

EXPERIMENTS ON THE INTERACTION OF *HOPLOLAIMUS UNIFORMIS* AND *FUSARIUM OXYSPORUM* F. *PISI* RACE 3 AND ITS IMPORTANCE IN "EARLY YELLOWING" OF PEAS

BY

R. E. LABRUYÈRE, H. DEN OUDEN and J. W. SEINHORST

Instituut voor Plantenziektenkundig Onderzoek, Wageningen, Netherlands

The disease of peas, which is now called "early yellowing" (Dutch "vroeg vergeling") was first mentioned by SCHREUDER (1951), who wrongly identified it with "St John's disease" of earlier authors. The Dutch name of the disease is derived from the symptoms in the overground parts of the plants: a yellowing appears early in the season in the lower leaves and spreads slowly to those higher up the stem. The yellowing starts at the edges of the leaflets and progresses inwards. Withering and shrivelling may soon follow the yellowing and occasionally under greenhouse conditions the leaves become very thin and flaccid before the yellowing is conspicuous. Plants with the symptoms described above are always seriously affected by root rot but not all plants with root rot in an affected field show overground symptoms. Favourable weather conditions in May and June seem to repress the development of the latter. Figure 1 shows the relation between the occurrence of root rot and overground symptoms in a field experiment. The root rot is considered the primary and most important symptom of the disease. The cortex of the diseased roots is blackened and necrotic. Thin lateral roots are often completely dead and break off easily. The vascular bundle of affected roots shows an orange discolouration, which often starts as more intensely coloured spots at points where damaged lateral roots are attached to thicker roots. Where the attack is especially severe even the vascular bundle in the stem may be orange. In the summary of the article by SCHREUDER (1951) this disease is wrongly called a pea *wilt* disease. Neither in the article nor in the Dutch summary is it described as such. Actually affected plants remain more rigid the more severely they are stunted. Unlike *Fusarium wilt* and "St John disease" (*Heterodera goettingiana*) "early yellowing" may affect peas

as early as the end of April and at low soil and air temperatures (hence the name). Such an early attack may stunt the plants severely. However, only rarely do they die before flowering. Usually they produce one or two small pods.

According to SCHREUDER (1951) this disease is closely associated with the occurrence in the roots of the diseased plants of a strain of

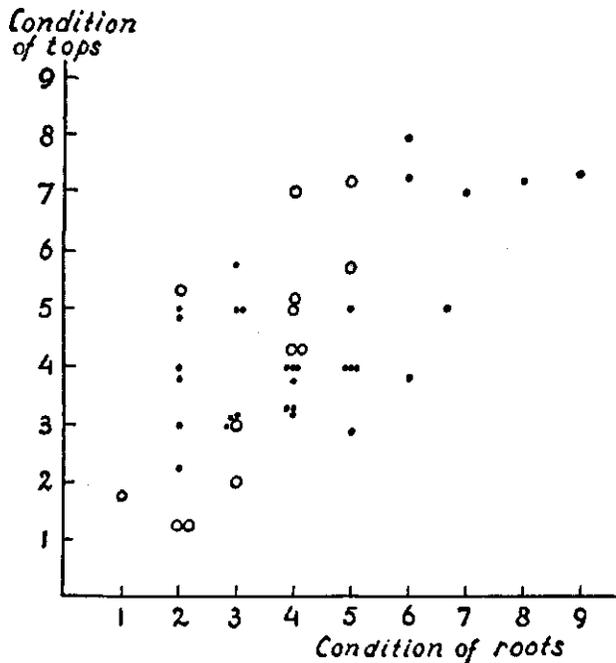


Fig. 1. Relation between the occurrence of root rot and symptoms of "early yellowing" in the overground parts of peas in a field experiment. Horizontal: degree of root rot; vertical: symptoms in overground parts. 1 = serious root rot and serious overground symptoms; 9 = almost healthy roots or tops; · on untreated soil and after different treatments with "Mylone". o after DD treatment (three dosages).

the fungus *Fusarium oxysporum* SCHL. em. SN. & H. f. *psii* LINF. She classified it as race 3 of this fungus, as its pathogenic characters were markedly different from those of the races 1 and 2.

However, no symptoms in either roots or overground parts could ever be obtained by growing peas in autoclaved soil, which had been inoculated with *F. oxysporum* isolated from plants suffering from "early yellowing". A very limited rot developed in pea roots, which

had been damaged by cutting them before bringing the plants in contact with the fungus (LABRUYÈRE & SEINHORST 1954).

With only one exception (after a DD treatment, see p. 342), fields, where the disease was found, contained medium to high populations of *Hoplolaimus uniformis* THORNE (300-3000 nematodes per 500 g of soil). It was never seen in fields on soil types which are known to be practically free from this nematode such as sandy loam and clay soils (LABRUYÈRE & SEINHORST, 1954). When nematodes were removed from soil, on which "early yellowing" occurred, either by mechanical means (elutriation) or by treatment with Mylone¹), peas grown on it in the field or in pots were not or only lightly attacked. However, peas grown in this treated soil after reinoculation with *Hoplolaimus uniformis* were severely attacked again (Table I, SEINHORST, 1954). Apparently there is a relation between medium to high population densities of *H. uniformis* in the soil and "early yellowing". The symptoms of the disease are also much more similar to those of a nematode disease than to those of attacks by *F. oxysporum*. However, "early yellowing" does not occur on all soils with high population densities of *H. uniformis*. Peas were healthy in a field at Rockanje (pop. density 300-650 nematodes per 500 g), in pot experiments with natural soil from different localities containing 100-3000 nematodes per 500 g, and in sand inoculated with large numbers of *H. uniformis*. In the last the nematodes were seen feeding on the roots in minute cracks in the cortex. In gardens at Ede and Bennekom and in several fields at Hoeven, "early yellowing" of the overground parts of peas occurred on an area much smaller than where medium to high population densities of *H. uniformis* were found. Some root rot occurred in healthy looking plants from both parts of the field. In one field the degree of attack was more severe where peas had been grown in the previous year than where they had not, although there was no material difference in eelworm population levels. The results of inoculation experiments with *H. uniformis* from fields on which peas had never been grown, or not grown for many years, do not suggest major differences in the capacity of different populations of this nematode to attack peas. Apparently "early yellowing" of peas is caused by the interaction of *H. uniformis* with one or more other factors. Such a factor could be *Fusarium oxysporum* f. *pisi* race 3 of SCHREUDER (1951), a fungus, isolated from almost all pea plants investigated which showed the typical symptoms of the disease.

¹) Active ingredient 3-5 dimethyl tetra hydro-1-3-5-2H-thiadiazine-2-thione.

TABLE I

Results of inoculation experiments on "early yellowing" of peas var. *Unica*. (1), 3), 4), and 6) after SEINHORST, 1954)

Treatment of soil	No. of pots	Population densities of <i>Hoplotisus uniformis</i> per 500 g of soil		Number of peaplants affected by "early yellowing"			Means of ratings per plant for size and disease symptoms
		beginning of experiment	end of experiment	severely attacked	lightly attacked	healthy	
1) Soil Ede	2	appr. 2000	?	6	0	0	4 (group rating)
2) Soil Hoeven	3	" 1600	2200	5	3	1	5 ¹⁾
3) As 1); nematodes removed mechanically	2	" 50?		0	0	6	8 (group rating)
4) 3) + <i>Tylenchorkhynchus</i> sp. and <i>Pratylenchus</i> sp.	2	" 50?	8	0	0	6	8 (group rating)
5) 2) treated with Mylone	4	" 22	22	1	2	12	7.7
6) 3) + <i>H. uniformis</i> from Ede	2	" 2000	700	6	0	0	4 (group rating)
7) 5) + <i>H. uniformis</i> from Hoeven	2	" 1500 in top 10 cm of pots	880	3	3	2	4.5
8) 5) + <i>H. uniformis</i> from Rockanje	2	the same	580	5	0	2	3

1) I severely stunted, only top leaves not withered 7, 8, 9 healthy, but different sizes.

INOCULATION EXPERIMENTS ON FOAM AGAR PLATES

As the root rot described above is considered the primary symptom of "early yellowing", attempts were made to reproduce this symptom experimentally. The "foam agar plate" technique (DEN OUDEN, 1958) was used because

1. Activities of the organisms and the development of symptoms could be followed from day to day.
2. Separate roots could be inoculated individually. As pea varieties are very homogeneous each inoculation can be considered as a replicate, so reducing the number of plants and the number of nematodes necessary in the experiments. As the latter had to be picked by hand from a mixture extracted from soil this is a great advantage.
3. Experiments cannot be done in partly sterilized soil as often *Hoplolaimus uniformis* does not long survive in it.

A drawback of the method was that the experiments had to be finished before the agar began to dry out. However, pea plants could be kept growing for more than four weeks at 15° to 18° C which was sufficient for the development of symptoms in the roots.

Three experiments were made with single pea plants (variety Unica) in foam agar cultures. The same treatments were used throughout: —

1. *Hoplolaimus uniformis* five to seven inoculations per bag with 50-100 specimens placed near to roots.
2. *Fusarium oxysporum* five to seven inoculations per bag with mycelium.
3. Inoculation with both organisms as in (1) and (2).
4. Controls, not inoculated.

In the first experiment, one plant was used per treatment, in the second four and in the third one. Altogether there were six plant replicates for each treatment. The results of the second experiment are given in Table II.

The plants inoculated with *H. uniformis* only developed a slightly grey discolouration of a limited length of root. Some inoculations with *Fusarium oxysporum* resulted in discolouration and superficial destruction of the root cortex near the inoculation sites. This was especially so where the inoculum had been placed in contact with the roots. Where it had been put a short distance from the roots as in the third experiment

the roots did not suffer visible damage although the mycelium grew through the agar and on the root surface (fig. 2).

However, most inoculations with both organisms resulted in dark brown discolouration often followed by complete decay of the root cortex near the inoculation site (fig. 3). Measurements of the length of discoloured root and counts of the number of inoculations resulting in decay of the cortex in the second experiment are shown in Table II. Three weeks after inoculation eight (out of twenty four) inoculations with *F. oxysporum* and twenty three (out of twenty eight inoculations)

TABLE II

Results of an inoculation experiment on foam agar plates with *H. uniformis* and *F. oxysporum* forma pisi race 3

Inoculated with:	Plant No.	Number of inoculations per plant	Two weeks after inoculation		Three weeks after inoculation	
			Length of discoloured root per inoculation in mm.	Condition of discoloured parts of roots	Number of inoculations which resulted in:	decay of cortex
A Not inoculated	1	—	0	—	—	—
	2	—	0	—	—	—
	3	—	0	—	—	—
	4	—	0	—	—	—
B <i>H. uniformis</i>	5	4	1	slightly grey		
	6	5	4	slightly grey	0	0
	7	5	1	slightly grey		
	8	4	0	slightly grey		
C <i>F. oxysporum</i>	9	7	5	light brown	8 ¹⁾	16 ³⁾
	10	6	3	" "	(12 mm	
	11	7	1	" "	per inocu-	
	12	4	6	very light	lation)	
D <i>H. uniformis</i> + <i>F. oxysporum</i>	13	7	4	brown to	23 ²⁾	5 ³⁾
	14	7	8	black	(16 mm	
	15	7	8	"	per inocu-	
	16	7	11	"	lation)	

1) Severest case as fig. 5.

2) Lightest case as fig. 5 more generally as fig. 4.

3) Difference between C and D highly significant ($P < 0.01$).

with *H. uniformis* and *F. oxysporum* had resulted in cortex decay. The mean length of affected root per inoculation in the latter was three and a half times that in the former. Three weeks after inoculation sections were made of attacked portions of roots from each treatment.

R. E. LABRUYÈRE *et al*: *Early yellowing in peas*



Fig. 2. Root system of pea plant four weeks after inoculation with *Fusarium oxysporum*.

R. E. LABRUYÈRE *et al*: *Early yellowing in peas*

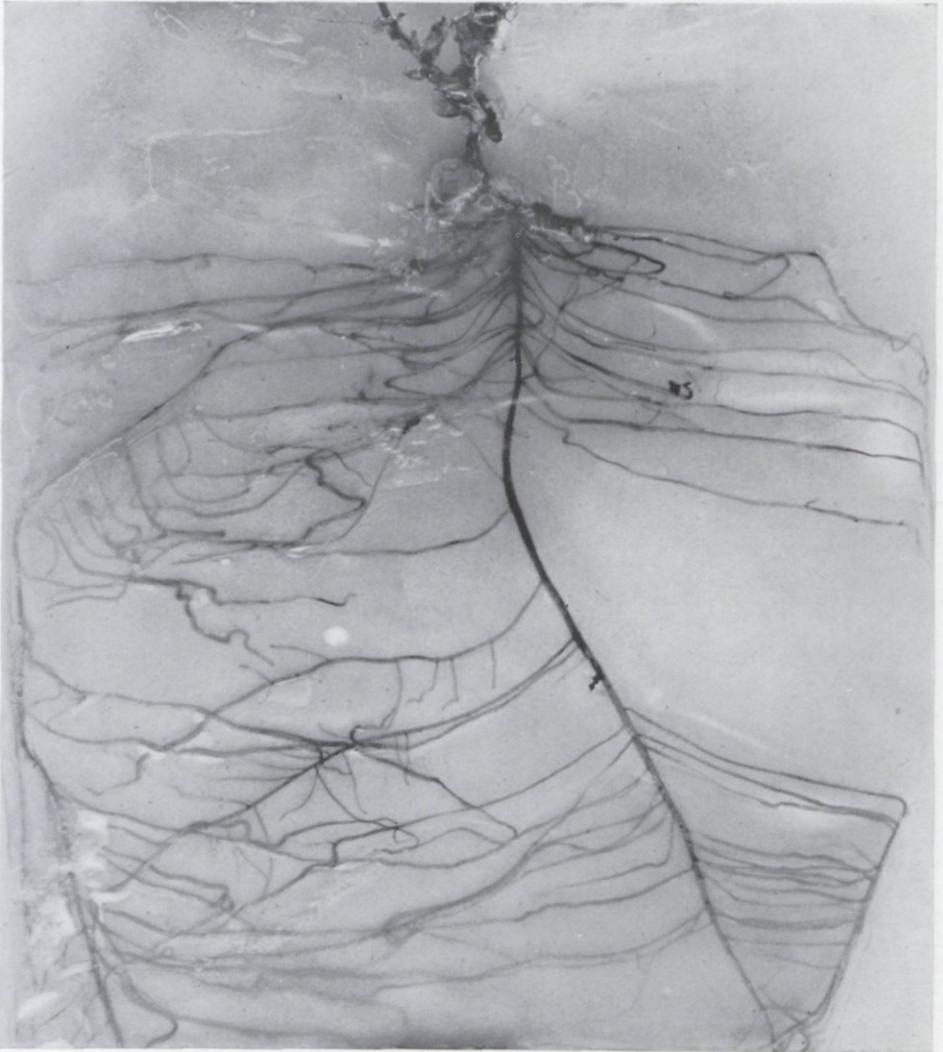


Fig. 3. Root system of pea plant four weeks after inoculation with *Hoplolaimus uniformis* and *Fusarium oxysporum*.

R. E. LABRUYÈRE *et al.*: *Early yellowing in peas*



Fig. 4. Section of a root three weeks after inoculation with *Hoplolaimus uniformis* and *Fusarium oxysporum*.

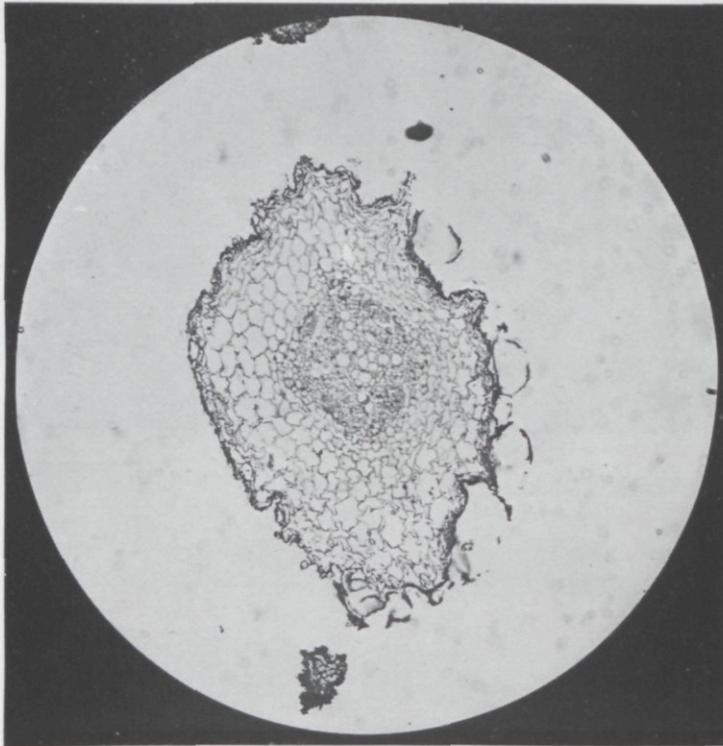


Fig. 5. Section of a root three weeks after inoculation with *Fusarium oxysporum*.

R. E. LABRUYÈRE *et al*: *Early yellowing in peas*

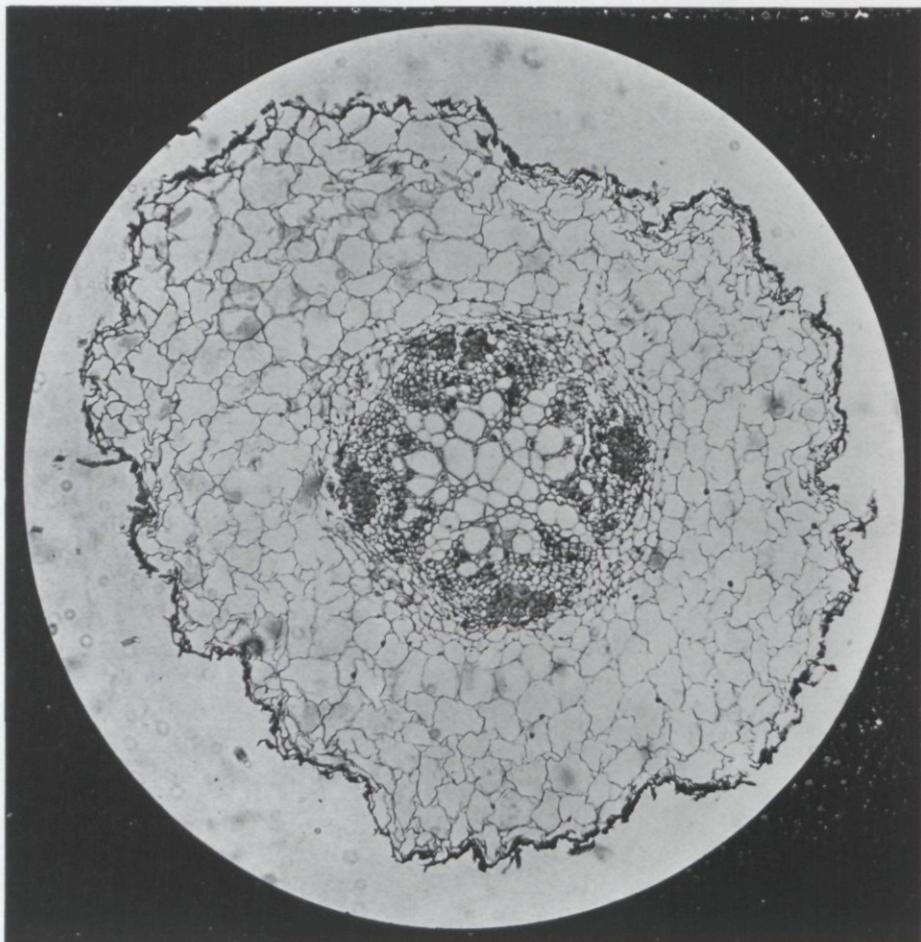


Fig. 6. Section of a root three weeks after inoculation with *Hoplolaimus uniformis*.

Inoculation with *H. uniformis* produced only cracks in the cortex (fig. 6). Eelworms had been seen active in these cracks. Inoculation with *F. oxysporum* caused destruction of the outer layer of the cortex in some cases (fig. 5) but this destruction never reached the vascular bundle. However, inoculation with both *F. oxysporum* and *H. uniformis* often resulted in complete destruction of the cortex (fig. 4). The worst attack by *F. oxysporum* was comparable to the lightest seen after inoculation with both *F. oxysporum* and *H. uniformis*. Therefore the ratio between severity of attack after inoculation with both organisms and that after inoculation with *F. oxysporum* only was much greater than the 3.5 to 1 mentioned above.

CONCLUSIONS

The experiments confirm the conclusion already drawn from other observations that neither *Fusarium oxysporum* SCHL. em. EN & *H. f. pisi* LINF. race 3 of SCHREUDER (1951) nor *Hoplolaimus uniformis* THORNE can cause serious root rot in peas when they operate separately. However, interaction of both organisms can lead to extensive decay of the root cortex which is the chief symptom in the roots of peas suffering from "early yellowing".

"EARLY YELLOWING" AFTER DD TREATMENT OF THE SOIL

One case of typical "early yellowing" was found on soil which was practically free from nematodes. In a field experiment (SEINHORST, BIJLOO & KLINKENBERG, 1956) peas were not only severely attacked by early yellowing on the untreated plots but also on those treated with DD seven months before sowing. It was unlikely that residual toxicity of DD in the soil alone had caused this as the symptoms were exactly the same as those on the untreated plots and did not appear until six weeks after sowing. Possibly traces of DD in the soil made the pea roots susceptible to attack by *Fusarium oxysporum f. pisi* race 3. Since the fungus was unable to attack pea roots in sterilized media, it is unlikely that the changed soil conditions after DD treatment would enable it to injure the peas directly. Also, since peas grown in the same soil after harvest remained completely healthy (eight pots, thirty plants), there did not appear to have been a change in pathogenicity.

The author wish to thank Miss A. Kits and Miss G. Engels for assisting in inoculating the plants and making microtome sections.

ZUSAMMENFASSUNG

Untersuchungen über die Wechselwirkung von Hoplolaimus uniformis und Fusarium oxysporum f. pisi Rasse 3 und ihre Wichtigkeit zur frühen Vergilbung von Erbsen.

In einigen Versuchen nach der Schaumagarplattenmethode von Den Ouden wird nachgewiesen, dass durch *Hoplolaimus uniformis* Thorne oder *Fusarium oxysporum* forma *pisi* Rasse 3 keine ernsthaften Krankheitssymptome an den Wurzeln von Erbsen hervorgerufen werden. Durch den Angriff beider Organismen zusammen wurde jedoch die Wurzelrinde von Erbsen ganz vernichtet. Auch trat hierbei eine rötliche Verfärbung des Zentralzylinders der angegriffenen Wurzeln auf. Die unterstützt die Auffassung, dass frühe Vergilbung bei Erbsen, wobei man immer *H. uniformis* im Boden und *Fusarium oxysporum pisi* in den faulenden Wurzeln findet, durch Zusammenwirken der beiden Organismen verursacht wird.

Frühe Vergilbung trat auch auf nach Bodenentseuchung durch DD. Wahrscheinlich hat Schädigung durch dieses Nematizid die Erbsenwurzeln hier für *Fusarium* anfällig gemacht.

REFERENCES

- LABRUYÈRE, R. E. & SEINHORST, J. W. (1954), Vroege vergeling bij erwten een aaltjesziekte. *Tijdschr. PlZiekt.*, **60**, 261-262.
- UDEN, H. DEN (1958), A new method for culturing plants enabling the observation of nematodes on growing roots. *Tijdschr. PlZiekt.*, **64**, 269-272.
- SCHREUDER, J. C. (1951), Een onderzoek over de Amerikaanse vaatziekte van de erwten in Nederland. *Tijdschr. PlZiekt.*, **57**, 175-206.
- SEINHORST, J. W. (1954), Een ziekte in erwten, veroorzaakt door het aaltje *Hoplolaimus uniformis* THORNE. *Tijdschr. PlZiekt.*, **60**, 262-264.
- SEINHORST, J. W., BIJLOO, J. D. & KLINKENBERG, C. H. (1956), Een vergelijking van de nematicide werking van DD en van 3-5-dimethyltetrahydro-1-3-5-2H-thiadiazine-2-thion. *Meded. LandbHoogesch., Gent*, **21**, 387-395.