STUDIES ON THE STERILITY MOSAIC DISEASE OF PIGEON PEA

I. Transmission of the Disease

By P. Narayanasamy and K. Ramakrishnan

(*Plant Pathology Division, Agricultural College and Research Institute, Coimbatore*-3)

Received January 21, 1965

(Communicated by Professor T. S. Sadasivan, F.A.SC.)

The viral nature of the sterility mosaic disease of pigeon pea was first established by Capoor (1952) who claimed that he obtained a small percentage of infection by sap transmission. The transmission of the disease by an eriophyid mite has been reported by Seth (1962). Kandaswamy and Ramakrishnan (1960) reported that the disease occurred in an epiphytotic form at Coimbatore and they observed that 176 varieties of pigeon pea were affected by this disease. The possibility of this disease being transmitted by nematodes was indicated by Narayanasamy *et al.* (1963). In the present paper some further observations on the transmission and epidemiology of the disease are presented.

Materials and Methods

The test plants were grown under insect-proof conditions. Sap inoculation was done using the usual methods and the modified methods of Hildebrandt (1956), Brunt and Kenten (1960) and Grant and Corbett (1960). The species of insects used for the transmission studies included *Empoasca kerri*, *Myzus persicae*, *Aphis craccivora* and an eriophyid mite *Tetranychus* sp. The acquisition feeding time and infection feeding time varied with the insects. Observations on the spread of PSMV were made in an irrigated crop sown for a spacing trial. The variety of pigeon pea grown was S.A. 1. The field consisted of 27 plots of 23 × 46 links each. There were nine different spacing with populations as indicated in the table on next page.

There were three replications for each of the treatments. The observations were started from 19–9–1960 and continued till 9–1–1961. On 19–9–1960 all the plots had already a high percentage of infection. The positions of these infected plants were marked on a field plan and thereafter weekly observations were made on the fresh infections taking place. The observations on the spread was continued till the crop reached maturity.

	Spacing	Number of plants
A	2′×1′	301
B	2′×2′	168
C	2′×3′	89
D	3′×1′	213
E	3′×2′	103
F	3′×3′	72
G	4′×1′	195
H	4′×2′	88
J	4′×3′	55

RESULTS

Sap inoculation was entirely unsuccessful to transmit the disease. Neither the insects tested nor the species of mite, transmitted the disease. Though dodder established well on both infected and healthy pigeon plants, the disease was not transmitted by this method.

Soil Transmission.—The attempts to transmit the disease by sap inoculation, insects and the dodder (*Cuscuta* sp.) did not yield positive results. Yet the disease was observed to spread in the field. The incidence of the disease in different fields in the Central Farm of the Agricultural College, Coimbatore, was recorded. In a field where pigeon pea had not been grown previously for several years, the plants were not affected by this virus disease at all. In another field where pigeon pea had been grown occasionally in the last few years up to 30 per cent. infection was noticed. In many fields in the campus pigeon pea is grown once annually in rotation with sorghum. In such fields the percentage of infected plants was as high as 90 per cent. These observations indicated that the soil or some agency present in the soil may be responsible for the spread of the disease in the absence of any insect vector. By growing pigeon pea frequently the virus inoculum appears to have been built up in the soil and this would probably account for the high level of infection in such fields.

The soil from the root zones of infected pigeon pea plants was therefore collected. Part of this soil was steam-sterilized and another lot left unsterilized. Pigeon pea seeds were sown in the unsterilized and sterilized soil in pots. The plants grown in the unsterilized soil were stunted and remained slightly chlorotic in the early stages. The symptoms of sterility mosaic disease appeared when the plants were 60 days old. Twenty-two plants out of sixty grown in the unsterilized soil showed the symptoms of sterility mosaic disease while the control plants remained healthy throughout the period of observation.

Soil samples were collected from fields where pigeon pea was grown. The nematode species were extracted by passing the soil suspension through 20, 100, 200 and 325 mesh sieves. Microscopic examinations revealed the presence of five species of nematodes. They were identified as *Helicotylenchus digonicus* Perry, 1959; *Tylenchorhynchus* sp., *Pratylenchus* sp. [close to *P. coffeae* (Zimmermann, 1898) Filipjev and Stekhoven, 1941], *Rotylenchulus reniformis* Linford and Oliveira, 1940 and *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935. All these nematodes belong to the order Tylenchida, Nematoda. The association of these nematode species with pigeon pea roots was consistently noticed.

A survey was undertaken to collect soil samples representing three districts of Madras State (Madurai, Ramanathapuram and Kanyakumari). The nematode population was screened by washing the soil.

All the samples collected at Periakulam, Kamatchipuram, Thirumangalam, Poovani, Thotiyapatti and Pechiparai contained the five species of nematodes the presence of which had already been recorded at Coimbatore. This constant association of nematode species with the root zones of infected plants appeared significant.

If any of the nematodes were responsible for the spread of the virus, it should presumably be possible to eliminate or reduce the spread of the disease by treating the soil with nematocides. To test this hypothesis, a preliminary observational field trial was laid out during 1962. There were ten plots of $10' \times 15'$ in size. Alternate plots were treated with the nematocide D.D. (Dichloropropene–dichloropropane) leaving five plots as controls. Each plot had 21 plants in three rows of seven plants each. Weekly observations were made on the incidence of the disease in the control and treated plot. The percentages of infection in treated and untreated plots are given in Table I. Growth measurements and counts of flowering branches were taken. Weights of grains and shoots of ten randomly selected plants in each plot were also recorded.

In D.D. treated plots the infection percentage was very much less than in the untreated plots. Since the percentage of disease incidence was reduced by nematocidal treatment of the soil it appears reasonable to assume that the disease in some way is associated with the presence of one of the species of nematodes in the soil.

TABLE I

Percentage of incidence of pigeon pea sterility mosaic disease in control and nematocide treated plots

Number of observation	Percentage of infection	
	Control	Treated with D.D.
1	16·7	0
2	17·8	0
3	10·2	1
4	5·6	4·4
5	4·5	9·4
6	1·4	3·4
7	4·6	0
8	4·6	1·0
9	3·6	0
10	1·4	0
11	1·2	0

In addition to reduction in the incidence of disease the uninfected plants were more vigorous. The height measurements (Table II) recorded showed that the plants in the treated plots had a better growth than those in the untreated plots. The number of flowering branches was reduced due to high percentage of virus infection in the untreated plots (Table II). When the average of the number of flowering branches produced by the plants in the control plots was considered, it was found that these plants formed only 63·4 per cent. of the number of flowering branches produced by the plants in the treated plots. A reduction of 36·6 per cent. in the number of flowering branches was caused by the virus disease.

The yields of ten randomly selected individual plants and the yield per plot were determined (Table II). The yield data showed that the yield of the plants in the untreated plots was brought down by the prevalence of very high percentage of infection. The per plant yield and the total yield per plot were reduced by 49·2 and 50·4 per cent. respectively. It can be seen that the shoot weight of the plants in the untreated plots was considerably reduced (Table II). By treating the soil with nematocides the incidence of the disease was very much reduced and with a possible stimulating effect of the nematocides, the plants in the treated plots were robust and gave higher yields.

To confirm the above results a second experiment was laid out during 1963. In addition to D.D., another nematocide nemagon (dibromochloropropane) was also included in the experiment. To eliminate the possibility of a fungus being responsible for the spread of the virus, Bordeaux mixture was included as one of the treatments. It was applied as soil drench twice at monthly interval. The plot size was 15′×6′ and there were six replications. Each plot had five rows with five plants in each row.

TABLE II

Growth of plants in the untreated and treated plots

Description	Untreated			Treated with D.D.		
	Oct.	Nov.	Dec.	Oct.	Nov.	Dec.
Height of plants in inches	21·2	55·1	61·1	27·7	62·7	69·2
Number of flowering branches	..	215	341	...	429	464
Weight of shoots per plant in grams	..	..	361·0	..	...	529·6
Yield of grains in grams	..	..	562·0	..	...	1131·0

TABLE III

Percentages of infection in control and treated plots

Treatment		Percentage of infection						Mean
		Replication						
		1	2	3	4	5	6	
1. Control	..	26·1	37·5	45·4	29·2	25·0	24·0	31·2
2. D.D.	..	0	0	0	5·5	5·5	5·2	2·7
3. Nemagon	..	5·5	5·9	22·2	18·2	18·1	4·0	12·3
4. Bordeaux mixture	..	5·2	17·4	38·5	33·3	31·8	36·3	27·1
S.E.	..	..	3·12	C.D. = 8·9				
D.D.		Nemagon,	Bordeaux mixture, Control					

D.D., Dichloropropene-dichloropropane.

The percentages of infection observed in the plots with different treatments are presented in Table III. D.D. treated plots had the lowest percentage of infection followed by nemagon, Bordeaux mixture and control. D.D. was found to be statistically superior to the other treatments in checking the disease. Nemagon also controlled the disease to some extent. Bordeaux mixture did not reduce disease incidence and was on a par with untreated control.

The nematode populations extracted from soil samples taken prior to and after treatment of soil with D.D., Nemagon, Bordeaux mixture and control were counted and are presented in the Table IV. The nematode population consisted of *Rotylenchulus reniformis*, *Tylenchorhynchus* sp., *Helicotylenchus digonicus*, *Pratylenchus* sp. and *Hoplolaimus galeatus* and the population counts were in the same descending order. After treatment with D.D., *H. digonicus* and *H. galeatus* were eliminated to a great extent. However, a very few numbers of *Pratylenchus* sp., *Tylenchorhynchus* sp. and *R. reniformis* were present in the post-treatment counts.

Nemagon was not as effective as D.D. in reducing nematode population. *Pratylenchus* sp. and *H. galeatus* were eliminated in the nemagon treated plots. But considerable numbers of *Tylenchorhynchus* sp., *R. reniformis* and *H. digonicus* were present even after treatment. In Bordeaux mixture treated plots, the number of *H. galeatus* was reduced. All other nematode species were present in large numbers as in control plots.

TABLE IV

Pre- and post-treatment counts of nematodes in treated and untreated plots

Name of the nematode	Control		D.D.		Nemagon		Bordeaux mixture	
	A	B	A	B	A	B	A	B
Tylenchorhynchus sp.	105	99	93	13	69	27	64	88
Rotylenchulus reniformis ...	281	412	182	11	218	205	339	522
Pratylenchus sp. ..	28	25	36	5	36	9	30	28
Helicotylenchus digonicus ...	114	62	102	1	131	40	142	47
Hoplolaimus galeatus ..	15	7	11	0	31	6	8	3

A = Pre-treatment. B = Post-treatment.

When the percentages of infection observed in different plots and the nematode population after treatment were considered, it was observed that the incidence of the disease was reduced if the population of nematodes was also reduced. Though *Hoplolaimus galeatus* and *Helicotylenchus digonicus* were eliminated by application of D.D. the presence of *Pratylenchus* sp., *Tylenchorhynchus* sp. and *Rotylenchulus reniformis* in small numbers was noticed. In nemagon treated plots *Hoplolaimus galeatus* and *Pratylenchus* sp. were absent. So the small percentages of infection in D.D. treated plots and a higher percentage of infection in nemagon treated plots could not probably be attributed to the presence of *Hoplolaimus galeatus, H. digonicus* and *Pratylenchus* sp. *Tylenchorhynchus* sp. or *R. reniformis* may be responsible for the incidence of the disease in D.D. and nemagon treated plots. A correlation between the numbers of *Tylenchorhynchus* sp. and *R. reniformis* and the percentage of infection was worked out. Positive correlations were obtained showing that the percentage of infection increased or decreased with increase or decrease in the number of *Tylenchorhynchus* sp. and *R. reniformis* (Text-Figs. 1 & 2).

SPACING AND SPREAD OF DISEASE

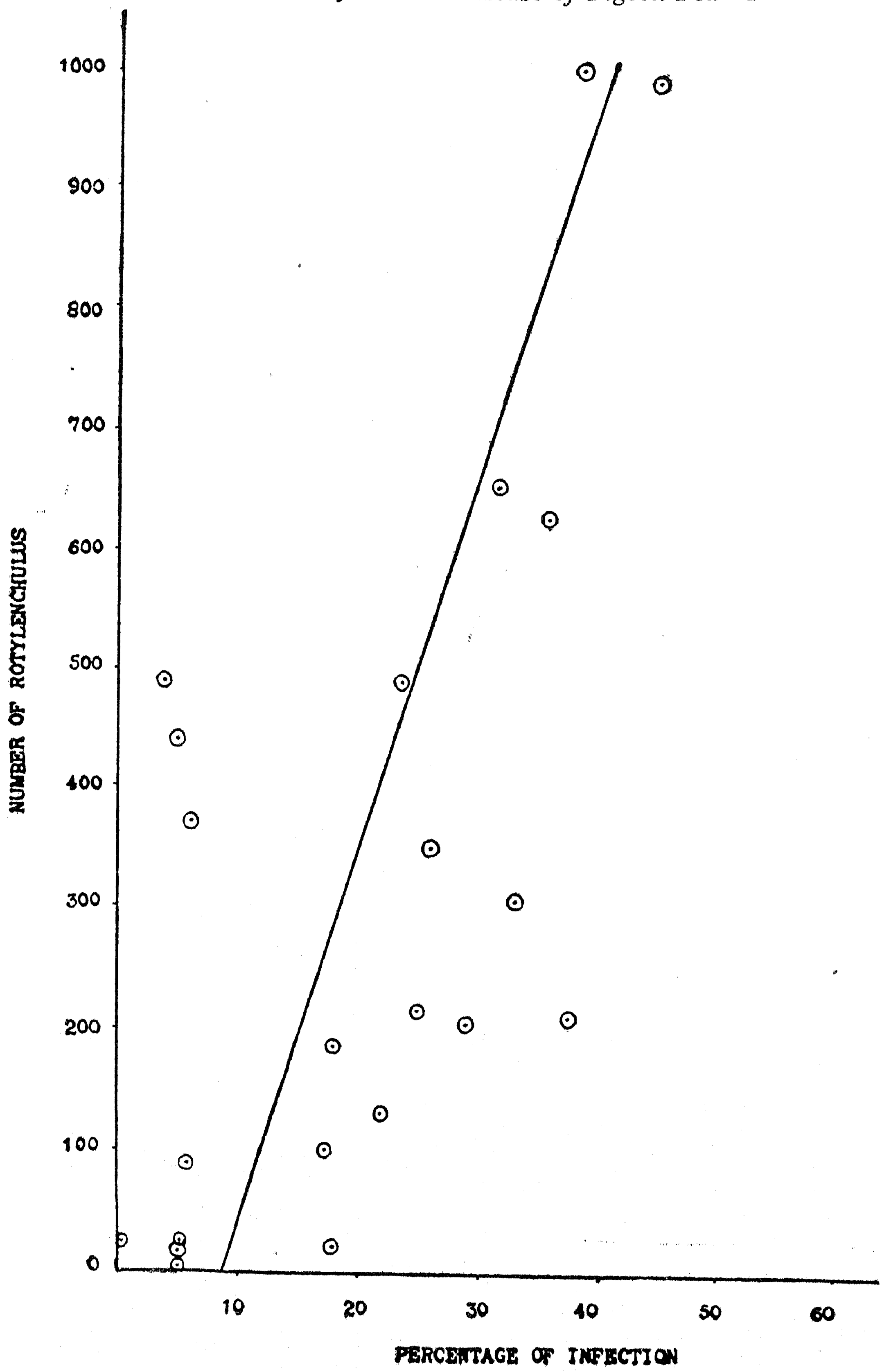
The final level of infection in the experiment on effect of spacing on disease spread are compared in Table V. It may be observed that there was a close negative correlation between the population of plants and percentages of infection. With increases of population there was corresponding reduction in total infection.

TABLE V

Percentage of incidence of disease in plots with different spacing

No.	Population	Percentage of infection
1	55	72·3
2	72	74·0
3	88	63·2
4	89	58·9
5	103	58·7
6	168	54·9
7	195	50·0
8	213	49·2
9	301	44·5

The observations on the incidence of the disease in twenty plots in another experiment showed that the disease was not noticed in the months of September and October. When the occurrence of disease in individual plots with seventy-five plants in each was considered maximum number of



TEXT-FIG. 1. Correlation between the numbers of *Rotylenchulus reniformis* and percentages of infection.

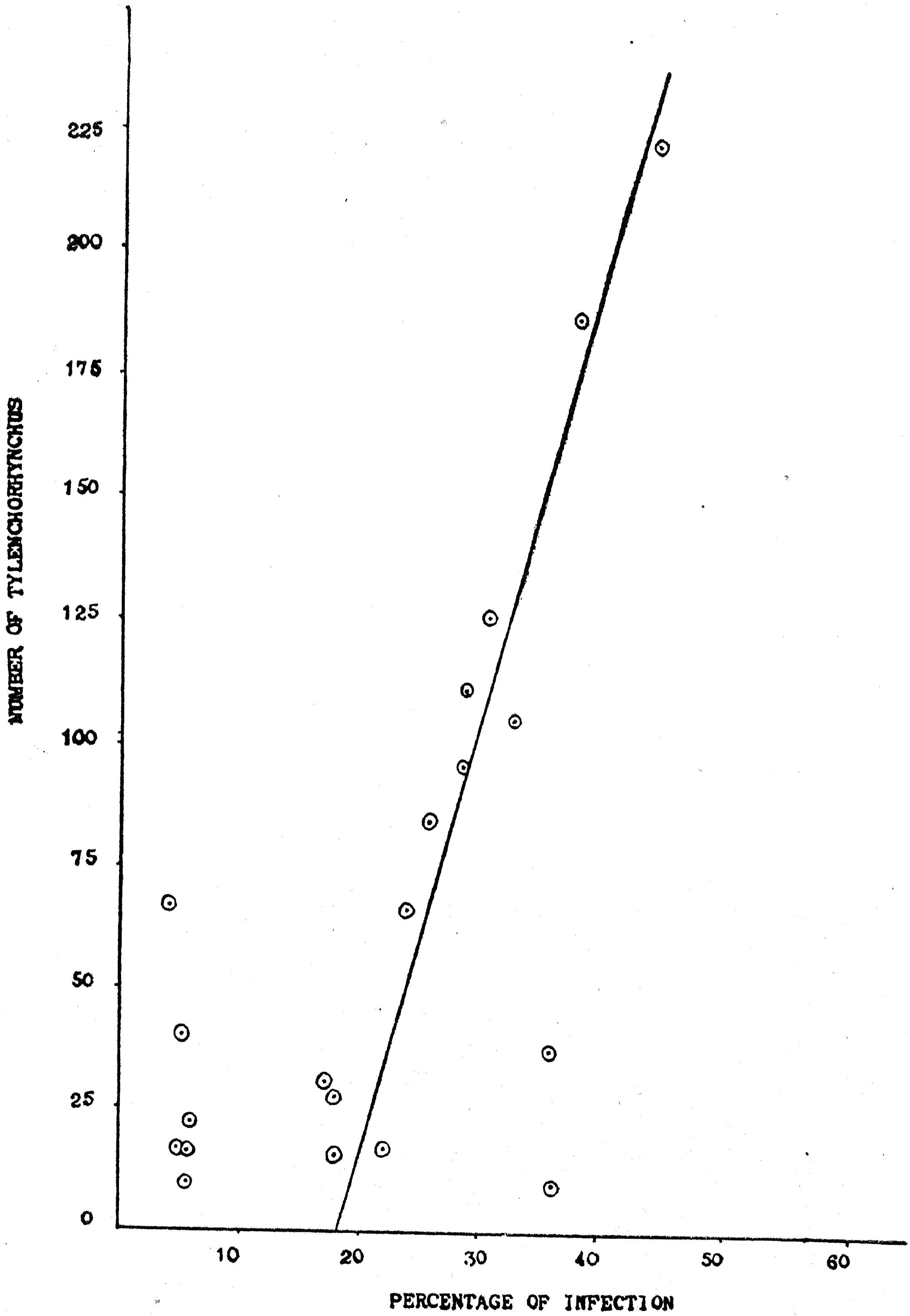
plants showing infection appeared in November. In a few cases the peak period extended to the first fortnight of December. This peak period of disease appearance was found to be the same when the mean of the percentages of infection of all plots was taken into consideration. This observation confirms the conclusion arrived at in the experiment conducted to study the pattern of disease spread in the previous year.

DISCUSSION

It was observed during the investigation that pigeon pea raised year after year in the same field showed a high percentage of infection amounting sometimes to 90 per cent. Fields which had never been cropped to this legume showed no disease when a crop was raised in 1961. These observations seemed to lend strong circumstantial evidence that the disease might be soil-borne. This observation was further confirmed when plants raised in pots on unsterilised soil taken from the root region of diseased plants in the field took infection, while those raised in sterilized soil remained healthy throughout.

Many soil-borne viruses have been observed to be transmitted by nematodes: grape fan leaf virus by *Xiphinema index* (Hewitt *et al.*, 1958; Raski and Hewitt, 1960), virus diseases of strawberry by *Xiphinema* sp. (Lister, 1958; Jha and Posnette, 1959) arabis mosaic virus by *Xiphinema* sp. (Harrison and Cadman, 1959), potato corky ringspot virus by *Trichodorus christei* (Walkinshaw *et al.*, 1961), a strain of tomato black ring virus by *Longidorus elongatus* (Harrison *et al.*, 1961), raspberry ringspot virus by *L. elongatus* (Taylor, 1962) and tobacco ringspot virus by *Xiphinema americanum* (Fulton, 1962).

It can be expected that the incidence of soil nematode borne diseases may be reduced if the vectors in the soil are eliminated by the application of nematocides. The two field experiments conducted during the present studies using nematocides indicated significant reduction of disease incidence due to the application of nematocides to soil. In the second experiment where two nematocides D.D., nemagon and one fungicide Bordeaux mixture were used indicated that the fungicide did not have any effect in reducing the incidence while both nematocides considerably reduced disease incidence. Of the two nematocides used D.D. was found to be more efficient. It was interesting to observe that the reduction in disease incidence was proportional to the reduction in population of two nematode species present in the soil, namely, *Rotylenchulus reniformis* and *Tylenchorhynchus* sp.



TEXT-FIG. 2. Correlation between the numbers of *Tylenchorhynchus* sp. and percentages of infection.

It, therefore, appeared that one or both of these nematodes may probably be responsible for the transmission of the virus.

In all virus diseases which have so far been demonstrated to be transmitted by nematodes, the vectors are members of the class Adenophoria, sub-class Aphasmidia represented by genera like *Xiphinema, Longidorus* and *Trichodorus*. In the present investigation, however, the possible vectors of the virus belong to class *Tylenchoidia*. It might be pointed out here, however, that among the insect vectors of plant viruses, a very large variety of insects are represented and a similar situation might very well exist among the nematodes also if only detailed investigations are carried out on this aspect.

A statistically significant negative correlation has been observed between the population of pigeon pea plants and percentage of infection by sterility mosaic virus. The finding of Van der Plank (1947) with regard to insect transmitted virus may form the basis of explanation of the result obtained in the case of the present virus. Closer spacing produced smaller plants and smaller root systems whereas wide spacing induced the production of larger plants and larger root system occupying larger area inside the soil. So it appeared that in a possibly soil-borne-nematode transmitted disease also, in thinly populated fields, the plants which become larger in size and had extensive root system are more likely to get infected than plants in a thickly populated field.

SUMMARY

The pigeon pea sterility mosaic was not transmitted by sap, insects or dodder. There were indications to show that probably the disease was soil borne. A consistent reduction in the disease incidence with reduction in nematode population by the application of nematocides D.D. and nemagon was observed. The reduction in disease incidence was greater in D.D. treated plots than in nemagon treated plots. A statistically significant positive correlation was established between the number of *Rotylenchulus reniformis* and *Tylenchorhynchus* sp. and the percentage of disease incidence. It is surmised that the disease is probably transmitted by *R. reniformis* and/or *Tylenchorhynchus* sp.

A negative correlation was obtained between the population of plants and percentages of infection by this disease.

ACKNOWLEDGEMENT

The authors are thankful to Dr. A. R. Seshadri, Nematologist, for his help in studies connected with the nematode transmission of the disease and to the Government of Madras for the award of a merit scholarship to the senior author during the tenure of which the study was undertaken. Thanks are due to the University of Madras for according permission to publish the doctoral thesis submitted by the senior author.

REFERENCES

Breece, J. R. and Hart, W. H. .. "A possible association of nematodes with the spread of peach yellow bud mosaic virus," *Plant Dis. Reptr.*, 1959, **43**, 989–90.

Brunt, A. A. and Kenten, R. H. "Mechanical transmission of Cacao swollen shoot virus," *Virology*, 1960, **12**, 328–30.

Capoor, S. P. .. "Observations on the sterility disease of pigeon pea," *Indian J. agric. Sci.*, 1952, **22**, 271–74.

Fulton, J. P. .. "Transmission of tobacco ringspot virus by *Xiphinema americanum*," *Phytopathology*, 1962, **52**, 375.

Grant, J. J. and Corbett, M. K. "Mechanical transmission of the infectious variegation virus of citrus," *Nature, Lond.*, 1960, **188**, 519–20.

Harrison, B. D. and Cadman, C. H. "Role of a dagger nematode (*Xiphinema* sp.) in outbreak caused by arabis mosaic virus," *ibid.*, 1959, **184**, 1624–26.

———, Mowat, W. P. and Taylor, C. E. "Transmission of a strain of tomato black ring virus by *Longidorus elongatus* (Nematoda)," *Virology*, 1961, **14**, 480–85.

Hewitt, Wm. B., Raski, D. J. and Coheen, A. C. "Nematode vector of soil-borne fan leaf virus of grape vines," *Phytopathology*, 1958, **48**, 586–95.

Jhildebrand, E. M. .. "Rapid inoculation techniques for mechanical transmission of sweet potato internal cork virus," *Plant Dis. Reptr.*, 1956, **40**, 527–30.

Ha, A. and Posnette, A. F. .. "Transmission of a virus to strawberry plants by a nematode (*Xiphinema* sp.)," *Nature, Lond.*, 1959, **184**, 962.

Kandaswamy, T. K. and Ramakrishnan, K. "An epiphytotic of pigeon pea sterility mosaic at Coimbatore", *Madras agric. J.*, 1960, **47**, 440–41.

Narayanasamy, P., Seshadri, A. R. and Ramakrishnan, K. "A preliminary note on suspected nematode transmission of red gram sterility mosaic virus," *ibid.*, 1963, **50**, 109–10.

Raski, D. J. and Hewitt, Wm. B. "Experiments with *Xiphinema index* as a vector of fan leaf of grape vines," *Nematologica*, 1960, **5**, 160–70.

Seth, M. L. .. "Transmission of pigeon pea sterility by an eirophyid mite," *Indian Phytopathology*, 1962, **15**, 225–27.

Taylor, C. E. .. "Transmission of raspberry ringspot virus by *Longidorus elongatus* (de Man) (Nematoda: Dorylaimidae)," *Virology*, 1962, **17**, 493–94.

Van der Plank, J. E. .. "The relation between the size of plant and spread of systemic disease. I. A discussion of ideal cases and a new approach to problems of control," *Ann. appl. Biol.*, 1947, **34**, 376–87.

Walkinshaw, C. H., Griffin, G. D. and Larson, R. H. "*Trichodorus christei* as a vector of potato corky ringspot (Tobacco rattle) virus," *Phytopathology*, 1961, **51**, 806–08.