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NEMATODES AND FUNGI ASSOCIATED WITH APPLE REPLANT DISORDER IN SAMPLED NEW YORK STATE ORCHARDS

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ABSTRACT
The factors associated with unthrifty growth of apple are not fully determined, resulting in inadequate diagnosis of apple replant disorder (ARD). The present study isolated and identified nematodes and fungi from ARD-infested soils to determine factors that interacted to cause ARD. Nematodes were extracted and identified in several orchard soil subsamples. The remaining soils were mixed to give composite soil. Transformed and non-transformed apple host seedlings were planted in the composite soil in a greenhouse, harvested after 10 weeks, and processed for isolation and identification of fungi. *Pratylenchus* and *Xiphinema* nematodes were present in the soils. Root-lesion nematode (RLN) frequency was always 100%, and dagger nematode (DN) frequency ranged from 40-70%. The RLN and DN ranged from 56-350 and 5-58 per 100 cm^3 of soil, respectively. Isolation frequency of *Pythium* species was highest (29%), followed by 10% for *Pythium cryptogea*, 4% for *Pythium cambivora* and *Pythium catorum*, 3% for *Pythium megasperma*, and 2% for *Phytophthora* species. Both transformed and non-transformed apple hosts were infested by at least several fungi. Of the six apple hosts tested, *Pythium* sp. and *F. oxysporum* colonized six each, *Pythium cryptogea*, *C. lucidum* and *C. destructans* colonized three each, *Pythium cactorum*, *Pythium cambivora* and *Pythium megasperma* two each, and *Phytophthora* sp., *F. solani*, *F. equiseti*, *F. acuminatum* and *Rhizoctonia* sp. one each. Consequently, the ARD symptoms observed on diverse apple hosts were associated with combined effects of RLN, DN and various species of *Pythium*, *Phytophthora*, *Cylindrocarpon*, *Fusarium* and *Rhizoctonia*. Whenever unthrifty growth is observed in soil especially that under perennial fruits and continuously cultivated annual crops, similar diagnostic isolation of multiple pathogens should be performed. In developing management strategies, germplasm for the crop in question should be evaluated in multiple sites or in composite soil in order to identify germplasm with broadspectrum resistance or tolerance to the pathogens.

KEY WORDS: Apple Replant Disease, *Malus* sp., *Pratylenchus*, *Xiphinema*, Transgenics

INTRODUCTION

Replant disorders refer to the poor growth of replanted perennial and annual crops and occur worldwide in fields, orchards and nurseries previously cropped or in fresh soils as intrinsic disorders termed "soil sickness" (Sewell, 1981). Apple replant disorders (ARD) cause widespread and serious difficulty in establishment of replanted apple trees (Hoestra, 1968; Utkhede and Smith, 1994). Symptoms of ARD appear as stunting, diminished root hair growth, root necrosis, sparse, browning and decaying fibrous roots, foliar nutrient deficiency, reduced productivity, and in the most severe cases, tree death within the first three years of planting (Jaffee *et al*., 1982b; Utkhede and Smith, 1992). Death of feeder roots account for impaired uptake of water and nutrients, whereas damage to root hairs (mycorrhizal penetration sites) partly accounts for the relative absence of Vesicular Arbuscular Mycorrhizae (VAM) in trees with ARD (Mai *et al*., 1970; Pinochet *et al*., 1996).

Many researchers have associated ARD with biotic and abiotic factors, acting individually or together. These factors include pathogenic actinomycetes (Westcott *et al*., 1987; Otto *et al*., 1993), bacteria (Utkhede *et al*., 1992; Dullahide *et al*., 1994), fungi (Sewell, 1981; Braun, 1995; Mazzola, 1997), dagger nematodes (*Xiphinema americanum* Cobb) (Sultan and Ferris, 1991; Lana *et al*., 1983, root lesion nematodes [*Pratylenchus penetrans* (Cobb) (Filipjev and Stekhoven] (Hoestra, 1968; Hoestra and Oostenbrink, 1962; Mai *et al*., 1970), phytotoxins (Benson *et al*., 1978), nutrient imbalance (Sadowski *et al*., 1988), and poor soil conditions (Mai and Abawi, 1981). However, the factors vary among apple growing regions and are not fully determined as evidenced by lack of sustainable control measures. Consequently, the list and interactions of agents associated with ARD increase with every new study (Dullahide *et al*., 1994). Most isolation studies have used soil from single orchards rather than composite soil obtained from more than one orchard and mixed up. Therefore, our immediate objective involved isolating and identifying nematodes and fungi from ARD-infested soils, similar to those previously used to evaluate apple germplasm for resistance or tolerance to ARD. The ultimate objective was to determine pathogens that interacted to cause poor growth of apple seedlings evaluated and in orchards that provided the ARD-infested soils. Separate orchard soils were used for nematodes that

may not survive well the compositing process (Merwin and Stiles, 1989). Composited soils were used for fungi under the assumption that fungal reproductive structures can withstand composting. Knowledge of these pathogens might lead to development of sustainable control measures such as plant resistance by screening apple germplasm in ARD-infested composite soil. Resistance or tolerance identified in composite soil rather than in single orchard soils is likely to be broad and durable.

MATERIALS AND METHODS

Apple Hosts

Microshoots of different apple hosts were obtained from the apple rootstock disease-resistance breeding programme at Geneva, Ney York. Different rootstocks were used to increase chances of isolating different fungi. The tested hosts include T348 'Royal Gala' and T565 'Marshall McIntosh' (MM), each with a chitinase gene, T286MM with a *gus* screenable marker gene, non-transformed MM, non-transformed M.7a, non-transformed 78M26R5 (CG11) and non-transformed *M. spectabilis*-1844. Transgenics and T286MM *gus* were included to determine effects of transgenes, *gus* gene, and transformation process on ARD pathogens. All microshoots were proliferated on modified Murashige and Skoog (1962) basal salts with minimal organics (Sigma Chemical Co., St. Louis, Missouri). Each litre of medium for proliferating non-transformed 78M26R5 (CG11) and non-transformed *M. spectabilis*-1844 was amended with 1 mg each of: thiamine, nicotinic acid, pyridoxine, N^6-benzyladenosine (BAP), indole-3-butyric acid (IBA), and gibberellic acid (GA_3); 100 mg myo-inositol, 30 g lab grade sucrose, and 7 g Difco Bacto agar. The medium for T565MM Chitinase and T286MM *gus* was amended per litre with 100 mg myo-inositol, 30 g sucrose, 7.5 g agar, 0.3 mg IBA, 1 mg BAP, 0.2 mg GA_3, 0.8 g mg thiamine, 1 mg pyridoxine, 1 mg nicotinic acid, 0.08 mg biotin, 4 mg glycine and 200 μg paromomycin. Non-transformed MM was proliferated on the same medium as for T565MM Chitinace, but without paromomycin. T348RG Chitinase was proliferated on the same medium as for T565MM Chitinase, except IBA and GA_3 were omitted, and 3 mg of kinetin and 0.1 mg 1-naphthalenacetic acid were added. The pH of all media was adjusted to 5.6, the media poured into glass jars or Magenta G7 boxes (Magenta Corp., Chicago, Illinois), and autoclaved for 18 minutes at 121°C and 100 kPa. Culture vessels were sealed with parafilm and placed in a room maintained at 24±2°C with about 70 μmol.$m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) for 16 hours per day. After proliferating for at least four weeks, microshoots were excised and rooted in peat: perlite (1:1, v/v) for about four weeks under mist in a greenhouse maintained at 25°C and 16 hours of light. Rooted plants were grown for 2 weeks with hand-watering before transplanting to ARD-infested soil.

Sources of ARD Soil

Based on previous ARD diagnostic bioassays, orchard surveys, and difficulties encountered in replanting young trees (Pruyne *et al*., 1994), ARD soil was collected from the same New York orchards on three occasions. The orchards ranged from old (established) to new (fallowed or recently replanted) sites of the Bartleson, Clarke, Crist, Grainger and Smith farms. Grass was scrapped off the surface of the alleyways in a random pattern; soil scooped with a hand shovel to a 30-cm depth at about 35 sites per orchard, bulked into black polythene bags and stored at 4°C until it was ready for use.

Nematode Isolation and Identification

Nematodes from diverse genera were extracted and identified from the soil. After taking soil samples, separate soils were homogenized (composited) in a motorized mixer and then immediately returned to a cold storage at 4°C. The composited soil was used to grow apple host plants from which fungi were isolated. Nematodes in soil subsamples were extracted for 7 days on modified Bearman pans, and then identified (Zuckerman *et al*., 1985; Mai and Mullin, 1996).

Isolation and Identification of Fungi

The fungal species targeted were for *Cylindrocarpon, Pythium, Fusarium, Rhizoctonia* and *Phytophthora* previously associated with ARD (Sonderhousen, 1965; Sewell, 1981; Jaffee *et al*., 1982b; Braun, 1995). About 10 plantlets of each clone were grown in a composite ARD soil, harvested after 10 weeks and processed for fungal isolation. All fungi were isolated and identified by the Plant Pathology Diagnostic Laboratory, except *Phytophthora* species which were isolated and identified by ourselves in the Laboratory at Cornell University, Ithaca, New York. To isolate species of *Cylindrocarpon, Pythium, Fusarium and Rhizoctonia*, roots of apple hosts were washed in running tap water for about two minutes and cut into 1-cm long pieces. Root pieces were surface-disinfected in 10% NaOCl solution for about 2 minutes and rinsed twice in sterilized distilled water. The roots were then blotted dry using a sterile paper, randomly selected and cultured onto selective isolation and identification media. *Pythium* species were isolated by culturing three root pieces from each host root replicate on water agar amended with rifampicin and primaricin (Sigma Chemical Co., St. Louis, Missouri; Jaffee *et al*., 1982a). Small agaral pieces of unique colonies were subcultured separately within three days on PDA or Corn Meal Agar (CMA) for identification up to genus level and recording isolation frequency (Jaffee *et al*., 1982a; Reeleder and Brammall, 1994). *Fusarium* and *Cylindrocarpon*, with simpler protocols, were subcultured for identification to species level. Single spores or hyphal tips were subcultured on carnation water agar or PDA for *Fusarium* and PDA for *Cylindrocarpon* (Samuels and Bryford, 1990). *Fusarium* species were identified based on the colour of sporochia (Nelson *et al*., 1983). *Cylindrocarpon* species were identified based on the presence or absence and size of microconidia, macroconidia and mycelial chlamydospores (Booth, 1966).

Phytophthora species Isolation and Identification

Roots of apple hosts were washed in running tap water for three minutes, surface-disinfected in 70% ethanol for two seconds, rinsed three times in sterilized distilled water,

blotted dry on sterilized paper and cut into 1-cm-long pieces. Nine pieces from each host root replicate were randomly selected and cultured on selective medium containing CMA, primaricin, ampicillin, rifampicin, PCNB and hymexazol (P_5ARPH) (Mircetich and Mathernon, 1976). After three days at 21^{o}C, emerging colonies were subcultured onto CMA and V-8 juice agar (V8A) for identification and recording isolation frequency (Wilcox and Ellis, 1989). Species were identified based on colony, sporangia and oogonia morphology on CMA and V8A (Wilcox, 1989; Wilcox and Ellis, 1989). Isolation frequency for all fungi was analyzed using the Chi-square test (StatXact, Cytel Software Corp., Cambridge, Massachusetts) to determine whether it varied significantly or not among the different fungi.

RESULTS AND DISCUSSION

Nematodes

Diverse genera of nematodes were present in the ARD soil collected from selected New York orchards (Table 1). The highest population densities were observed for non-parasites, as reported in other studies (Hoestra, 1968; Vrain and Rousselle, 1980; Vrain and Yorston, 1987). Root-lesion nematodes (RLN) and dagger nematodes (DN), both pathogenic to apple were among the nematodes isolated. The frequency of occurrence (10/10, 9/9 and 9/9) for RLN was always 100% (Table 1), whereas (6/10, 6/9 and 4/9) for DN indicated absence of DN in some of the orchards sampled. The presence of RLN in all and of DN in some New York orchards surveyed has been reported by other researchers (Mai and Abawi, 1978; Rosenberger *et al*., 1983; Driel *et al*., 1990). Therefore, plant resistance to these two nematodes should be included in strategies designed to combat ARD.

The number of RLN and DN varied by season and orchard (Table 1). In 100 cm^3 of separate soil samples, average RLN and DN populations ranged from 56 to 350, and 5 to 58, respectively (Table 1). This variation was consistent with previous reports on seasonal distribution and habits of these nematodes (Constante *et al*., 1985; Jaffee *et al*., 1987; Kotcon, 1990; Vrain *et al*., 1997). Populations within these ranges have frequently been observed in orchards exhibiting ARD (Mai and Abawi, 1978; Vrain and Rousselle, 1980; Merwin and Stiles, 1989; Utkhede *et al*., 1992).There is no commonly accepted economic injury threshold for RLN and DN in apple. Reported thresholds vary with apple-growing region and other soilborne pathogens acting in association (Hoestra, 1968; Sitepu and Wallace, 1974; Jaffee *et al*., 1982b; Utkhede and Li, 1988; Dullahide *et al*., 1994; Utkhede *et al*., 1992). One estimated threshold ranged from 20 to 50 RLN per 100 cm^3 of soil (Hoestra and Oostenbrink, 1962). Hoestra (1968) considered orchards with 50 RLN per 100 cm^3 of soil and poor growth of replanted trees as heavily infested. In contrast, injury thresholds for DN can be as low as 1 per 100 cm^3 of soil, because of its potential to transmit tomato ringspot virus to apple (Rosenberger *et al*., 1983; Kotcon, 1990). Therefore, RLN and DN populations in most orchards sampled were relatively high. Based on the substantial stunting that other researchers have reported for these population densities, we associated RLN, DN and other pathogens in the composite soil with the ARD symptoms observed among apple hosts.

Fungal Species

Fusarium, Cylindrocarpon, Pythium, Rhizoctonia, Mortierella, Trichoderma, Penicillium, Chaetonium, Rhizopus and *Mucor* were isolated from ARD-infested apple roots (Table 2). However, this paper will focus on the pathogenic *Fusarium, Cylindrocarpon, Pythium* and *Rhizoctonia* previously that have associated with replant disorders (Sewell, 1981; Jaffee *et al*., 1982a; Mai and Abawi, 1987; Braun, 1995). The frequency of isolation across hosts varied significantly among fungi (P<0.05) (Table 2). *Pythium* species had the highest isolation frequency (29%), followed by four species of *Fusarium* (26%), two *Cylindrocarpon* spp. (12%) and lastly by *Rhizoctonia* sp. (1%) (Table 2). Among the four *Fusarium* species, *F. oxysporum* was the most prevalent (22%) (Table 2). The frequency of *C. lucidum* was higher (7%) than that of *C. destructans* (5%) (Table 2).

Therefore, *Pythium* species may be more aggressive in colonizing apple roots than the other fungi identified. The number of hosts colonized by each fungus varied and was 6 of 6 each for *Pyhtium* species and *F. oxysporum*, 3 of 6 each for *C. destructans* and *C. lucidum*, and 1 of 6 each for *Pyhtium* species and *F. acuminatum* and *Rhizoctonia* sp. (Table 2). Thus *Pythium* species and *F. oxysporum* colonized all hosts tested, suggesting that they can cause disease in a wide range of apple rootstocks (Sharma and Gupta, 1989). The eight pathogenic fungi isolated were: *Pytium species, F. equiseti, F. oxysporum, F. solani, F. acuminatum, C. lucidum, C. destructans* and *Rhizoctonia* sp. (Table 2). These fungi varied per host and were 2 of 8 on T348RG Chitinase, 3 of 8 on T565MM Chitinase, 5 of 8 on T286MM *gus*, 4 of 8 on 78M26R5 (CG11), 6 of 8 on 'Marshall McIntosh', and 3 of 8 on *M. spectabilis*-1844 (Table 2). Similar variation in fungal infection has been observed among apple rootstocks (Jones and Aldwinckle, 1990). It appears diverse hosts and the composite soil enabled us to isolate different fungi. Both transgenic and non-transgenic apple hosts were infested by several fungi. Endochitinase, the *gus* gene, and gene-transfer process did not prevent fungal infection. Several *Fusarium* species, including *F. equiseti, F. oxysporum* and *F. solani* isolated have been associated with ARD in the United States (Utkhede and Smith, 1994). *F. acuminatum*, which has not been associated with ARD before was also isolated. However, *Fusarium* species cause considerable damage in nursery plants (Mazzolla, 1997). *Cylindrocarpon* species previously associated with ARD include *C. lucidum, C. scoparium, C. radicicola, C. heteronema* and *C. destructans* (Jaffee et al., 1982a; Braun 1995). *Cylindrocarpon lucidum* caused lower biomass, discolouration, black lesions and extensive cortical decay symptoms on roots; these symptoms were more severe when *P. irregulare* interacted with *C. lucidum.*

TABLE 1: Population of nematodes extracted from soils for sampled New York orchards

		Orchard										
Year 1 Season 1 (Spring)	Frequency	1[x]	2[x]	3[x]	4	5	6	7	8	9[x]	10	Average ± SD
Non-parasites	10/10	478	601	1443	1794	960	624	476	280	306	266	723±521
Pratylenchus penetrans	10/10	40	39	51	78	72	72	68	84	73	38	62±18
Heterodera sp.	8/10	52	0	0	26	72	120	34	28	18	152	50±51
Pratylenchus sp.	8/10	82	26	45	26	192	0	34	0	124	76	61±60
Criconemella sp.	7/10	0	173	102	26	24	0	0	280	180	76	86±97
Filenchus sp.	7/10	54	26	15	0	120	48	0	84	18	0	37±40
Predators	6/10	14	108	17	0	144	24	0	0	11	0	32±51
Xiphinema americanum	6/10	40	0	17	0	192	0	0	224	65	38	58±83
Coslenchus sp.	4/10	232	18	32	0	0	0	0	0	54	0	34±72
Aphelenchus sp.	4/10	0	18	17	0	24	48	0	0	0	0	11±16
Psilenchus sp.	3/10	176	0	60	78	0	0	0	0	0	0	31±59
Helicotylenchus sp.	2/10	0	0	0	15	0	0	0	0	0	0	4±10
Tylenchus sp.	4/10	0	0	0	0	24	0	34	140	126	0	32±54
Tylenchorhynchus sp.	2/10	0	0	0	26	0	0	0	56	0	0	8±19
Year 1 Season 2 (Fall)												
Pratylenchus penetrans	9/9	115	254	132	318	352	120	635	550	678		350±222
Xiphinema americanum	9/9	20	21	0	49	64	0	18	0	7		20±23
Year 2 Season 2 (Fall)		1[v]	2[v]	3	4[w]	5[v]	6[v]	7[v]	8[w]	9[v]		56±36
Pratylenchus penetrans	9/9	61	24	39	71	77	49	10	134	36		5±7
Xiphinema americanum	4/9	0	0	0	15	6	0	0	5	18		

[z]1 = Bartleson I, 2 = Bartleson II, 3 = Clarke, 4 = Crist I, 5 =Crist II, 6 = Grainger I, 7 = Grainger II, 8 = Smith II, 9 = Smith I, 10 = Trapani. I = fallowed or recently planted. II = Established. SD = Standard Deviation

[y]Extracted by sucrose floatation, the rest by modified Bearman pie pans (Zuckerman et al., 1985)

[x,w,v]Average of two, three and four samples, respectively

TABLE 2: Isolation frequency (percentage ±standard deviation) of fungi infesting roots of various apple hosts grown in a composite ARD-infested soil

Fungi	Apple hosts							
	T348RG Chitinase	T565MM Chitinase	T286MM *gus*	78M26R5 (CG11)	Marshall McIntosh	*M. spectabilis* 1844	% Frequency/ fungus	Hosts infested
Pythium species[z]	43±40[y]	31±39	33±15	46±31	6±10	13±22	29	6 of 6
Fusarium oxysporum[z]	32±16	6±10	40±11	5±8	26±23	25±8	22	6 of 6
Mortierella sp.	17±29	17±17	0±0	21±18	22±9	25±8	17	5 of 6
Cylindrocarpon lucidum[z]	0±0	0±0	5±8	15±15	6±10	22±25	7	3 of 6
Trichoderma sp.	0±0	11±19	5±8	0±0	11±10	6±10	6	4 of 6
C. destructans[z]	0±0	14±13	0±0	0±0	9±9	0±0	5	3 of 6
Unknown	8±14	6±10	0±0	0±0	9±9	0±0	4	3 of 6
Penicillium sp.	0±0	6±10	11±10	0±0	6±10	6±10	3	3 of 6
F. equiseti[z]	0±0	0±0	0±0	0±0	0±0	0±0	2	1 of 6
Chaetomium sp.	0±0	11±19	0±0	0±0	0±0	0±0	2	1 of 6
Fusarium solani[z]	0±0	0±0	7±12	5±8	0±0	0±0	1	1 of 6
Fusarium acuminatum[z]	0±0	0±0	0±0	0±0	0±0	0±0	1	1 of 6
Rhizopus sp.	0±0	0±0	0±0	8±14	0±0	0±0	1	1 of 6
Rhizoctonia sp.[z]	0±0	0±0	0±0	0±0	6±10	0±0	1	
Mucor sp.	0±0	0±0	0±0	0±0	0±0	0±0	1	1 of 6
Pathogenic fungi	2 of 8	3 of 8	5 of 8	4 of 8	6 of 8	3 of 8	*[w]	

[z]Indicates fungi pathogenic to apple
[y]Each frequency is a mean of three replicate root systems. Total frequency is 100 for each host
[x]Percentage isolation frequency for each fungus is average of six hosts
[w]Indicates significant difference in percentage isolation frequency among fungi (Chi-square test, P<0.05)

TABLE 3: Isolation frequency (percentage ± standard deviation) for *Phytophthora* species infesting roots of various apple hosts in a composite ARD-infested soil

Fungi	Apple hosts							
	T348RG Chitinase	T565MM Chitinase	M.7a (Control)	78M26R5 (CG11)	'Marshall McIntosh'	*M. spectabilis*-1844	% Frequency/ fungus[x]	Hosts infested
None	[y]67±58	66±34	55±51	33±0	33±33	22±19	46	6 of 6
Other fungi	11±19	33±33	33±58	22±19	55±39	33±0	31	6 of 6
Phytophthora cryptogea[z]	5±9	0±0	0±0	33±33	0±0	22±38	10	3 of 6
Phytophthora cactorum[z]	0±0	0±0	0±0	0±0	11±19	11±19	4	2 of 6
Phytophthora cambivora[z]	11±19	0±0	0±0	11±19	0±0	0±0	4	2 of 6
Phytophthora megasperma[z]	5±9	0±0	0±0	0±0	0±19	11±19	3	2 of 6
Phytophthora sp.[z]	0±0	0±0	11±19	0±0	0±0	0±0	2	1 of 6
Phytophthora spp.	3 of 5	0 of 5	1 of 5	2 of 5	1 of 5	3 of 5	*[w]	

[z]Indicates *Phytophthora* species pathogenic to apple
[y]Each frequency is a mean of three replicate root systems. Total frequency is 100 for each host
[x]Percent isolation frequency for each *Phytophthora* species is average of six hosts
[w]Indicates a significantdifference in percentage isolation frequency among species (Chi-square test P <0.05)

Pythium species that have been isolated from orange-coloured lesions on apple roots previously grown in ARD soil varied with the geographic region and included *Pythium irregular, Pythium sylvaticum, Pythium ultimum, Pythium intermedium* and others (Sewell, 1981; Dullahide et al., 1994; Braun, 1995). It is possible that the *Pythium* isolates included some of these species. *Pythium ultimum* has been associated with collar rot of apple (Sharma and Gupta, 1989).

A few *Rhizoctonia* species have been isolated from ARD soils and roots. Mazzolla (997) reported that *R. solani* isolates in anastasmosis groups 5 and 6 were the most dominant and pathogenic in Washington State soils and they caused severe symptoms of ARD in artificial inoculations. Since *Rhizoctonia* sp. was isolated from only one root system of 'Marshall McIntosh', it may be less prevalent in New York orchards. These results agree with observations that *Rhizoctonia* is primarily a damping-off and root rot fungus in seed beds and nurseries (Mazzola, 1997).

***Phytophthora* species**

Phytophthora cryptogea, *Phytophthora cactorum*, *Phytophthora cambivora*, *Phytophthora megasperma* and *Phytophthora* sp., were isolated from apple hosts tested (Table 3). The isolation frequency of *Phytophthora cryptogea* was highest (10%) followed by *Phytophthora cambivora* and *Phytophthora cactorum* (4% each), *Phytophthora megasperma* (3%) and lastly *Phytophthora* sp. (2%) (Table 3).

Phytophthora cyrptogea was also isolated from 3 of 6 hosts, while each of the other species was found on 2 of 6 hosts. *Phytophthora* sp. occurred on 1 of 6 hosts (Table 3). Although *Phytophthora cryptogea* was the most prevalent, all the *Phytophthora* species isolated have previously been isolated from ARD-infested roots (Sitepu and Wallace, 1974; Utkhede et al., 1992). They also cause crown rot in apple (Jeffers et al., 1982; Jones and Aldwinckle, 1990).

The number of species per host was zero on T565MM Chitinase, 1 of 5 each on M.7a and 'Marshall McIntosh' controls, 2 of 5 on 78M26R5 (CG11), and 3 of 5 on T348RG Chitinase and *M. sectabilis*-1884 (Table 3). Similar variation has been observed among apple rootstocks, where M.9 was the most resistant, M.7 and M.7a were moderately susceptible and M.26 was moderate to very susceptible to *Phytophthora* (Jones and Aldwinckle, 1990).

CONCLUSION

The ARD soil from New York orchards had both fungi and nematodes, some of which are known to be pathogenic to apple. These pathogens and others not isolated most likely interacted to cause ARD symptoms in the apple hosts. Therefore, composite soil from ARD-infested apple orchards are a good source of inoculum for screening *Malus* germplasm for resistance or tolerance to mixed ARD pathogens. Resistance to a combination of pathogens may be more durable than resistance to single pathogens.

RECOMMENDATIONS

Whenever unthrifty growth is observed in soil especially that under perennial fruits and continuously cultivated annual crops, similar diagnostic isolation of multiple pathogens should be performed. In developing management strategies, germplasm for the crop in question should be evaluated in multiple sites or in composite soil in order to identify germplasm with broadspectrum resistance or tolerance to the pathogens. Another technique than can accelerate development of broad-spectrum resistance is gene transfer.

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REFERENCES

Benson, N. R., Covey, Jr. R. P. and Haglund, W. (1978) The apple replant problem in Washington State. J. American Society for Horticultural Science. 103, 156-158.

Booth, C. (1966) The genus *Cylindrocarpon*. Mycological Papers 104(14), 1-56.

Braun, P. (1995) Effects of *Cylindrocarpon* and *Pythium* species on apple seedlings and potential role in apple replant disease. Canadian Journal of Plant Pathology. 17, 336-341.

Caruso, F. L., Neubauer, B. F. and begin, M. D. (1989) A histological study of apple roots affected by replant diseases. Canadian Journal of Botany 67, 747:749.

Costante, J. F., Mai, W. F., Aleong, J. and Klein, R. M. (1987) Effects of apple rootstocks and nematicides on *Pratylenchus penetrans* populations and apple trees growth. Journal of American Society for Horticulture Science. 112, 441-444.

Dullahide, S., Stirling, G. Nikulin, A. and Stirling, A. (1994) The role of nematodes, fungi, bacteria and abiotic factors in etiology of apple replant disorders in the granite belt of Queebsland. Australian Journal of Experimental Agriculture 34, 1177-1182.

Hoestra, H. (1968) Replant disease of apple in the Netherlands. Meded. Landbouwhogesh. Wageningen 68, 13.

Hoestra, H. and Ooestenbrink, M. (1962) Nematodes in relation to plant growth IV. *Pratylenchus penetrans* (Cobb) on orchard trees. Netherland J. Agricultural Science 10, 286-296.

Jafee, B. A., Abawi, G. S. and mai, W. F. (1982a) Fungi associated with roots of apple seedlings grown in soil from replant site. Plant Disease 66, 942-944.

Jafee, B. A., Abawi, G. S. and Mai, W. F. (1982b) Role of microflora and *P. penetrans* in apple replant disease. Phytopathology 72, 247-251.

Jones, A. L. and Aldwinckle, H. S. (1990) Compendium of apple and pear diseases. APS Press, St. Paul, Minnesota.

Kotcon, J. B. (1990) Distribution, frequency and population density of nematodes in West Virginia peach orchards. J. Nematology 22 (4S), 712-717.

Lana, A. Perterson, J., Rousselle, G. and Vrain, T. (1983) Association of tobacco ringspot virus with union incompatibility of apple (*Xiphinema americanum* and *Pratylenchus penetrans* nematode vectors). Journal of Phytopathology 106, 141-148.

Mai, W. F. and Abawi, G. S. (1978) Determining the cause and extent of apple, cherry and pear replant diseases under controlled conditions. Phytopathology 68, 1540-1544.

Mai, W. F. and Abawi, G. S. (1981) Controlling replant disease of pome and stone fruits in northeastern United States by preplant fumigation. Plant Disease 65, 859-864.

Mai, W. F. and Abawi, G. S. (1987) Interaction among root-knot nematodes and *Fusarium* wilt fungi on host plants. Annaul Review of Phytopathology 25, 317-338.

Mai, W. F. and Mullin, P. G. (1996) Plant-parasitic nematodes: A pictorial key to genera. 5th Edition. Cornell University Press, Ithaca, New York.

Mai, W. F., Parker, K. G. and Hickey, K. D. (1970) Populations of *Pratylenchus penetrans* and growth of apple in response to soil treatment with nematicides. Plant Disease Reports 54:792-795.

Mazzola (1997) Identification and pathogenicity of *Rhizoctonia* spp. isolated from apple roots and orchards soils. Phytopathology 87, 582-587.

Merwin, I. A. and Stiles, W. (1989) Root-lesion nematodes, potassium deficiency and prior cover crops as factors in apple replant disease. Journal of American Society for Horticultural Science 114, 724-728.

Mircetich, S. and Matheron, M. (1976) *Phytophthora* root and crown rot of cherry trees. Phytopathology 66, 549-558.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum 15, 473-497.

Nelson, P. E., Tousson, T. A. and Marasas, W. F. O. (1983) *Fusarium* species, an illustrated manual for identification. Pennsylvania State University Press, University Park, Pennsylvania.

Otto, G., Winkler, H. and Szabo, K. (1993) Investigations of the cause of infestation of rootlets of apple seedlings by root pathogenic actinomycetes in soils with specific apple replant disease. Zentrablatt fuer Mikrobiologies 148, 467-476.

Pinochet, J., Calvet, C., Camprubi, A. and Fernandez, C. 1996. Interactions between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in perennial crops. Plant and Soil 185, 183-190.

Pruyne, P. T., Merwin, I. A., Mullin, P. G. and Gibson, D. M. (1994) Diagnosis of apple replant disorders in New York orchard soils and evaluation of nematode-suppressive cover crops. Acta Horticulturae 363, 211-228.

Reeleder, R. D. and Brammall, R. A. (1994) Pathogenicity of *Pythium* species, *Cylindrocarpon destructans* and *Rhizoctonia solani* to ginseng seedlings in Ontario. Canadian Journal of Plant Pathology. 16, 311-316.

Rosenberger, D. A., Harrison, M. and Gonsalves, D. (1983) Incidence of apple union necrosis and decline, tomato ringspot virus and *Xiphinema* vector species in Hudson valley orchards. Plant Disease 67, 356-360.

Sadowski, A. Scibisz, K, Tomala, K., Kozanecka, T. and Kepka, M. (1988) Negative effects of excessive nitrogen and potassium fertilization in a replanted apple orchard. Acta Horticulturae 233, 85-94.