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## **Pythiaceous Fungi and Plant-Parasitic Nematodes in California Pear Orchards**

### **I. Occurrence and Pathogenicity of Pythiaceous Fungi in Orchard Soils**

**Carl W. Nichols, S. M. Garnsey, R. L. Rackham,  
S. M. Gotan, and C. N. Mahannah**

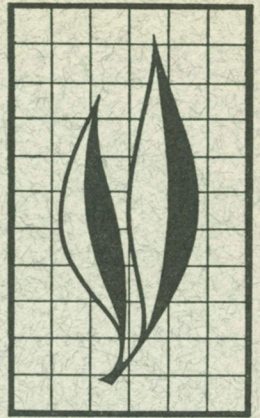
### **II. Incidence and Distribution of Parasitic Nematodes in Orchard Soils**

**Alex M. French, B. F. Lownsbery, S. M. Ayoub, A. C. Weiner,  
and N. El-Gholl**

### **III. Effect of Reduction of Nematode Populations by Soil Fumigation on Subsequent Growth of Pear Seedlings**

**B. F. Lownsbery, J. T. Mitchell, and S. M. Paracer**





### Pythiaceous Fungi and Plant-Parasitic Nematodes in California Pear Orchards

I. During a year's survey of 126 California pear orchards with varying degrees of pear-decline damage, a total of 3,586 isolates of soil-borne fungi of the genera *Phytophthora* and *Pythium* were recovered, and, where possible, identified as to species.

A direct relationship was found between the severity of pear-decline damage in an orchard and the percentage of isolates from the orchard that were *Pythium* spp.; and a corresponding inverse relationship between pear-decline damage severity and the percentage of isolates that were *Phytophthora* spp.

Isolates of *Phytophthora cactorum* and *P. cryptogea* were found to be pathogenic on *Pyrus communis* L. hort. var. Winter Nelis and *Pyrus serotina* Rehd. seedlings. These pathogens were found infrequently, and it was concluded that pythiaceous fungi were not the primary cause of pear decline.

*Continued on inside back cover*

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## II. Incidence and Distribution of Parasitic Nematodes in Orchard Soils<sup>1</sup>

### INTRODUCTION

Deterioration of the root system and sparseness of healthy feeder roots, noted in the first observations of pear-decline disease in the Pacific Northwest, suggested the possibility that plant-parasitic nematodes were involved as a causal factor (Blodgett *et al.*, 1955). Early studies of the etiology of the condition included examination for nematodes in and about the pear roots (Woodbridge *et al.*, 1957; Blodgett, 1958; Blodgett and Aichele, 1959). Although these studies revealed the presence of several types of parasitic nematodes in some orchards in the Northwest (Blodgett and Aichele,

1959), they failed to provide any evidence of nematode involvement with the disease (Batjer, 1958; Anon., 1960; Chiarappa, 1960).

After pear decline was recognized in California (Nichols *et al.*, 1960), a nematode survey of pear orchards was initiated as part of a broad research program under the auspices of the University of California Research Committee on Pear Decline (Nichols *et al.*, pp. 577-610, this issue; Stout, 1961). This report concerns the methods used in this survey and the information it has provided.

### METHODS

Soil samples were collected each month for one year beginning in April, 1961, from the upper root zones of the same trees in the same 126 randomly selected pear orchards throughout California described by Nichols *et al.* (1964).

Each sample was thoroughly mixed in the polyethylene bag in which it was collected, and the nematodes present in 250cc of the soil were extracted by a combination of screening and Baermann-funnel techniques. Four screens (mesh sizes 20, 100, 200, and 325) were employed in series, and the residue from the three finest screens was

washed into a beaker and placed on the tissue of a Baermann funnel for 3 to 5 days.

At the time of the first soil sample collection (April, 1961), samples of feeder roots also were gathered from the pear trees. These and portions of the first month's soil samples were examined for nematodes at the University of California Agricultural Experiment Station at Davis. Weighed samples of feeder roots were subjected to a mist-extraction process (Lownsbery and Serr, 1963) and the nematodes extracted during two weeks were identified and counted. The soil was processed

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by two methods: (1) A 50cc sample from each orchard was placed directly into a Baermann funnel and extracted for one week; and (2) a 250cc sample was suspended in water and passed through 5 screens (mesh sizes 20, 100, 100, 200, and 200) in a racked series. Aliquots of the Baermann funnel extract and of the residue washed from the last four screens were examined and nematodes of each type counted.

The nematodes were in most cases identified to species. Exceptions included the root-knot and cyst nematodes (*Meloidogyne* and *Heterodera*

species); specific identification of these could not be made, since only larvae were at hand. Another exception was certain spiral nematodes (*Helicotylenchus* species), whose taxonomy at the time of the survey was being revised.

It should be emphasized that the nematodes present in soil samples collected in this survey were not necessarily parasitic on the pear trees. Many of the orchards were non-tilled and very weedy during most or all of the year, and weeds and grasses undoubtedly supported part of the nematode populations.

## RESULTS

Repeated sampling of the same orchards throughout the year and nematode extraction of one set of samples by the four different procedures described above provided a comprehensive picture of the parasitic nematode fauna of each orchard included in the survey.

The types of plant-parasitic nematodes most frequently found in the pear orchards (table 1) will be discussed separately in order of their prevalence.

### 1. *Paratylenchus* species — the pin nematodes

Members of this genus of small ectoparasitic nematodes were observed in samples from 121 (96 per cent) of the orchards. *Paratylenchus hamatus* Thorne and Allen was the only species found which could be definitely identified, and it was recorded in 85 of the orchards.

Pin nematodes were observed in samples from all of the 18 counties included in the survey and in all seasons. Population densities as great as 2,500 pin nematodes in 250cc of soil were noted. The number of these ectoparasites recovered from root samples was usually small. In one case, however, they were recovered at the rate of 143 per gram of root. This recovery from pear roots is a good indication that pin

nematodes are parasites of pear, and not merely of orchard cover crops.

### 2. *Xiphinema americanum* Cobb—the dagger nematodes

This species occurred in 118 (93 per cent) of the orchards sampled. The only member of the genus found during the survey, it was present in every orchard sampled except five in Santa Clara County and one each in Placer, El Dorado and Kern Counties. The greatest population found of *Xiphinema americanum* was about 200 nematodes in 250cc of soil. The number of orchards in which this species was reported remained about the same through the year. Indications that Bartlett pear is a host for *X. americanum* are presented by Lownsbury (1964).

### 3. *Tylenchorhynchus* species — the stunt nematodes

One or more members of this genus of ectoparasitic nematodes were found in 95 (75 per cent) of the orchards. As many as 700 individuals were recovered from 250cc of soil.

Species of *Tylenchorhynchus* and the number of orchards in which they were found were:

<i>Tylenchorhynchus brevidens</i> Allen . .	79
<i>Tylenchorhynchus clarus</i> Allen . . .	22
<i>Tylenchorhynchus capitatus</i> Allen . .	12



TABLE 1  
OCCURRENCE OF PLANT-PARASITIC NEMATODES IN PEAR ORCHARD SOILS  
IN CALIFORNIA

Nematode	COUNTIES																			TOTAL
	Contra Costa	El Dorado	Glenn	Kern	Lake	Los Angeles	Mendocino	Napa	Placer	Sacramento	San Benito	San Diego	San Joaquin	Santa Clara	Solano	Sonoma	Tuolumne	Yuba		
	Number of orchards surveyed																			
	5	13	1	1	16	1	8	3	20	18	1	1	1	19	8	7	2	1		
	Number of orchards in which specimens were found																			
<i>Criconemoides</i> spp.....	1	8			2		1		20	3				1		1	1		38	
<i>Helicotylenchus</i> spp.....	2	13			4		4	1	20	13	1		1	14	4	3		1	81	
<i>Heterodera</i> spp.....	3	6			4		2		3	4		1	1	7	3	1			35	
<i>Meloidogyne</i> spp.....		10	1	1	2		2		13	4				2		2	2		39	
<i>Paratylenchus</i> spp.....	5	13	1	1	16	1	7	2	19	18	1	1	1	18	7	7	2	1	121	
<i>Pratylenchus</i> :																				
<i>neglectus</i> .....		8			5	1	1		12	10		1	1	6		2	1		48	
<i>thornei</i> .....		3			8		3		1	1				3	1	2	1		23	
<i>vulnus</i> .....		2		1		1	1		2	8			1	1		2			19	
<i>crenatus</i> .....		5							9										14	
<i>penetrans</i> .....		5							3	2									10	
<i>zeae</i> .....									10										10	
other species.....	1				3				5	4	1	1		2	1	2			20	
<i>Trichodorus</i> spp.....		4			2	1	2		15	5			1			3			33	
<i>Tylenchorhynchus</i> :																				
<i>brevdens</i> .....	4	4	1	1	14	1	4	3	3	9	1	1	1	19	5	6	2		79	
<i>clarus</i> .....	1		1		2	1			9				1	6	1				22	
other species.....	2	1			1	1	1		9	2				1		2	1		22	
<i>Xiphinema americanum</i> .....	5	12	1		16	1	8	3	19	18	1	1	1	14	8	7	2	1	118	

*Tylenchorhynchus nanus* Allen .... 3  
*Tylenchorhynchus acutus* Allen .... 2  
*Tylenchorhynchus claytoni* Steiner.. 1  
*Tylenchorhynchus cylindricus* Cobb .1  
*Tylenchorhynchus striatus* Allen ... 1

Although *Tylenchorhynchus brevidens* was rather generally distributed, it was notably less prevalent in the foothills of El Dorado and Placer Counties than in the San Francisco Bay and coastal mountain areas. *T. capitatus* was the prevalent stunt nematode in Placer County. Five species of *Tylenchorhynchus* (*T. acutus*, *T. brevidens*, *T. clarus*, *T. claytoni*, and *T. cylindricus*) were identified from the one orchard surveyed in Los Angeles County.

The number of pear orchards from which stunt nematodes were recovered

showed some seasonal fluctuation. The nematodes appeared to be most prevalent from August to December, and this tendency was most evident in the case of *Tylenchorhynchus brevidens*, which was found in 37 to 40 orchards each month during the fall period and in only 15 to 25 orchards the remainder of the year. If more extensive sampling had been done in each orchard, this nematode possibly would have been found in all seasons, since stunt nematodes can be exceedingly variable in their distribution within an orchard.

No stunt nematodes were recovered from any pear root samples by the mist-extraction technique.

4. *Pratylenchus* species — the root-lesion nematodes

In the literature, the only *Pratylenchus* species which has been associ-

ated with pear disease is *Pratylenchus penetrans* (Oostenbrink, 1954; Decker, 1960).

One or more *Pratylenchus* species were recovered from 91 (72 per cent) of the orchards sampled, and species which are not known as economic pests were the most prevalent. The species of *Pratylenchus* and the number of orchards in which each was found are as follows:

<i>Pratylenchus neglectus</i> (Rensch)	
Chitwood and Oteifa .....	48
<i>Pratylenchus thornei</i>	
Sher and Allen .....	23
<i>Pratylenchus vulnus</i>	
Allen and Jensen .....	19
<i>Pratylenchus crenatus</i>	
Loof .....	14
<i>Pratylenchus penetrans</i> (Cobb)	
Chitwood and Oteifa .....	10
<i>Pratylenchus zeae</i>	
Graham .....	10
<i>Pratylenchus scribneri</i>	
Steiner .....	1
<i>Pratylenchus</i> spp.	
undetermined .....	17

*Pratylenchus neglectus*, *P. thornei*, and *P. vulnus* were of widespread occurrence in all pear-growing areas of the state, but *P. crenatus* and *P. penetrans* were not found outside the foothills and contiguous valley area in El Dorado, Placer, and Sacramento Counties.

Only *Pratylenchus vulnus* and *P. penetrans* were recovered from pear roots. Pear has previously been reported to be a host of both these species (Oostenbrink, 1954; Lownsbey and Serr, 1963).

The upper extremes of population densities recorded for lesion nematodes were 115 *Pratylenchus vulnus* per gram of roots from trees in a Sacramento County orchard and 625 *Pratylenchus penetrans* in 250cc of soil in a Placer County orchard. No seasonal cycle of prevalence of lesion nematodes, either

as a group or as individual species, was observed.

Of special interest was the discovery of *Pratylenchus zeae*—a nematode pest not known to be generally distributed in California—in soil samples from 10 of the 20 pear orchards surveyed in Placer County. Normally a pest of corn, milo, and sugar cane (Ayoub, 1961; French, 1961), this species has not been reported on pear, and its association with the trees is a subject for further investigation. Root samples were collected from grasses and weeds surrounding trees in the infested orchards, but this nematode could not be consistently associated with the roots of any particular plant.

#### 5. *Helicotylenchus* species—the spiral nematodes

Eighty-one (64 per cent) of the orchards were found to be infested with one or more members of this genus of ectoparasitic nematodes. An important revision in the taxonomic treatment of this group was taking place at the time of the survey. Some of the types most frequently encountered have not yet been described or named.

The identifiable species and the number of orchards in which they were found are:

<i>Helicotylenchus dihystrera</i>	
(Cobb) Sher .....	38
<i>Helicotylenchus digonicus</i> Perry ..	35
<i>Helicotylenchus erythrinae</i>	
(Zimmerman) Golden .....	7

Spiral nematodes were present in every orchard sampled in Placer and El Dorado Counties, and also were widespread in other areas of the state. Populations up to 1,600 per 250cc of soil were recovered.

#### 6. *Meloidogyne* species—the root-knot nematodes

Thirty-nine orchards (31 per cent) were found to be infested with root-knot nematodes. These could not be identified to species because no mature



female nematodes were recovered by the procedures used.

Orchards infested with *Meloidogyne* species were most numerous in the foothill areas of El Dorado and Placer Counties. As many as 300 larvae per 250cc of soil were found in some orchards. Very likely, weeds in the orchards were supporting these nematodes, since pear is resistant to the common species of *Meloidogyne* (Day and Tufts, 1944).

7. *Criconemoides* species — the ring nematodes

Members of the genus *Criconemoides* were found in 38 (30 per cent) of the orchards. *Criconemoides xenoplax* Raski occurred in 20 orchards, *C. mutabile* Taylor in 11, and unidentified species in 7 orchards. Few orchards infested with ring nematodes were found outside Placer and El Dorado Counties. One sample from Placer County contained 580 *C. xenoplax* per 250cc of soil. Since Bartlett pear trees are not a host of *C. xenoplax* (Lownsbery, 1964), this nematode presumably was parasitizing weeds.

8. *Heterodera* species — the cyst nematodes

Larvae of cyst nematodes were found in 35 orchards, although pear is not known to be a host of any species of *Heterodera*. Since mature cysts were not recovered by the methods used, specific determinations could not be made. Possibly the nematodes were existing on weed hosts.

9. *Trichodorus* species — the stubby-root nematodes

Ectoparasitic nematodes of the genus *Trichodorus* were present in 33 pear orchards. They were especially prevalent in Placer County, where *Trichodorus christiei* Allen occurred in 12 orchards and *T. porosus* Allen in six.

10. Other nematodes

An undescribed species related to the genus *Radopholus* was found in four monthly soil samplings from one orchard in Santa Clara County and is being given further taxonomic study.

Larvae and juvenile females of *Trophonema arenarium* Raski were found in the December, January, and February soil samples from a pear orchard in Placer County.

## RELATIONSHIP BETWEEN PEAR DECLINE AND NEMATODES

Evidence of a relationship between pear-decline incidence and occurrence of the various nematode types was sought, employing pear-decline ratings assigned during the cooperative survey by Rackham *et al.* (1964).

The ratings of 108 orchards were divided arbitrarily into three severity groupings as follows: light (ratings 1.00 to 1.40); medium (ratings 1.41 to 1.90); and heavy (ratings above 1.90). Charting the percentage of orchards in each of these groupings from which various genera of nematodes were recovered (fig. 1) indicated no association between disease expression and prevalence of nematodes except possibly in

the case of *Meloidogyne*, *Criconemoides*, and *Trichodorus* spp. Since pear is not generally affected by *Meloidogyne* or *Criconemoides* and since none of these types occurred in more than 45 per cent of the orchards with serious pear decline, this apparent relationship was not considered significant.

When plant-parasitic nematode populations were determined quantitatively in April, 1961, population levels of any parasitic species greater than one per cent of soil were found in approximately 9 of the 126 orchards. Six of these more heavily infested orchards were in Lake and Santa Clara Counties, where incidence of pear decline has been relatively

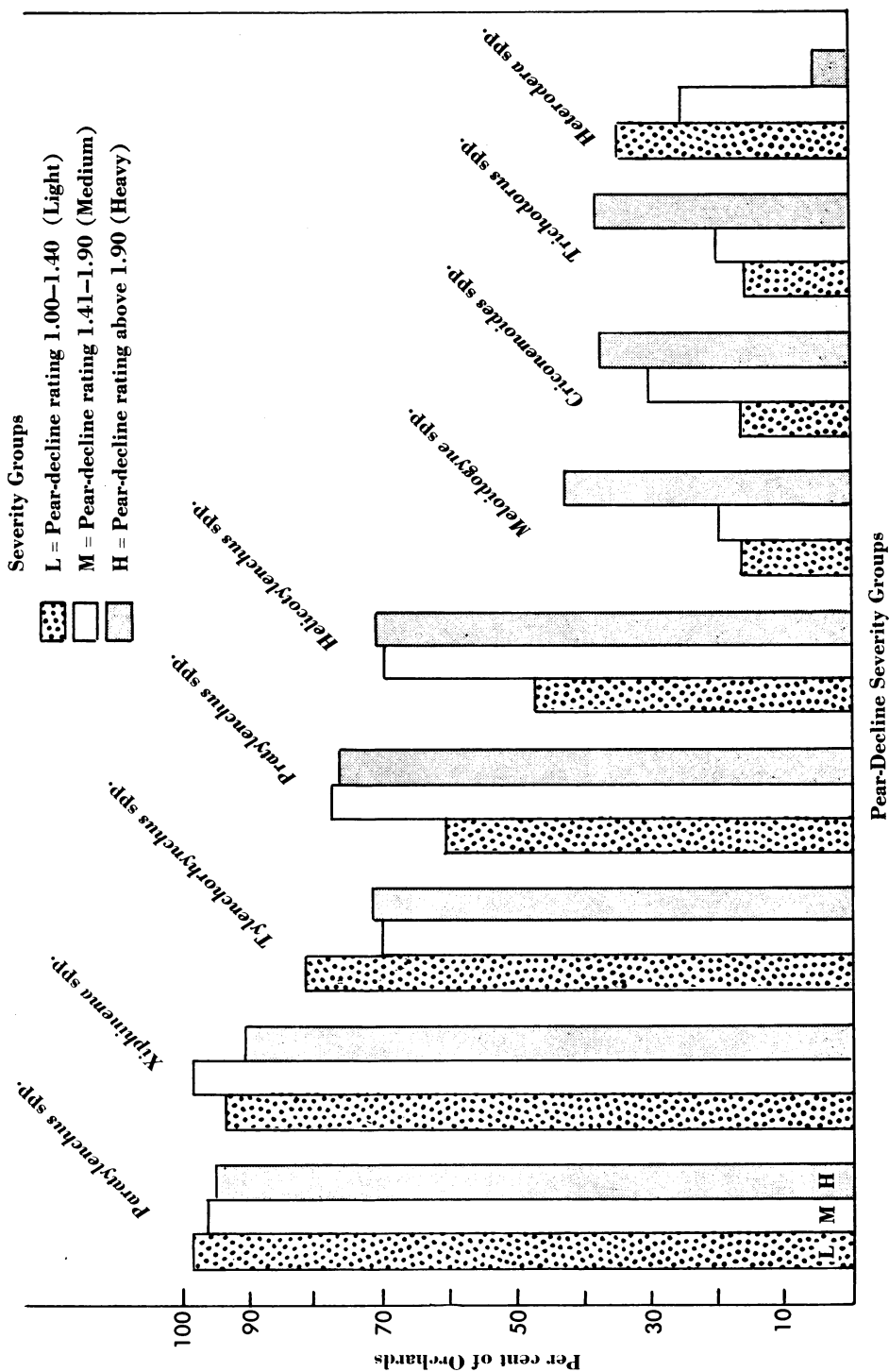


Fig. 1. Occurrence of nematode types in pear orchards with different pear-decline severity levels.



low (Nichols *et al.*, 1960). When pear-decline ratings for these more heavily infested orchards were paired with those of neighboring, less heavily infested orchards, mean ratings for the two kinds of orchards did not differ significantly (Student's *T* test).

No relationship could be shown between the occurrence of any of the more important species of nematodes and such factors as irrigation, cultivation, or rootstock type. In the foothill orchards of Placer and El Dorado Counties,

where the soil is generally a relatively shallow, reddish clay loam, plant-parasitic nematodes were most prevalent. *Pratylenchus penetrans*, *P. crenatus*, and species of *Helicotylenchus*, *Meloidogyne*, *Criconemoides*, and *Trichodorus* were more numerous in this area than in the deeper alluvial silty loams and black-clay soils of the valley and coastal counties. Climatic differences as well as soil type could be involved in these variations in nematode population.

## SUMMARY AND CONCLUSIONS

At least one type of plant-parasitic nematode was present in soil samples collected from the upper root zones of pear trees in each of 126 pear orchards in California. The orchards were sampled at monthly intervals during 1961–1962 in connection with investigations of pear decline.

*Xiphinema americanum* and *Pratylenchus hamatus* occurred in more than 90 per cent of the orchards in all pear-growing areas of the state. Other nematodes frequently found were *Tylenchorhynchus brevidens*, *Pratylenchus neglectus*, *Helicotylenchus* species, *Meloidogyne* species, and *Criconemoides*

species. The lesion nematodes *Pratylenchus vulnus* and *P. penetrans* were recovered in considerable numbers from both soil and roots of some orchards. *Pratylenchus zeae* was found for the first time in northern California, apparently feeding on weed grasses in 10 pear orchards in Placer County.

Only the stunt nematodes (*Tylenchorhynchus* spp.) showed seasonal fluctuations in their incidence in various orchards; they were most prevalent from August to December.

No direct relationship was indicated between pear decline and nematodes in the orchard soils.

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Pear fruit and pear and apple seedlings trapped more *Pythium* spp. than the fruit of apple, lemon, and avocado, while few *Phytophthora* spp. were trapped in seedlings. *Phytophthora* spp. isolates were trapped most often in pear and apple fruit. Lemon and avocado fruit yielded a fair number of both genera.

II. Plant-parasitic nematodes of various types are prevalent in the upper root zone of pear trees in California. Monthly samplings over a 12-month period, from the same 126 orchards mentioned above, showed that the dagger nematode *Xiphinema americanum* and the pin nematode *Paratylenchus hamatus* occurred in more than 90 per cent of the orchards and in all pear-growing areas of the state. Other nematodes frequently found were species of *Tylenchorhynchus*, *Pratylenchus*, *Helicotylenchus*, *Meloidogyne*, and *Criconemoides*. No direct relationship was indicated between pear decline and nematodes in orchard soils.

III. Reduction of populations of pin nematodes, spiral nematodes, and stunt nematodes in orchard soil by fumigation with ethylene dibromide did not improve the growth of Bartlett or Oriental pear seedlings. The results suggest that these nematodes are not an important factor limiting growth of pears in California.



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