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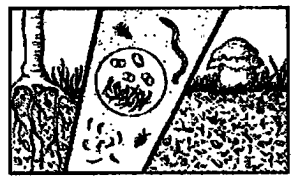
SOME INVESTIGATIONS ON SCUTELLONEMA BRADYS A NEMATODE PARASITIZING ON YAM



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Some investigations on *Scutellonema bradys*, a nematode parasitizing on yam

Rapport de stage

par H.M.VENNE

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Acknowledgements

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Introduction

Scutellonema bradys (Steiner and Le Hew, 1933) Andrassy, 1958, is among the nematodes parasitizing on Dioscorea sp. one of the most important species. It was first described by Steiner and Le Hew in 1933 as Hoplolaimus bradys, when found in yams from Jamaica and Puerto Rico. Since then severae names have been given to it but in 1963 Sher synonimized them and now there is only one valid name.
After it was discovered in yams from Jamaica and Puerto Rico it was found in several other places, usually in close connection with Dioscorea species : In Nigeria (Goodey, 1935, Steiner 1937 b, Phillips 1956, Caveness 1961, 1965, 1967), In the Ivory Coast (Baudun 1956, Smit 1966, 1972), Brazil (Lotdello 1959) and Cuba (Decker, H., R. Casamayer Garcia and Dario Bosch, 1967).

The damage done to a yam plant by an infection of Sc.bradys alone is difficult to estimate. However, being an endoparasite, they facilitate the growth of fungi causing the " dry-rot " disease and, dependent on the climatic or storage conditions, bacterial wet-rot. In this way they cause severe losses during the growth stage of the plant while the entire tuber may be lost during the storage period which usually follows. As more tham 10 million tons are harvested in West Africa alone, and probably the bigger part is stored for at least a few months the yam nematode can be considered as economically important.

Most of his lifetime the nematode spents as an endoparasite in underground parts of plants. The eggs are laid in roots of tubbers by the female. From these second, third and fourth stage juveniles develope successively, the latter differentiating to males and females. This life-cycle is very favorous on the yam plant, because the tubers form excellent feeding sites for colonies of nematodes. They occur mainly in the outer layer, where they can reach densities of several thousands per gram of tissue. Propagation goes on during the storage of the tuber and when the latter of part of it is used as a seedyam the new plant will be heavily attacked from the beginning.

In the tuber the nematodes show their presence by a yellowish brown discolouration of the tissue just under the skin. The dry-rot which can follow this causes a loss of weight and locally the skin shrinks and wrinkles. However as it comes clear in this report the absence of obvious dry-rot symptoms does not mean that there is no infestation.

Within the genus Dioscorea, D.alata and D.esculenta are the most susceptible to attack (Caveness 1965 ; Baudin 1956) but many others are reported as host plants under which D.cayenensis and D.rotundata which are the major food yams in Western Africa. Outside this genus other cultivated plants may act as hosts. This might be important as yam cultivation is usually part of a rotational system and mixed cultures are common too.

To examine further the host range of Sc.bradys was one of the objects of my stay at the ORSTOM. Other things which were to be examined under the guidance of Mr.Smit were methods of estimating nematode populations in yam tubers, the usual methods for roots being not very satisfactory, the process of infection during the growth stage by means of an inoculation of the soil and the utility of a hot-water treatment for infested seed yams.

I - Hot-water treatment of infected tubers

A - Object of the experiment

The killing of Scutellonema bradys which have invaded tubers of Dioscorea sp. by a treatment with hot water.

Different times and temperatures are tested as well as a pretreatment with a wetting agent on tubers of different degrees of infestation.

The examination of the germinal force of the treated tubers and the development of a possible surviving population of nematodes after using the tubers as seed-yams.

B - Materials

In august 1972 109 tubers of Dioscorea cayenensis var. Lokpa were bought on the local market.

The tubers were immediately divided into tubers which showed symptoms of infection by Sc.bradys and tubers which did not show such symptoms.

This was checked by scraping a small piece of the skin from the tuber on places where it showed small cracks or felt spongy. A yellowish-brown discolouring of the tissue or the beginning of dry-rot points to the presence of Scutellonema bradys.

In the first half of february 1973 the tubers were again examined in that way.

The tubers with symptoms in august proved to be heavily affected by dry-rot in february. About 30% of the tissue of every such tuber was rotten.

The tubers without symptoms in august could again be divided. The tubers which had developped symptoms by now were in a much better condition tham the former category, not more than 10% of each tuber was rotten.

On this basis we could make a division in 3 groups :

no symptoms : unaffected in february
40 tubers

slight symptoms : symptoms in february, not yet in august
19 tubers

heavy symptoms : symptoms in august
50 tubers.

Plate I gives an impression of the condition of a tuber from the category " slight symptoms ".

For the hot-water treatment was further used a reservoir of about 200 liters provided with a thermostat and heating.

As a wetting-agent was used concentrated liquid house hold soap in a dilution of 3 : 1000.

C - Methods of extraction used in this experiment.

Soil samples were as soon as possible extracted in the Seinhorst elutriator. The obtained suspension was poured on a cottonwool filter, which was placed in a petri-dish. After 24 hours the catch was counted. The cottonwool filter was often replaced by a paper handkerchief.

Tubers were analysed by peeling and adding to the skin all the discoloured tissue. This mixture was cut into pieces of one to one and a half square cm and after that macerated in a domestic blender for 2 seconds (8000 r.p.m.). 10 grams of tissue in 200 ml.water every time.

The obtained mixture of tissue and nematods was poured over a nest of sieves to remove the starch and put under the mistifier. After one week was counted.

See for this extraction method also the experiment on this subject, described in this report.

Roots were cut into pieces of 1 1/2 cm and extracted under the mistifier. After 2 weeks, the catch was counted.

D - The treatments

1)- The hot-water treatment

Before beginning any treatment some tubers from every category were taken at random and analysed.

For the treatment the following combinations of times and temperatures were chosen.

Temperature		Time
52° C	-	15 min.
52° C	-	30 min.
50° C	-	15 min.
50° C	-	30 min.
46° C	-	15 min.
46° C	-	30 min.
no treatment		

After the treatment the tubers were immediately put into water of room-temperature for 15 minutes.

2)- The pre-treatment

With a hot-water treatment it is important that the air is removed entirely from the surface of the tuber for a maximum transport of heat. Affected tubers have much air in their corky skin. That's why half of the tubers was laid in a soapy solution 24 hours before the hot-water treatment also half of the tubers which got no hot-water treatment was pretreated with soap to examen the effect of soaps alone. This tubers were also rinsed in water of room temperature for 15 minutes to minimize soap-residues and to keep the effect of the soap comparable with the other soap-treated tubers.

The other half of the tubers which got no hot-water treatment was not pretreated.

After all, there 14 different treatments : the 7 hot-water treatments each combined with soap or no soap.

3)- The cutting and planting.

After the treatments the tubers were left drying for 10 days and then were cut into pieces appropriate for planting.

The division of the tubers in groups for every treatment was at random, however in such a way that :

a - In every group were tubers with no symptoms which could together be cut into 5 pieces.

b - In every group were tubers with slight symptoms which together could be cut into 3 pieces

c - In every group were tubers with heavy symptoms which together could be cut into 5 pieces.

The pieces varied in weight from 150 to 250 grams. Of every group one piece of every category was analysed immediately after the cutting.

The remainder of the tubers, 10 pieces for every treatment was planted after somedays in buckets with a pierced bottom, filled with sterilized soil. After 2,3,6 and 7 weeks soil samples of 100 cc were taken.

After 12 weeks a soil sample of 250 cc is taken from the soil surrounding the old tuber and the nwoly formed roots, tuber and roots are analysed.

E - Results and conclusions

1)- The immediate influence of the treatments

The original population can be estimated from two series of tubers : the 8 tubers which were extracted before the treatments and the 3 pieces of tuber which received no treatment and were analysed after the cutting of the tubers. See table I.

The number of nematodes found in the first 5 tubers in this table could be reduced by some trouble with the water-supply and the mistifier.

With a non-parametric test (Kruskall-Wallis) no significent difference was found between the 3 categories, neither was found a difference in the distribution over stages.

However, if the tubers and pieces, are arranged according to the number of extracted nematodes it appears that in the bigger populations the percentage of the larvae in higher than in the smaller populations and that the percentage of the males is lower (Spearman rank correlation, $\alpha = 0,05$ for both percentages).

From table II the following conclusions can be drawn. No treatment killed all the nematodes.

The pretreatment with soap had a favorable effect, 24 hours in soap water hills a big part of the nematodes even without hot-water treatment.

Without pretreatment with soap more nematodes stayed alive in the tubers with heavy symptoms than in the tubers without symptoms. This was probably caused by the air in the big corky pieces of the tubers with heavy symptoms for between the soap-treated tubers this difference was not significant (Mann-Whitney test).

The surviving number of nematodes consisted for a bigger part of adults than the original population. This effect was stronger as the surviving number was smaller. This difference was significant with the treatments without soap ($\alpha < 0,05$), just not significant with the soap-treatments. (Spearman rank correlation).

Comparing soap-treatments and treatments without soap it shows that the female can stand the soap better than the male and also better than the larval stages (Mann-Whitney test $\alpha = 0,05$).

Table I Untreated tubers

The number of extracted nematodes and their distribution over adults and larval stages

symptoms	number of nematodes	distribution in percentages					quotient	
		♀	♂	L_4	L_3	L_2	♀/♂	Ad./juven.
no	15.381	22.5	25.1	9.8	34.1	8.5	0.90	0.91
no	1.585	28.5	37.4	5.7	23.9	4.4	0.76	1.93
no	3.056	40.4	29.2	3.4	22.2	4.7	1.38	2.29
slight	39.268	32.2	29.7	7.4	27.0	3.6	1.08	1.62
slight	54.980	33.8	24.3	8.7	29.3	3.9	1.39	1.39
heavy	41.050	33.5	30.8	4.0	27.8	3.8	1.08	1.80
heavy	46.950	26.7	28.5	6.0	35.0	7.8	1.09	1.05
heavy	61.700	30.9	29.6	5.3	27.9	6.1	1.04	1.53
no	173.600	12.2	13.4	4.3	35.3	34.8	0.91	0.34
slight	34.950	31.7	22.5	6.2	34.7	4.9	1.41	1.18
heavy	13.867	35.1	33.4	3.6	26.1	1.8	1.05	2.17

Table II Treated tubers

The number of extracted nematodes of different stages

without soap

treatment	sympt.	♀	♂	L_4	L_3	L_2	tot.
52°C - 15 min	no	11	15	0	0	1	27
	slight	16	24	0	1	0	41
	heavy	16	15	3	17	0	51
52°C - 30 min	no	2	1	0	0	0	3
	slight	2	0	0	0	0	2
	heavy	500	229	18	209	35	991
50°C - 15 min	no	3	7	0	0	0	10
	slight	3.729	2.728	281	2.033	175	8.946
	heavy	562	386	89	395	21	1.455
50°C - 30 min	no	2	2	0	0	0	4
	slight	5	3	0	2	0	10
	heavy	4.605	4520	185	1.330	255	10.895
46°C - 15 min	no	21.890	21.175	1.785	10.430	975	56.255
	slight	3.600	3.675	550	8.250	3.450	19.525
	heavy	14.820	6.195	1.310	9.070	1.085	32.480
46°C - 30 min	no	218	134	9	37	3	401
	slight	102	204	2	9	0	317
	heavy	2.435	2.910	160	1025	300	6830
no treatment	no	21.200	23.250	7.500	61.300	60.350	173.600
	slight	11.100	7.850	2.150	12.150	1.700	34.950
	heavy	4.862	4.627	501	3.625	252	13.867

with soap

treatment	sympt.	♀	♂	L_4	L_3	L_2	tot
52°C - 15 min	no	3	0	0	1	0	4
	slight	0	2	0	1	0	3
	heavy	5	6	1	1	0	13
52°C - 30 min	no	2	0	0	0	0	2
	slight	0	0	0	0	0	0
	heavy	2	3	0	1	0	6
50°C - 15 min	no	12	9	3	7	3	34
	slight	8	3	0	3	0	14
	heavy	11	5	1	5	0	22
50°C - 30 min	no	2	1	0	0	0	3
	slight	84	67	0	4	0	155
	heavy	13	7	0	3	0	23
46°C - 15 min	no	587	332	42	486	15	1.462
	slight	1.904	721	82	888	150	3.745
	heavy	284	146	8	190	4	632
46°C - 30 min	no	6	3	0	2	0	11
	slight	18	8	0	3	0	29
	heavy	11	3	1	6	0	21
no treatment (only soap)	no	2 870	1.099	101	972	52	5.902
	slight	3 457	2.708	157	997	151	7.470
	heavy	2.010	396	102	1.231	50	3.709

The treatments of 30 min. were better than those of 15 min. (Mann-Whitney α = 0,05)

2)- The surviving nematodes after planting of the tuber.

Table III gives the number of nematodes which were extracted from the soil samples per 100 cc soil, from the old tubers per tuber and from the newly-formed roots per root-system. In the numbers given for the soil, all the buckets are equally represented. In the numbers for tubers and root-systems only the germinated tubers are represented because the ungerminated tubers were entirely rotten and had formed no roots.

Again no treatment has killed all the nematodes. Looking at the numbers found in the soil the soap makes again a significent difference (Mann-Whitney α = 0,05) in the killing of the nematodes. The same is true for the numbers in the newly grown roots. The difference is not present between the numbers in the old tubers.

The pretreatment with soap is efficient enough to limit infection of the new roots if used with a hot-water treatment. The combination 52° C + soap proved effective in this respect, after the other treatments with soap so many nematodes were still found in the old tuber and in the soil that infection after 12 weeks might be possible.

Again the numbers of the females have decreased less than the numbers of the males, the numbers of the larvae have decreased most (Mann-Whitney test α = 0,05).

No connection was found between the coming above ground-level of the vine on the one side and the found numbers in roots, soil, tuber or their mutual proportions on the other side.

3)- The influence of the treatments on the germinal force.

Of the 140 pieces of tuber which were planted 79 germinated within 12 weeks. The other 61 were rotten without exception.

Table IV gives the distribution over the different catagories and treatments.

The following conclusions can be drawn.

The tubers with heavy symptoms germinate rarely. In this respect there is no difference between treated and untreated tubers.

Only for the germination of the tubers with heavy symptoms the pretreatment with soap was unfavourable (Fisher-test α = 0,05). The pretreatment with soap seemed to cause also a delay in the germination but this just failed to be significent at α = 0,05.

The duration of the hot-water treatment, 15 wether 30 minutes, had no effect on the germination.

F - Discussion

The growing of yams in buckets showed the disadvantage, that the flow of the rainwater through the holes in the bottom, was not sufficiently large. This caused inundation and this again promotion of the rotting of tubers. In at least 3 cases was observed that the vine had partly died because of inundation.

Table III

treatment	not soap-treated			soap-treated		
	soil 100 cc	tuber	roots	soil 100 cc	tuber	roots
52°C – 15 mim	1.04	2.75	3.63	0.20	0.0	0.0
52°C – 30 min.	3.45	0.0	0.83	0.02	0.0	0.0
50°C – 15 min	8.17	0.0	55.7	0.22	0.25	0.25
50°C – 30 min	11.06	0.0	3.00	0.04	45.8	0.5
46°C – 15 min	9.14	16.7	73.9	3.34	4.60	0.0
46°C – 30 min	9.47	4.83	19.3	0.15	0.0	0.0
	17.89	1.50	26.6	1.47	1.50	97.3

Table IV

soap – treated

Symptoms	after each treatment planted	52° 15 min	52° 30 min	50° 15 min	50° 30 min	46° 15 min	46° 30 min	no treatm.	total
no	4	3	2	4	4	4	4	4	25
slight	2	2	2	0	2	1	2	2	11
heavy	4	1	0	0	0	0	0	0	1
total	10	6	4	4	6	5	6	6	37

not soap-treated

symptoms	after each treatment planted	52° 15 min	52° 30 min	50° 15 min	50° 30 min	46° 15 min	46° 30 min	no treatm.	total
no	4	4	4	2	4	4	4	4	26
slight	2	2	1	2	1	2	2	1	11
heavy	4	2	1	0	0	1	0	1	5
total	10	8	6	4	5	7	6	6	42

It might be better with such experiments in a wet climate to pierce the bucket also just above ground-level, to shorten the periods of inundation.

Little could be learnt about the development of the surviving populations. That was caused by :

1)- The bad germination of the tubers with symptoms. Some 8 pieces were probably rotten through when planted and contained for that reason no living nematodes any more. This has probably reduced the number of infected buckets. Anyway the number of new plants was severily reduced.

2)- The bad results of the soil-samples.
In the case of small numbers of nematodes the taking of 100 cc - soil-samples proves to give no good indication of nematodes which have survived in the tuber. In several cases nematodes had infested the new roots without having been found in the soil samples. Even 250 CC - soil samples which are taken from the soil directly surrounding roots and tubers, do not give a good impression of a surviving population in the tuber or a new one in the roots.

Probably the nematodes can move quickly from the tuber to the roots because the roots grow partly along the sides of the old tuber. This is easy for the part of the nematodes which leave the tuber only after sometime. After 12 weeks considerable numbers of nematodes were still found in the tubers.

3)- The relative success of most of the treatments resulting in such small numbers of nematodes that no conclusions could be drawn from the development of the numbers.

II - The distribution of nematodes among different parts of roots, tubers and soil.

A - Object of the experiment

The density of nematodes is very unequally in different parts of the tuber. That renders the taking of samples difficult and makes the estimation of the infestation a laborous job.

To get some insight in the process of infestation and the distribution of nematodes this experiment was undertaken.

B - Materials

81 kg of tubers of Dioscorea alata were bought on the local market. The eventual nematodes in the tubers were killed or mostly killed by a pretreatment of 24 hours in soap-water followed by a 30 min.bath in water of 52° C.

The nematodes for the inoculation were extracted from infested tubers and from soil in which Vigna Sp. had been grown.

The tubers were placed in buckets with a pierced bottom and filled with sterilized soil.

C - The planting and inoculation

The tubers were all planted at the same depth. (See fig.I) the buckets were randomly divided into 3 equal groups. Every

groups was inoculated at a certain depth. This was realised by making 3 holes of the wanted depth in every bucket immediately before the inoculation.

The object was to inoculate with two different numbers of nematodes, but this was not possible by lack of nematodes.

Every week part of the buckets would be examined by scooping out and separation into 3 parts to count the nematodes in roots, tuber and soil on different depths.

D - The virus

Two weeks after planting the yams, several plants were stunted to about 30 cm. height. The leaves were crinkled and showed a mosaïc of light/dark green.

Other plants showed a bulging of the leaves along the principal ribs. Still others showed the beginning of a mosaïc on the older leaves.

Before the first examination of the buckets big differences in growth rates became apparent and not a single plant was free of symptoms. That's why the experiment was stopped.

For the symptoms, see also plate II and plate III.

III - Methods of extraction of nematodes from tubers.

A - Material and preliminary treatment

Some well-infested tubers, like the one on plate I, were peeled with a knife and all the discoloured tissues were added. Then this was cut into pieces not bigger than on and a half cm^2. This pieces are mixed thoroughly and each time 10 grams are taken for the next treatment.

As a blender is used a domestic blender with 3 different speeds filled with 200 CC of water.

speed III	topspeed	14.000 r.p.m.
" II	"	8.000 r.p.m.
" I	"	5.000 r.p.m.

B - Experiment I, 1)- Compared methods.

Extraction by the mistifier was compared with the use of a cotton-wool filter on a petridish.

Both were combined with blending with diffent speeds of the blender.

Used were the high and the moderate speed (speed III en II).

For a better comprehension of the process of extraction the suspension from the blender was seperated into two components by pouring it over a coarse sieve. The pieces of tissue, were washed, the solution of starch and nematodes together with the wash-water were poured over a nest of four 50 - u sieves with extra water to diminish the amount of starch.

Every treatment was 10 times repeated.

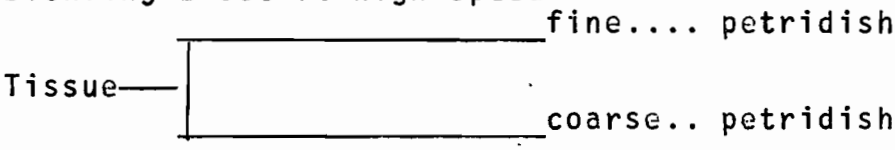
The fine fraction was counted after one day, the coarse fraction from under the mistifier after one and two weeks, the

coarse fraction from the petridish after, 4, 9 and 15 days.

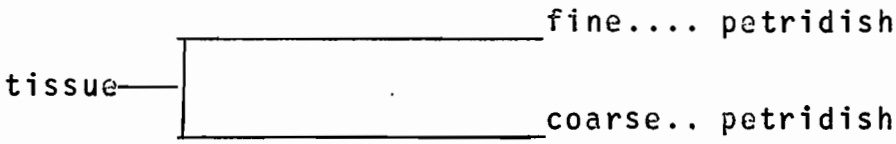
So we arrive at the following scheme.

Method A : no blending, tissue under the mistifier

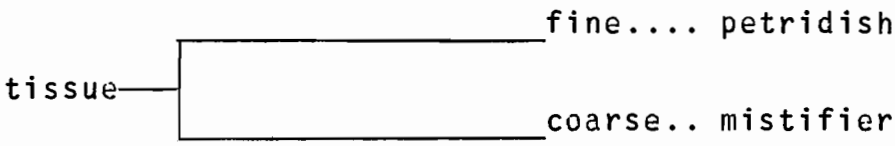
Method B : blending 2 sec at high speed



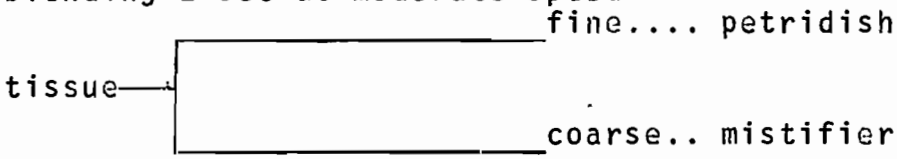
Method C : blending 2 sec at moderate speed



Method D : blending 2 sec at high speed



Method F : blending 2 sec at moderate speed



2)- Statistical manipulations and results

At first the results were only compared with non parametric tests. This was necessery because the results were clearly not normally distributed.

To find out if the methods differed significantly the kruskall-Wallis test was used and when this proved to be the case, the methods were compared with the Mann-Whitney-test. This gave the following result (see table V)

Table V significant differences between methods of extraction

Treatments	♀	♂	L4	L3	L2	Tot.
D - B	+	0	0	+	+	+
F - C	+	+	+	+	+	+
F - D	+	+	+	+	+	+
F - A	+	+	+	+	+	+
D - A	0	0	+	+	+	0

0 means : no difference
+ means : the first mentioned treatment is the best.

To make manipulations possible which are more quantitative a transformation of the results was necessary. The presumption was made that the logarithms of the extracted numbers were normally distributed. To test this presumption at first the average of every 10 repetitions was calculated after transformation as well as the variance of groups of mutual independent results. See table VI this groups consisted of 50 observations of the coarse fraction or of coarse and fine fraction together, however method A had no fine fraction so here the groups consisted of 40 observations.

The methods Dand B are identical as to the fine fraction. A t-test did not give any difference either, so we took them together to get more accuracy. The same is true for methods Fand C.

Next, for three groups of 50 independent observations the quotient $\frac{\underline{X} - \overline{X}}{\underline{S}}$ was calculated

If the 50 observations are normally distributed then this quotient should have the some distribution as $\underline{X}$, If $\underline{x}$ is the mean of the ten observations of each series and $\underline{s}$ is the estimation of the square root of the variance.

The first group consisted of the sum of nematodes extracted from each sample.

The second and third group consisted of samples taken at random from the other series. These samples consisted of :

Group two : A - σ°, B (c+f) - L3,C(f)-L4,D(c)-tot.,F(f)-tot.
Group three:A - L2,B(c)-tot,C(f+c)-L2,D(f)-tot,F(f)-L4

The quotients of the three groups did not prove to have another distribution as $\underline{X}$. This was tested by the X^2-test. For an exemple of the preceding calculations see fig.II.

It is not easely possible to compare the means and variances of different stages and different fractions which come from the same samples, because these are not independent observations. For each sample the distribution among adults and different larval stages was computed, however the logarithms of the percentages proved to be not normally distributed so they were compared by the nonparametric tests of Kruskal-Wallis and Mann-Whitney.

Table VII gives some computed quotients of extracted numbers for the observations of the fine and the coarse fraction together.

Table VIII gives the same but for the observations split up between the two fractions.

3)- Conclusions

Table V shows that the mistifier is superior to the petridish, and that the use of the blender at moderate speed is better than its use at high speed or nouse atall. Use of the blender at high speed has only for the larval stages significent advantages above treatment without blender.

Table VII gives the same conclusions but here are the results quantificated.

Table VI

		A	B	C	D	F	S
♀	f	—	3,2988	3,5191	3,2988	3,5191	0,138
	c	3,6677	3,6776	2,7899	3,2687	3,5440	0,193
	f+c	3,6677	3,4053	3,6179	3,5733	3,8363	0,140
♂	f	—	3,3027	3,4388	3,3027	3,4388	0,171
	c	3,5014	2,5622	2,6607	3,0611	3,3352	0,201
	f+c	3,5014	3,4119	3,5419	3,4798	3,6879	0,158
L_4	f	—	2,6049	2,8766	2,6049	2,8766	0,259
	c	2,5558	1,7249	1,8218	2,3812	2,4653	0,189
	f+c	2,5558	2,8053	2,8491	2,7389	3,0799	0,198
L_3	f	—	3,1797	3,4336	3,1797	3,4336	0,158
	c	3,4217	2,2957	2,4687	3,2687	3,5092	0,219
	f+c	3,4217	3,2065	3,5230	3,5661	3,7636	0,164
L_2	f	—	2,5182	2,7735	2,5182	2,7735	0,200
	c	2,6380	1,4540	1,8082	2,4489	2,7111	0,284
	f+c	2,6380	2,5192	2,8687	2,8538	3,0521	0,171
tot.	f	—	3,8120	4,0117	3,8120	4,0117	0,123
	c	4,0612	3,0583	3,1915	3,7453	3,9949	0,178
	f+c	4,0612	3,8755	4,0983	4,0872	4,3028	0,125

Table VII, Quotients of numbers
fine + coarse together

effects	of mistifier		of blender		
stages	D/B	F/C	F/D	F/A	D/A
♀	1,47	1,65	1,83	1,47	0,80*
♂	1,17*	1,40	1,61	1,54	0,95*
L_4	0.86*	1,70	2,19	3,34	1,52
L_3	2,29	1,74	1,58	2,20	1,39
L_2	2,16	1,53	1,58	2,59	1,65
total	1,55	1,60	1,64	1,74	1,06*

Table VIII Quotients of numbers
fractions separated

effects	of mistifier		of blender			
fractions	coarse		coarse			fine
stages	D/B	F/C	F/D	A/F	A/D	F+C/D+B
♀	3,90	5,68	1,88	1,33*	2,51	1,66
♂	3,15	4,73	1,88	1,47	2,76	1,37
L_4	4,53	4,40	1,21*	1,23	1,49	1,87
L_3	9,40	11,0	1,74	6,82*	1,42*	1,79
L_2	9,88	8,00	1,83	0,85*	1,55*	1,80
total	4,86	6,36	1,78	1,16*	2,07	1,58

* this quotients do not differ significantly from 1 with $\alpha = 0,05$.

The use of the blender with high as well as moderate speed gives a change in the composition of the number of nematodes in favour of the larval stages.

Table VIII proves the bad extraction of the coarse fraction on a petridish compared with the extraction under the mistifier.

Blending with high speed gives less nematodes than with moderate speed in both fractions.

Blending with moderate speed gives, compared with no blending, less adults and L 4, but not less L 3 and L 2 in the coarse fraction.

4)- Discussion

In view of the results we can say that there are many nematodes in the unblended pieces of tissue, that are not extracted within two weeks under the mistifier.

The question arises how many nematodes still stay in the blended tissue after two weeks under the mistifier. To extract these, blending with a higher speed proves not to be efficient. Blending longer than 2 seconds could be the solution perhaps at less speed. However, this might cause the killing of a bigger part of those nematodes which have been freed from the tissue already.

To examen all this the experiments II and III were done.

Moreover was in experiment II examined if the use of the nest of sieves could be abandoned at a moderate or low speed of the blender. For with these speeds much less starch is freed from the tissue than with the high speed.

C - Experiment II

1)- Materials and treatments

As material was used a tuber which was freed from nematodes by a bath of 30 min. in water of 52° C preceded by a bath of soap-water during 24 hours. After this the tuber was handled as described under A 50 grams of the so-obtained pieces were extrated according to method F in the former experiment. No nematodes were found.

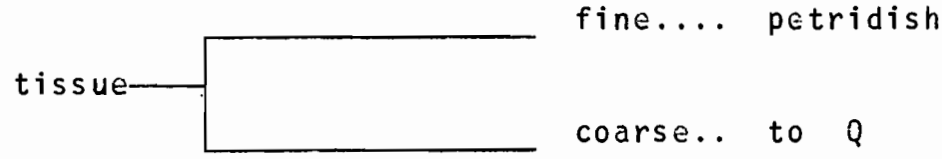
The blending went as follows :

At first the blender with 200 CC of water was started and when he had reached a stable velocity after a few moments 10 grams of tissue were added together with 15 ml. of a suspension of nematodes in water. After the blending the coarse fraction was separated by the fine by a coarse sieve and once again treated in the blender with water and nematodes. In this way each time 2 fine fractions were obtained. These were poured on a cottonwool filter after 20 minutes test without use of the nest of sievesand counted after one day.

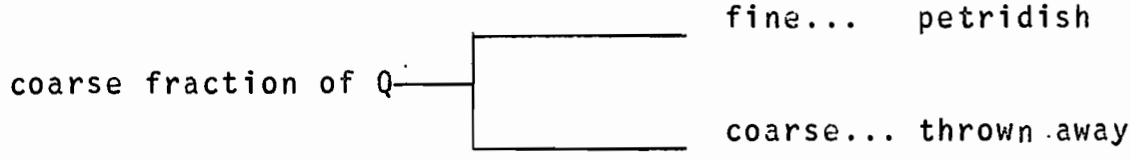
Every treatment was repeated 9 times.

The following scheme was used :

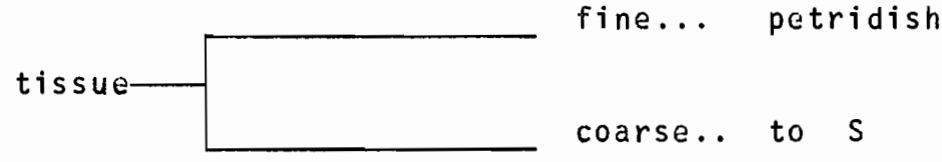
treatment P : blending 5 sec. at low speed



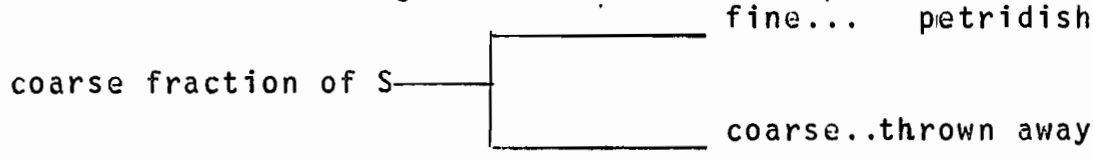
treatment Q : blending 5 sec. at low speed



treatment R : blending 2 sec. at moderate speed



treatment S : blending 2 sec. at moderate speed



treatment T : suspension of nematodes without blending on a petridish.

2)- Results and conclusions

Table IX gives the mean numbers of extracted nematodes and their estimated standard deviation.

Table IX

Treatm.	♀	♂	L 4	L 3	L 2	Tot.
P	1154	647	59	670	42	2572
Q	1232	693	39	696	38	2699
R	1152	629	40	741	34	2597
S	1230	659	22	735	52	2699
T	1349	622	39	775	33	2818
S	160	92.0	15.2	95.4	15.9	289

When we test the differences wether with a non parametric test or with a t-test only a few of them prove to be significant with $\alpha = 0,05$, namely :

T > P for ♀ , L 3 and total.

T > R for ♀

T > S for L 4

If we assume that the observations are normally distributed we can compute that with aprobability of 95% the loss of nematodes by one of the treatments is not bigger than 16% in the first 2 seconds and 13% in the last two seconds.

From this we can conclude that not many nematodes which are not embedded in the tissue are killed in the blender and that the starch which is freed under this circumstances does not prevent the nematodes from passing the cottonwool filter.

D - Experiment III

1)- Materials and Methods

The materials and the preliminary treatment as well as the handling of the fine and coarse fractions were the same as in Experiment I with the exception of the nest of sieves, which was not used. The fine fraction with the washing-water was after 15 min. poured on the cottonwool.

Blended was according to this scheme :

Blending at moderate speed.

- F tissue
  - 2 sec.
    - fine..... petridish F (f)
    - coarse..... mistifier F (c)
- G tissue
  - 2 sec. fine -1.... petridish G (f 1)
  - 2 sec.
    - coarse
      - fine-2 petridish G (f 2)
      - coarse..mistifier G (c)
- H tissue
  - 4 sec.
    - fine..... petridish F (f)
    - coarse.... mistifier H (c)

Blending at low speed

- K tissue
  - 5 sec.
    - fine..... petridish K (f)
    - coarse... mistifier K (c)
- L tissue
  - 3 sec. fine 1..... petridish K (f 1)
  - coarse
    - 3 sec. fine 2...petridish K (f 2)
    - coarse.. mistifier K (c)

2)- Statistical manipulations and results

At first the means and standard deviations were calculated after transformation to logarithms, see table X

With the estimation of the different variances we had to reckon with some series of observations which had a bigger variance than the others.

For them a separate estimation of their variance is made, see table XI

Table XI, Estimation of S

	H (f)	K (f)	L (f 1)	F (f+c)
♀	0.950	1.080	0.625	0.456
♂	1.044	1.145	0.504	0.447
L 4	0.677	0.869	0.688	0.627
L 3	0.886	1.121	0.544	0.466
L 2	1.033	0.950	0.779	0.718
Tot.	0.916	1.101	0.553	0.458

This made it sometimes necessary touse a non-parametric test.

Like in experiment I the distribution of the observations after transformation was compared with the normal distribution.

For this purpose two samples of independent observations were takenat random.

Group one : F (f)-L3, G(f1)- ♀, H (f+c)-L2,K(f+c)-tot.,L(f1)-L4

Group two : F (f)- ♀,G(f1) - tot., H (f)-L2, K (f)- ♂,L(d)- ♀

For group one $\underline{X}^2$= 3,4 for group two $\underline{X}^2$= 7,4 which is not significant for $\alpha = 0{,}05$.

The F (f 1) and the G (f) have got the same treatment. As there proved to be no significant out come the two series of observations were taken together in the calculations for a better estimation.The same is true for G (c) and H (c).

To compare the composition of the extracted numbers of nematodes the percentage of adults and larval stages was computed for each sample. As their logarithms were not normally distributed they were compared by the Kruskall-Wallis test and the Mann-Whitney test. Table XIV gives the mean percentages after different treatments, table XV gives the significance and the direction of changes in the composition.

3 - Conclusions

Table XII shows the superiority of method G : 2 x 2 sec. blending at moderate sped. Method H, 4 sec. blending without tapping after 2 seconds gives much worse results, it gives even

Table X

		F	G	H	K	L	S
♀	f_1	2,8775	2,8175	2,1329	1,2756	2,0215	0,266
	f_2		2,6362			2,5101	0,285
	f_1+f_2		3,1440			2,8196	0,222
	c	2,5219	2,5395	2,5395	2,1199	2,3502	0,311
	f+c	2,9895	3,2378	2,8179	2,1668	3,0281	0,191
♂	f_1	2,8526	2,8526	2,1495	1,4801	2,0040	0,233
	f_2		2,6041			2,4183	0,252
	f_1+f_2		3,0969			2,6941	0,204
	c	2,4260	2,4302	2,4302	2,1336	2,4135	0,218
	f+c	2,9577	3,1886	2,8071	2,8204	2,9780	0,206
L_4	f_1	1,9485	1,9485	1,5680	0,7896	1,2044	0,229
	f_2		1,9072			1,4368	0,407
	f_1+f_2		2,2273			1,8630	0,251
	c	1,3369	1,0851	1,0857	0,2254	0,9112	0,372
	f+c	2,1060	2,2749	1,1728	1,4341	1,9523	0,300
L_3	f_1	2,8513	2,8573	2,2239	1,4661	2,1120	0,211
	f_2		2,1928			2,7387	0,211
	f_1+f_2		3,2169			2,9582	0,242
	c	2,4990	2,4220	2,4220	2,6237	2,5479	0,303
	f+c	2,9769	3,2639	2,8099	2,7250	5,1261	0,212
L_2	f_1	2,4430	2,4430	1,7096	0,8243	1,1030	0,215
	f_2		2,3889			1,8657	0,341
	f_1+f_2		2,7235			2,1953	0,275
	c	1,4166	1,0583	1,0583	1,5842	1,4303	0,563
	f+c	2,5285	2,7369	2,0306	1,7147	2,2050	0,406
tot.	f_1	3,4202	3,4202	2,7545	1,9764	2,6030	0,213
	f_2		3,2600			3,1376	0,256
	f_1+f_2		3,6919			3,3598	0,191
	c	2,9974	2,9507	2,9507	3,2657	3,0756	0,282
	f+c	3,5235	3,7707	3,3443	3,2774	3,5635	0,200

Table XII fine + coarse fractions

Quotients of numbers

	G/F	F/H	G/H	L/F	G/L	L/K	G/K
♀	1,77	1,48	2,63	1,09*	1,62	>1 *	>1
♂	1,70	1,41*	2,41	1,05*	1,62	>1 *	>1
L_4	1,47*	2,15	3,18	0,70*	2,10	>1 *	>1
L_3	1,94	1,47	2,84	1,41*	1,38*	>1 *	>1
L_2	1,62*	3,15	5,08	0,48	3,40	>1 *	>1
tot	1,77	1,51	2,67	1,10*	1,61	>1 *	>1

Table XIII fine and coarse separated

Quotients of numbers

	G(f1+2)/H(f)	G(f1+2)/F(f)	L(f0+2)/K(f)	L(f1+2)/F(f)	L(f2)/F(f)	G+H(c)/F(c)	K(c)/F(c)	L(c)/F(c)	K(c)/L(c)
♀	2,27	>1	>1	1,08*	0,61*	1,04*	1,58*	1,07*	1,48*
♂	2,05	>1	>1*	0,81*	0,49	1,01*	2,05	1,54*	1,33*
L_4	1,66	>1	>1	0,72*	0,27	0,56*	0,77*	0,43	1,80*
L_3	3,14	>1	>1	1,51*	0,91*	0,84*	1,33*	1,12*	1,16*
L_2	1,75	>1	>1	0,52*	0,24	0,44*	1,47*	1,03*	1,43*
tot	2,19	>1	>1	1,00*	0,60	0,90*	1,62*	1,20*	1,35*

* this quotients do not differ significantly from 1 with $\alpha = 0.05$

Table XIV. The perceived composition of extracted numbers

stage	♀		♂		L_4		L_3		L_2	
treatment	mod.	slow	mod.	slow	mod.	slow	mod.	slow	mod.	slow
fine 1	29	26	27	25	3,4	4,0	27	37	11	3,2
coarse 1x blended	34	–	27	–	2,2	–	32	–	2,6	–
fine 2	24	27	22	22	4,4	2,0	34	40	14	5,3
coarse 2x blended	39	31	30	35	1,4	1,0	30	30	1,3	2,3
coarse, method K		33		34		1,0		26		2,4

Table XV Significant differences in percentages

differences	♀	♂	L4	L3	L2
<u>fine and coarse</u>					
moderate:					
fine 1 - coarse 2 sec.	−	o	+	−	+
fine 2 - coarse 4 sec.	−	−	+	o	+
fine 1 - coarse 4 sec	−	−	+	o	+
slow:					
fine 1 - coarse 6 sec	o	o	+	o	o
fine 2 - coarse 6 sec	o	−	+	+	+
<u>between fine</u>					
moderate:					
fine 1 - fine 2	+	+	o	−	−
slow:					
fine 1 - fine 2	o	o	+	o	o
slow and moderate:					
all slow - all mod.	o	−	o	+	−
<u>between coarse</u>					
moderate:					
coarse 2 sec - coarse 4 sec	o	−	+	o	+
slow:					
coarse 6 sec - coarse 5 sec	o	o	o	o	o
slow and moderate:					
6 sec. slow - 4 sec. mod.	−	o	−	o	o
5 sec slow - 4 sec. mod.	o	o	o	o	+
6 sec. slow - 2 sec. mod.	−	+	−	o	o
5 sec. slow - 2 sec. mod.	o	+	−	o	o

\+ The first mentioned is bigger

− The first mentioned is smaller

o No significant difference

less nematodes than method F.

Method L is about as best as method F except for the extraction of L 2.

The comparaison of method K with other ones is difficult because of its big standard deviation. This does not say much for the use of this method. Anyway its results are worse than those of L or G (Mann-Whitney test $\alpha = 0,05$).

From table XIII we candraw the following conclusions.

The 2 sec.additional blending at moderate speed do not give a significant decline of the number of nematodes which are extracted from the coarse fraction. But they do give an additional fine fraction with about as much nematodes as in the first fine fraction. However the blending four seconds at a stretch reduces the number from the fine fraction considerably.

The slow blending of 5 sec. at a strech without tapping (method K) is in the fine fraction significantly worse than 2 x 3 sec. slow blending (method L). In the coarse fraction it seems to give a some what higher number of nematodes.

Generally speaking, the differences between the coarse fractions are not very big, the differences appear in the fine fractions.

The composition of the extracted numbers as to adults and larval stages can change much with the use of an other method. This is shown by table XIV and table XV? These changes can be summarized as follows :

a The blender at moderate speed.
When we reapeat the blending then the numbers of :
♀ and ♂ from coarse increases
and from fine decreases,
their numbers from coarse are bigger then those from fine.

L4, L3 an L2 : The inverse effects, for L3 the difference between fine and coarse is smaller, for L2 very big.

b The blender at low speed.
The same tendency as under a but almost never significant

c When we blend at low instead of at moderate speed we get :
in the fine fraction less ♂ and L2
and more L3

in the coarse fraction more ♂and L2
and less ♀and L4

4)- Discussion

a - The small number of nematodes which were extracted from the fine fraction when their had been nothing tapped halfway the blending seems contrary to the results of Experiment II.

The big variances of this fractions point to the possibility that the extraction on cottonwool did not work well. Maybe the amount of starch was still too big here for filters with a diameter of 9 cm. For, the amount of starch was here twice as big as in the other fine fractions, and also twice as big as in Experiment II.

b - The results of the treatment according to G let us suspect, that a third blending might improve the extraction yet. As to this, the extraction with the blender at low speed should be examined further, also in view of the big differences in composition between the fractions after blending with a higher speed.

IV - Host plants of Scutellonema bradys

A - Methods and Materials

No different plant species were tested, for the bigger part cover crops and more over some plants that after form a mixed culture together with yams. The young plants were each put in a plastic beaker of 500 CC with sterilized soil. Shortly there after the soil was inoculated with a counted suspension together with a series of pots without plants each series consisted of 10 pots.

After 45 days the 10 pots without a plant were extracted in the seinhorst elutriator almost immediately after that some pots with series of plants which did not grow very well. Their roots were coloured with lactophenol-cotton blue and examined under the dissecting microscope.

The other series of plants were left growing as time permitted. Their soil was extracted in the above mentioned way and their roots were cut into pieces and extracted for one week under the mistifier.

B - Results

Fig.III Shows for every series of plants the mean number of nematodes that was extracted per pot from the soil and the roots together as well as the percentage from the total that was found in the roots. The latter is given only were a reasonable quantity was recovered. Moreover is noted wether second stage juveniles were found in the roots.

As can be seen in fig.III it is arbitrary to draw a line between host plants and others. However, it can be said that Crotolaria usaramoensis, Vigna - 140, Desmodium biarticulatum and Tephrosia ehrenbergiana are good host-plants. Problably Lycopersicon esculentum var. Roma and Vigna rouge are host plants too, but here them results are unclear by the bad condition of the plants. In bad condition were also Lycopersicon esculentum var.Ronita and Hibiscus esculentus, but here the other results pointed to an unsuitibility as host plants.

The numbers extracted from mais, millet, Desmodium lasiocarpum, Fleuringia congesta, Stylosanthes gracilis, St.humilis, st.mucronata and Clitoria rubinochinosa are not different from the numbers that were to be expectfrom " fallow " pots at their moments of counting.

Of the rest can be said, that the numbers of nematodes might be decreasing, but not as quickly as without any plants. From suitable to less suitable hosts the orderis about as follows : Cassia hirsuta, Phaseolus calcaratus, Pueraria phaseoloïdes, Desmodium tortuosum, Desmodium heterocarpum, Indigofera trita.

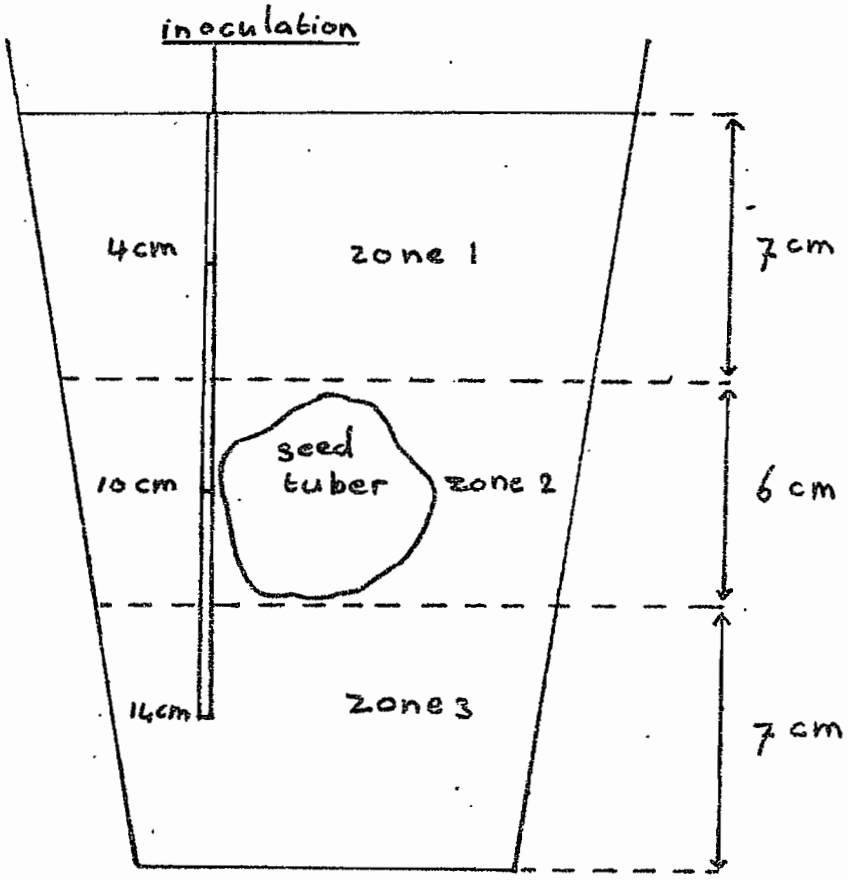


Figure I Inoculation depths

Figure II, Example of calculations

F(f)-L_4 : The numbers L_4 which were extracted from the fine fraction after treatment according to F

number	X = log number	$\frac{X - \bar{X}}{S}$
500	2.6990	-0,972
950	2.9777	0,104
950	2.9777	0,104
1150	3.0607	0,425
1400	3.1461	0,754
1200	3.0792	0,496
850	2.9294	-0,082
1050	3.0212	0,272
550	2.7404	-0,812
750	2.8751	-0,292

$\bar{X} = 2,9507$

$\Sigma X^2 = 87,250.323.89$ together

with Σx^2 from B(f)-L_4, D(f)-L_4 and F(f)-L_4

this gives $S^2 = 0,067237$ and $S = 0,259$

The same was done for the other series, this gave the following distribution of $\frac{X - \bar{X}}{S}$

X	%	ideal distribution	perceived distribution Group I	Group II	Group III
$-\infty$	0				
-1,282	10	5	7	1	3
-0,842	20	5	4	6	5
-0,524	30	5	5	6	6
-0,253	40	5	5	5	6
0	50	5	7	7	5
+0,253	60	5	2	9	7
+0,524	70	5	4	5	6
+0,842	80	5	5	3	6
+1,282	90	5	7	3	3
$+\infty$	100	5	5	5	4
total	100	50	50	50	50
χ^2			4,6	9,2	3,4

χ^2 is 16.92 for $\alpha = 0,05$ so H_0 is not rejected

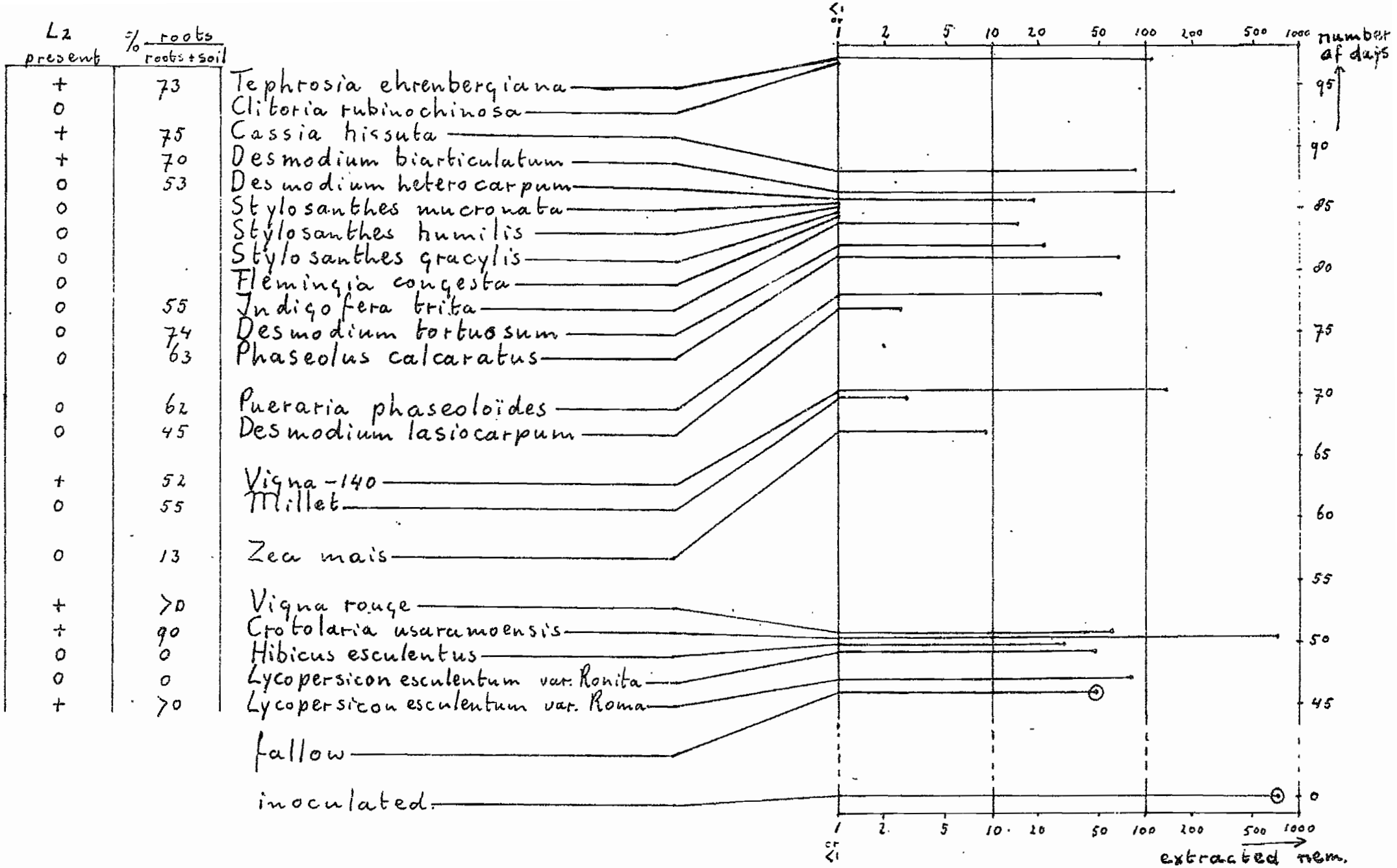


Fig. III Some data concerning the suitability of plants as hostplants.

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