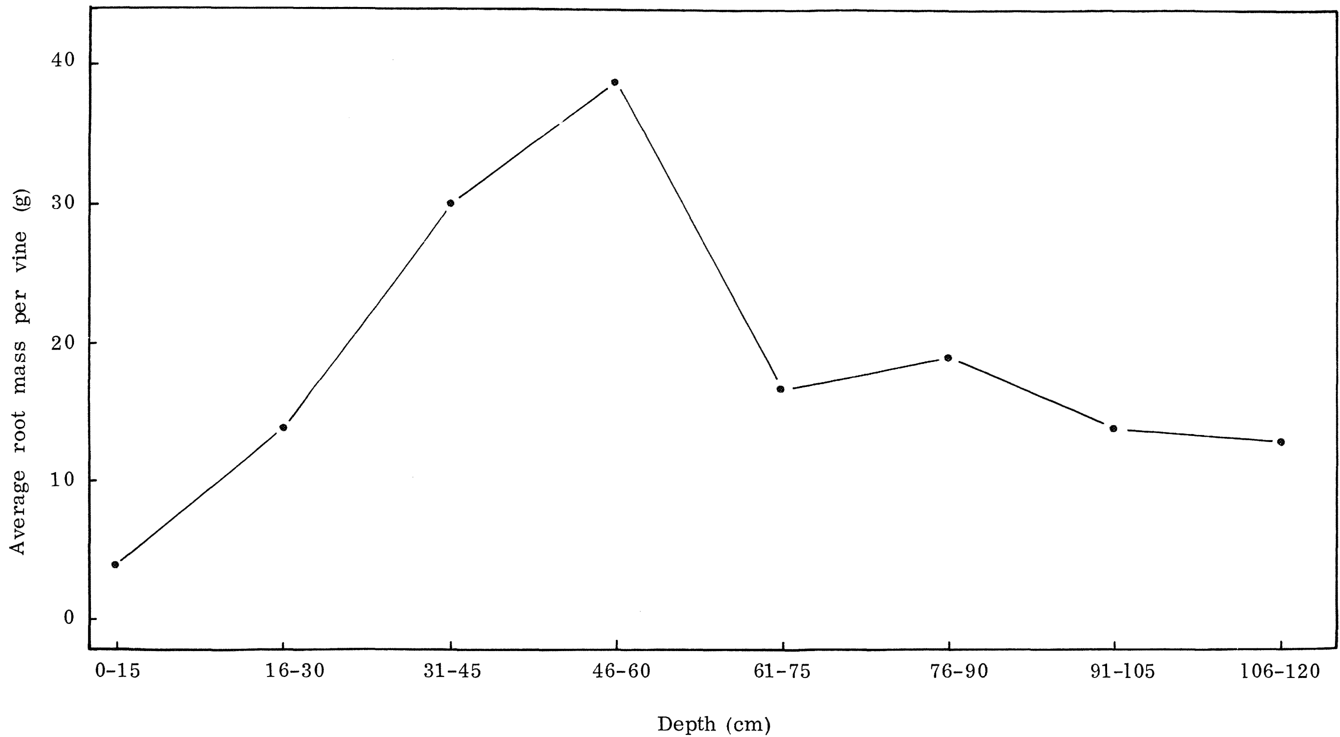


FIG. 6
Average root mass per vine at various depths to 1,2 m sampled at Vredendal during January 1976.

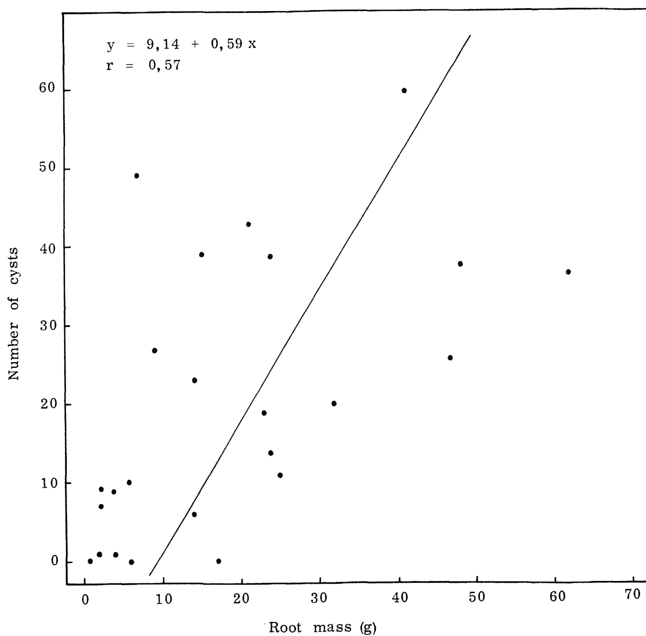
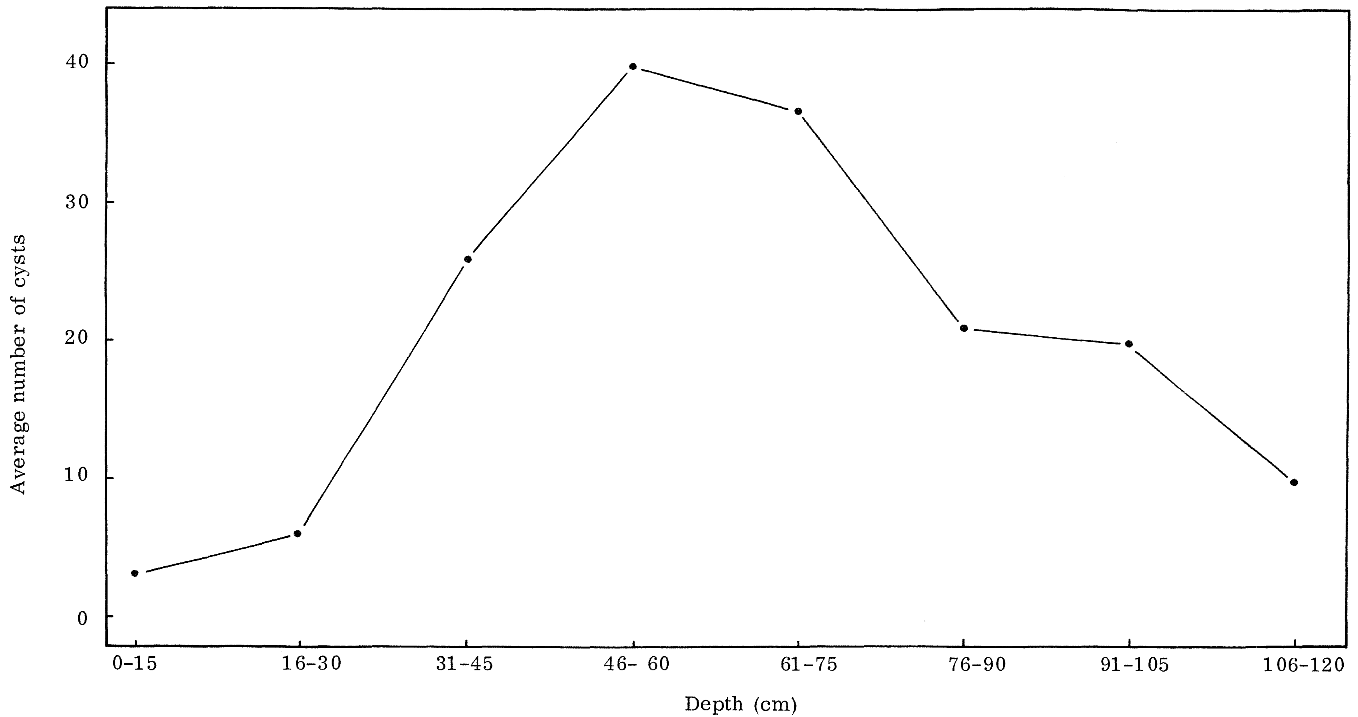


FIG. 7
Correlation between number of cysts of *M. vredendalensis* and root mass.

Empty cysts: The total number of empty cysts (all sizes) at the various depths in the soil is shown in Figure 8. Empty cysts occurred in very low numbers in the upper 15 cm of soil, increasing gradually to a peak at a depth of 46–60 cm. In the deeper layers, to a depth of 1,2 m, their numbers decreased again. A comparison between Figure 4 and Figure 8 indicates that the vertical distribution of live cysts and that of empty cysts followed almost the same pattern with a peak at a depth of 46–60 cm.

Adult females: The total number of adult females obtained at the three vines investigated and at the various depths in the soil, is shown in Figure 9. Their numbers were low in the first 45 cm of soil, increasing to a peak at a depth of 61–75 cm. In the deeper layers their numbers decreased again, and at a depth of 106–120 cm it was at a low level.

The number of females and the percentage of each of the various soil fractions were analysed statistically, and a linear regression coefficient determined. No significant correlation at the 5% level was found between the number of females and any of the soil fractions. No significant ($P < 0,05$) correlation was found between the number of females and the distribution of roots according to root mass, soil moisture or even the number of cysts and the number of empty cysts.



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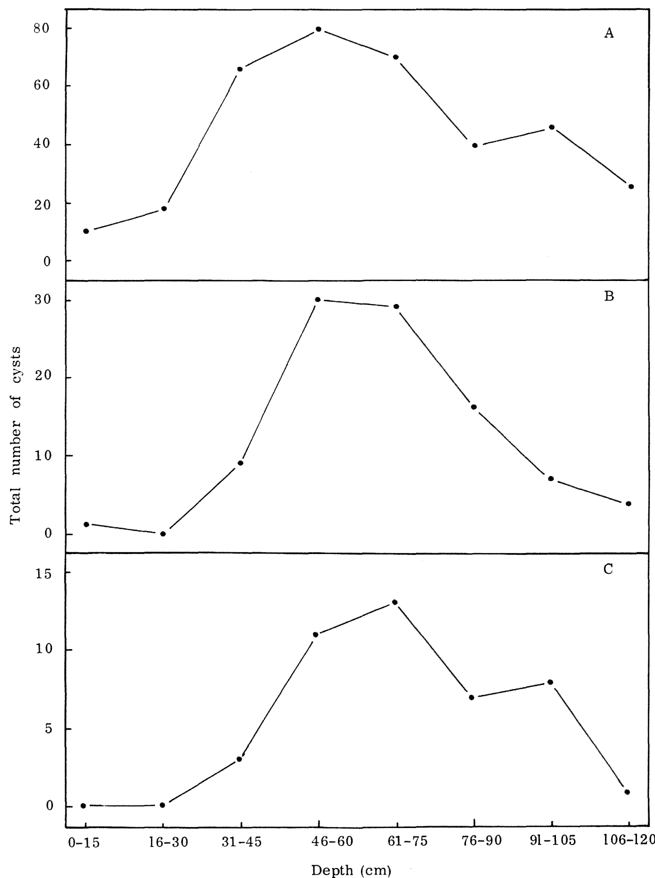

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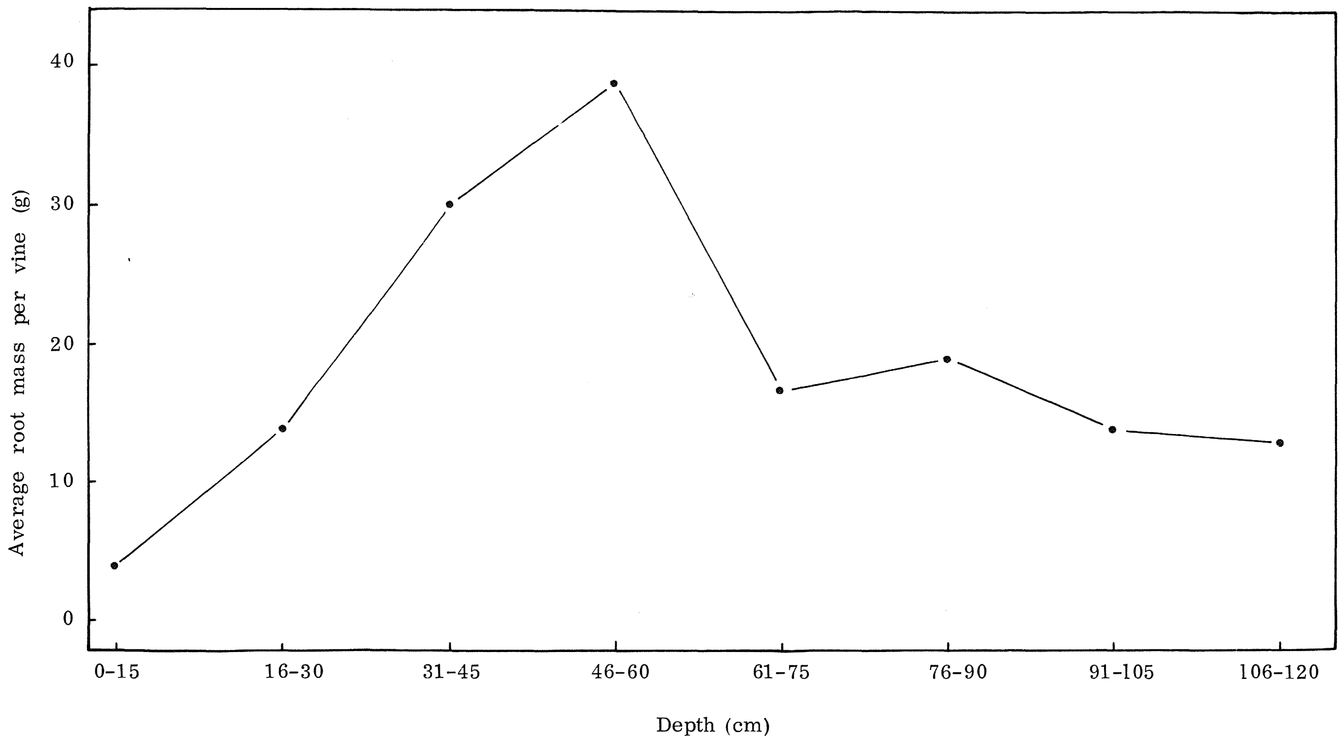
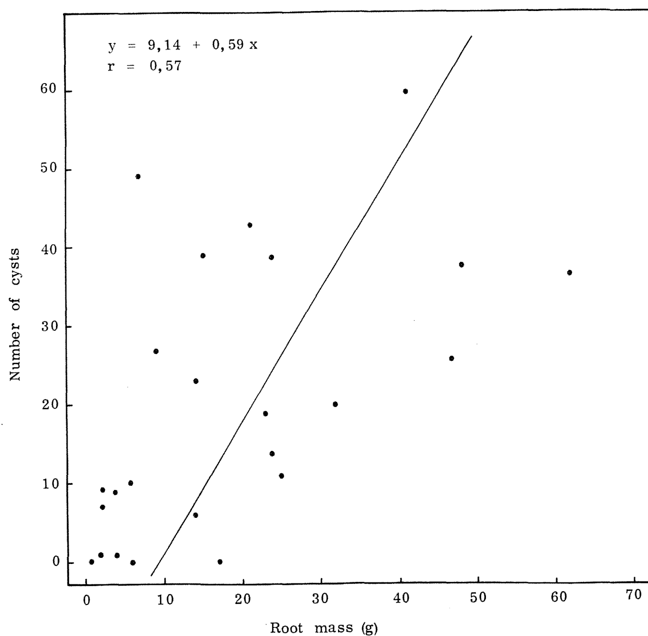


FIG. 6
Average root mass per vine at various depths to 1,2 m sampled at Vredendal during January 1976.



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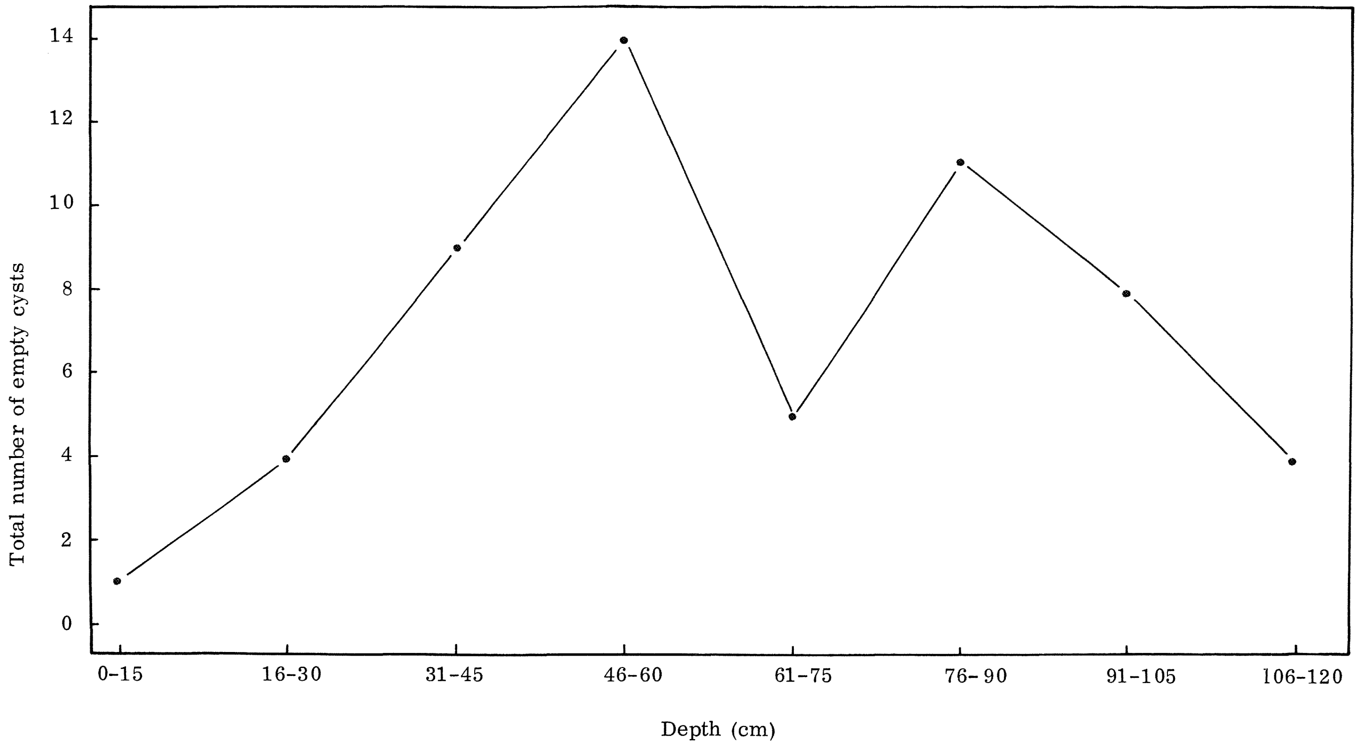

FIG. 8
Total number of empty cysts per vine of *M. vredendalensis* at various soil depths sampled at Vredendal during January 1976.

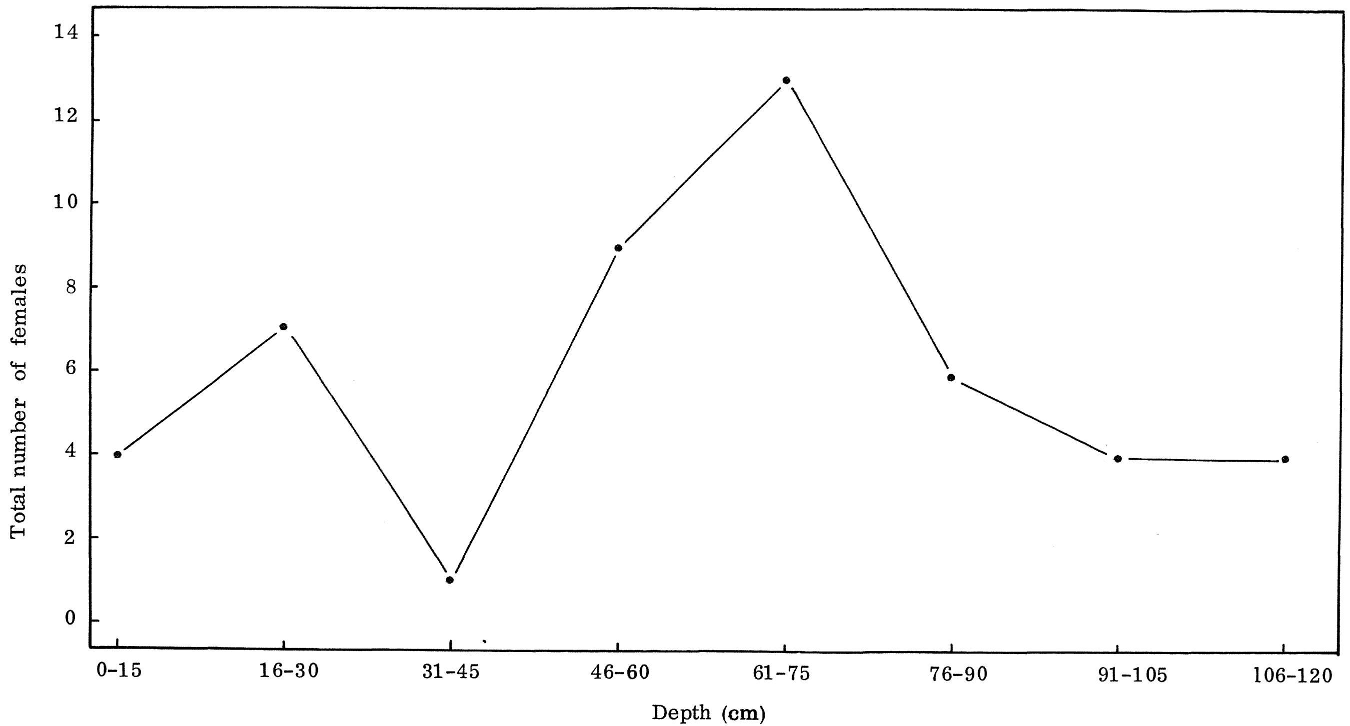

FIG. 9
Total number of adult females per vine of *M. vredendalensis* at various soil depths sampled at Vredendal during January 1976.

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