Olpidium brassicae, tobacco necrosis virus, and Pythium spp. in relation to rusty root of carrots in Ontario and Quebec

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Olpidum brassicae, Pythium sulcatum, and *Pythium irregulare* were frequently isolated from roots of carrots grown in soils from Ontario and Quebec carrot fields regardless of the presence of carrot rusty root. Although there were physiological strains of these fungi, there were no apparent differences in morphology or pathogenicity of isolates collected from the Holland-Bradford and Keswick marshes in Ontario, where rusty root disease is a problem, and isolates from non-problem soils. Tobacco necrosis virus was frequently found in problem soils in association with diseased carrots but it was only detected once in a non-problem soil. It is suggested that in addition to Pythium spp.. tobacco necrosis virus and its vector *Olpidium brassicae* are involved in rusty root etiology.

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Olpidium brassicae, *Pythium sulcatum* et *Pythium* irregulare ont ete frequemment isoles de carottes cultivees dans des echantillons de sol provenant de champs de carottes de l'Ontario et du Quebec frappes ou non par la rousselure. Bien que l'on ait identifie les souches des champignons on n'a releve aucune difference apparerite entre la morphologie et la pathogenicite des micro-organismes isoles des echantillons de terre noire de Holland-Bradford et de Keswick (Ontario), oh la rousselure pose un probleme, et celles des micro-organismes isoles de sol libres de la maladie. Le virus de la necrose du tabac a ete frequemment isolé des carottes malades provenant de sols problèmes alors qu'on ne l'a trouve qu'une seule fois dans les carottes cultivees ailleurs. Nous pensons que le virus de la necrose du tabac et son vecteur, *Olpidium brassicae,* avec *Pythium* sp. jouent un rôle dans l'étiologie de la rousselure.

Rusty root, an economically important disease of carrots *(Daucus carota* L. var. *sativa* DC) in Ontario, was first reported in the Holland-Bradford Marsh in 1962 (Fushtey and Filman 1968). Although the disease is widespread there and in the nearby Keswick Marsh, it has not been reported in other carrot-growing areas in Ontario and Quebec.

Sutton (1973, 1975) concluded from his work on this disease that several species of *Pythium* cause rusty-root in Ontario carrots and stated that the disease is similar to the lateral-root diseases caused by *Pythium* spp. in muck-grown carrots in British Columbia (McElroy et al. 1971), Wisconsin (Mildenhall et al. 1971), and Florida (Pratt and Mitchell 1973).

The possible involvement of other biotic agents, particularly non-filamentous fungi and soil-borne viruses, in soils associated with the rusty-root disease syndrome, appears to have been overlooked or ignored. One of us **(W.G.K.)** has frequently found tobacco necrosis virus (TNV) and its fungus vector *Olpidium brassicae* (Wor.) Dand. associated with carrot roots with typical rusty root symptoms in the Holland-Bradford Marsh. Subsequently, in 1973 a survey of randomly selected carrot fields was carried out in the major muck soil areas in southern and southwestern Ontario and in the Napierville-Sherrington-Ste. Clotilde area of Quebec to determine possible relationships between the presence of *Olpidium brassicae,* TNV, *Pythium* spp., and the rusty root disease. In addition, because of the possibility that a specific carrot strain of *O. brassicae* was introduced into the marsh areas, a survey of wild carrots was made at the same time; wild carrots *(Daucus carota* **L.)** are abundant in eastern, southern, and southwestern Ontario as well as in parts of Quebec, but in marshes they occur only along roadsides.

Materials and methods

Composite samples **of** soil from carrot fields were assayed for *Olpidium brassicae,* TNV, and *Pythium* spp. by a bait-plant technique. Carrot (Nantes type) seed was sown in about 2-3 cm of each test soil over 4-5 cm of sterilized white sand (24 mesh) in 7.5 cm (3 inch) diam clay pots. After 3-4 weeks growth at 20°C, roots that penetrated the sand were examined for necrosis and cultured for fungi. Sap from small fragments of carrot root grown in each soil was also indexed for TNV by mechanical inoculation to leaves of *Gomphrena globosum* **L.** and/or *Chenopodium amaranticolor* Coste & Reyn.

O. brassicae was isolated from the pot-grown carrots by sprinkling fragments of washed, air-dried roots of the

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carrot bait plants around roots of carrot seedlings. The inoculated seedlings were then potted in sterile sand, watered daily with Hoaglands solution (1/3 concn), and after 3-4 weeks at 20°C the roots were examined for the fungus. Dried roots from the second or third carrot generation were used as inoculum in O. *brassicae* host range tests. Several isolates of O. *brassicae* from lettuce (*Lactuca sativa* L. cv. Great Lakes) and one from dandelion *(Taraxacum officinale* Weber) maintained in dried roots of these plants were also tested.

Pythium was isolated by placing washed roots from test carrots grown in pots onto Emerson's YpSs agar (Difco) containing neomycin sulfate (200 ppm). *Pythium* mycelium growing from the roots was transferred to 2.4% V-8 juice agar (2% agar), and later to autoclaved hemp seed in water for critical morphological examination.

The origin of soil samples from Ontario is shown in Fig. 1. The soil type, whether organic (muck) or sand, is also recorded. In addition, the location of four collections of

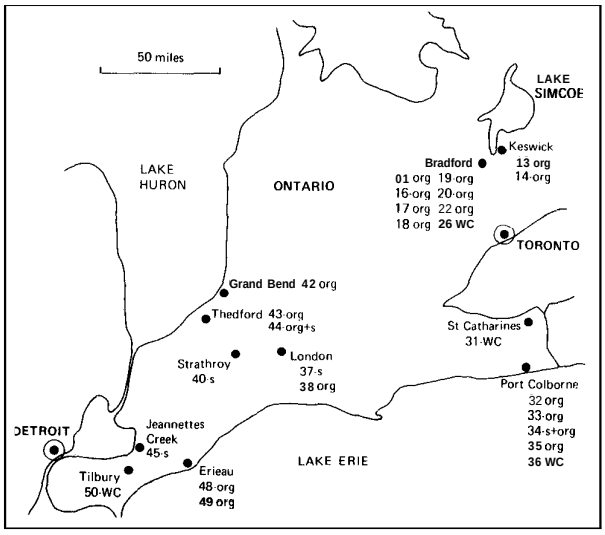

Figure 1. Collections of carrot made in southern and southwestern Ontario in 1973: org - organic (muck) soil, s - sandy soil from carrot producing areas, WC - wild carrot.

wild carrot is shown. Soil collections from Quebec (Nos. 52-62) originated from the carrot-growing region approximately 25 miles south of Montreal; all Quebec soils examined were organic.

Results

Presence of Olpidium brassicae, TNV and Pythium spp. in soils

Both root necrosis and severe infection by O. *brassicae* occurred in carrots grown in most soil samples from fields in which carrots had been grown frequently in recent years or less frequently over many years. These soils were collected from both the Holland-Bradford and Keswick marshes where rusty root is a problem, and from elsewhere in Ontario and Quebec where the disease has not been reported (Table 1 and Fig. 1).

In 1973, TNV was detected in soils only from farms in the Holland-Bradford Marsh where rusty root occurred. However, in 1974 additional checks were made of some of the 1973 locations and the virus was detected in a soil from the London Marsh.

Pythium spp. were found in all soils in which carrot root necrosis occurred (Table 1). Both *P. sulcatum* Pratt & Mitchell and *P. irregulare* Buisman were recovered from plants with severe root necrosis; the former species was isolated more frequently. *P. sulcatum* was also isolated from apparently healthy roots of test plants grown in some soil samples; these isolates failed to induce necrosis in carrot. Other *Pythium* spp. were associated with mild or moderate necrosis.

O. brassicae was abundant in test plants grown in some of the soil samples and often infected about 90% of the epidermal cells (Fig. 2). In spite of the abundance of this fungus in roots there was often no necrosis; when necrosis occurrred *Pythium* spp. were always present. Repotting of these *Olpidium*/*Pythium* infected plants usually facilitated the spread of *Pythium* and root necrosis to new roots while resulting in a marked decline of detectable O. *brassicae* in the roots of these plants.

Survey of wild carrot for Olpidium brassicae

O. brassicae was found on wild carrots collected from widely distributed areas including some in eastern Ontario remote from commercial carrot production. It was also present on wild carrots by a roadside next to a newly opened marsh area near Port Colborne, Ontario, (No. 36) but not in cultivated carrots grown in the laboratory in muck soil from an adjacent carrot field (No. 35). In four samples (Fig. 1) wild carrots were severely infected and the fungus was easily maintained on wild or cultivated carrots in the laboratory; however, in other cases, the chytrid died-out after carrots were grown in the laboratory under conditions favorable for fungus multiplication.

Morphology of Olpidium brassicae isolates

O. brassicae from wild and cultivated carrots grown in a number of Ontario and Quebec soil samples were compared morphologically for varietal differences; none were observed. Sporangium size varied with host cell size and the number of infective units per cell but was not characteristic of a specific isolate; the smallest sporangia were spherical and 10 μm diam whereas the largest filled the host cell and measured 35 x 80 to 12 x 110 μm. Discharge tube length varied among isolates; they were usually short, up to 5-6 μm in length but in some isolates they were longer, ranging to 45 μm. Resting spores (Fig. 3) were more uniform than sporangia in size; they were always stellate, generally spherical, 12-18 μm diam, but occasionally as small as 9 μm or as large as 18 x 28 μm. Some isolates, however, produced few resting spores in carrot. Zoospores were spherical to subspherical and varied in size from 3.5 to 4.5 (-5.5) μm, with flagellum lengths, including the fine whiplash end, of 16 to 22 μm. Critical examination of flagellum length showed that within many isolates the

Table 1. Presence of root necrosis, *Olpidium* ***brassicae,*** and ***Pythium*** spp. in carrots grown in samples of soil from carrot fields in Ontario and Quebec

Location* and No.	Root necrosis	*Olpidium brassicae*	*Pythium* species isolated
Holland-Bradford & Keswick marshes, Ontario			
13†	severe	abundant	***P, sulcatum*** & ***P. irregulare***
14	none	trace	none
16†	severe	abundant	***P. sulcatum***
17†	severe	abundant	***P. sulcatum*** & ***P. irregulare***
18†	severe	abundant	***P. sulcatum***
19	moderate	abundant	***P.*** sp.
20†	mild	abundant	***P. sulcatum***
22†	moderate	abundant	***P. sulcatum***
Other Ontario locations			
32	severe	light	***P. sulcatum*** & ***P. sylvaticum***
33	moderate	trace	***P.*** sp.
34	severe	abundant	***P. irregulare***
35	none	none	none§
37	none	none	none
38	severe	abundant	***P. sulcatum***
40	moderate	trace	***P.*** sp.
42	trace	none	***P.*** sp.§
43	severe	abundant	***P. sulcatum***
44	moderate	trace	***P. sulcatum***
45	trace	none	***P. sulcatum*** & ***P.*** sp.
48	moderate	abundant	***P. sulcatum***
49	severe	trace	***P. sulcatum***
Quebec locations			
52	severe	abundant	***P. sulcatum*** & ***P. irregulare***
53	none	trace	***P. sulcatum***
54	trace	none	***P. sulcatum***
56	trace	abundant	***P. irregulare***§
57	moderate	abundant	***P. sulcatum***
60	trace	abundant	***P. irregulare***
61	severe	abundant	***P. irregulare*** & ***P. ultimum***
62	severe	abundant	***P. sulcatum*** & ***P. irregulare***

* See Figure 1 for location of field samples.

† Samples from fields with history of rusty root disease.

§ ***P. sulcatum*** was subsequently isolated from other hosts grown in the same soil sample: 35-celery, 42-celery, and 56-dill.

length varied by only 1.5 to 2.0 μm among individual zoospores and the length remained constant when the isolate was grown on different hosts. In conclusion, the morphological differences among isolates were slight, being confined to discharge tube length and minute differences in flagellum length. More important, there were no discernible morphological differences between isolates from the Holland-Bradford and Keswick marsh soils and those from other organic or mineral soils in Ontario and Quebec. Nor could differences be found in isolates from wild or cultivated carrots.

Host range of Olpidium brassicae

O. ***brassicae*** isolates were grouped into four types based on their growth habit on carrot and lettuce roots (Table 2). Type 1 isolates all grew well on carrot and wild carrot and some, in addition, on dill, but none grew on lettuce or other nonumbelliferous hosts. Type 2 isolates all grew well on carrot and lettuce. Type 3 isolates grew well on lettuce but poorly on carrot and usually died out after a few weeks on this host. Type 4 isolates grew on lettuce but not on carrot.

When 18 hosts were tested it was soon apparent that variation within types was considerable (Table 2). This variation was likely due, at least in part, to mixtures of O. ***brassicae*** strains within the original isolates. Repeated transfers through lettuce or carrot indicated that many

Table 2. Type and host range of isolates of *Olpidium brassicae* from plants grown in soils from different locations in Ontario and Quebec

Location* and No.	Original host	Isolate type†	Notes on host range
Holland-Bradford & Keswick area, Ontario			
01§	carrot	1	Grew only on carrot and wild carrot
01§	lettuce	4	Grew on parsley & celery but not carrot
13§	carrot	2	Wide host range
14	carrot	3	Grew on lettuce
16§	carrot	2	Grew only on carrot, dill, lettuce, and spinach among 15 hosts tested
17§	carrot	2	Wide host range
18§	carrot	2	Wide host range
19	carrot	2	**
20§	carrot	2	**
22§	carrot	2	**
26	wild carrot	1	Grew only on carrot and wild carrot
Other Ontario locations			
31	wild carrot	1	Grew only on carrot and wild carrot
32	carrot	3	Grew on lettuce
33	dandelion	4	Grew on dill, parsley, celery, and lettuce
34	carrot	2	**
35	lettuce	4	Not found on carrot in this soil
36	wild carrot	1	**
37	lettuce	4	Not found on carrot in this soil
38	lettuce	2	Wide host range including carrot
40	carrot	2	Grew only on carrot, lettuce, ***Plantago,*** and ***Setaria*** among 15 hosts tested
42	—	—	Not found on carrot or lettuce in this soil
43	lettuce	2	Wide host range including carrot
44	carrot	3	Grew on lettuce
45	—	—	Not found on carrot or lettuce in this soil
48	carrot	2	**
49	carrot	3	Grew on lettuce
50	wild carrot	1	Only grew on carrot and dill
Quebec locations			
52	carrot	1	No apparent growth on lettuce**
52	lettuce	3	Poor growth on carrot
53	carrot	3	Grew on lettuce
56	carrot	2	Wide host range
57	carrot	2	Wide host range
60	carrot	2	**
61	carrot	1	**
62	carrot	1	**

* See Figure 1 for location of fields samples.

† Type 1 - good growth on carrot but none on lettuce; type 2 - good growth on carrot and lettuce; type 3 - poor growth on carrot but good growth on lettuce; type 4 - non carrot isolates which did not grow on carrot.

§ Samples from fields with history of rusty root disease.

** Presence of ***Pythium*** spp. with inoculum prevented thorough testing.

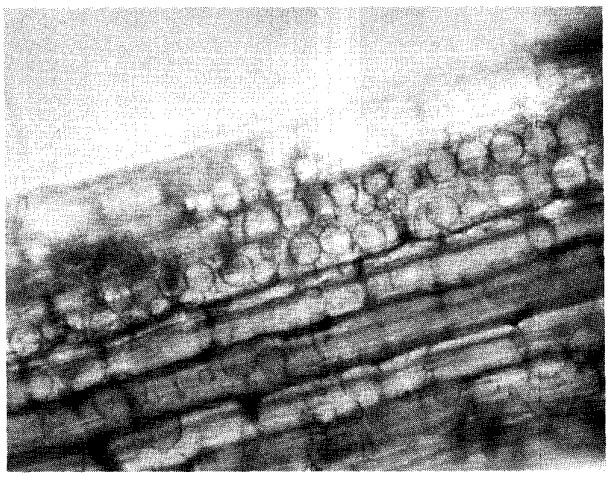

Figure 2. Carrot root containing numerous globular sporangia of *Olpidium brassicae.* ,X200.

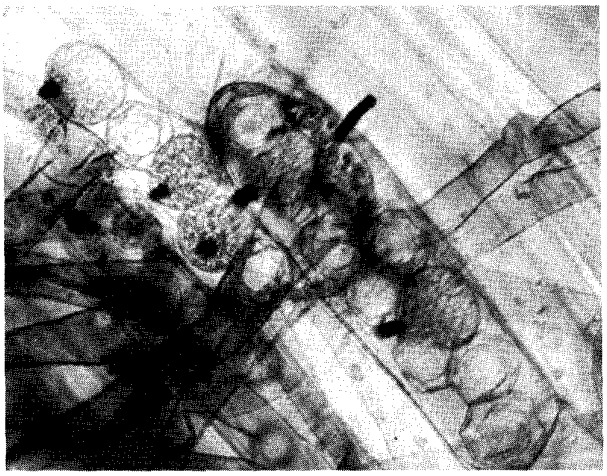

Figure 3. *Olpidium brassicae* in carrot root cells: stellate resting spores (arrows) and globular sporangia with darkly stained discharge tubes., X470.

isolates classed initially as type 2 were mixtures of types 2 and 3; ability to infect carrot being irretrievably lost after successive transfers through lettuce. Furthermore, at least one soil (No. 01) had types 1 and 4. Unfortunately, largely owing to the presence of virulent strains of *Pythium* in the inoculum, it was not possible to test all isolates on all 18 hosts; these results are, therefore, summarized as the number of isolates which grew on a host over the number of isolates tested on a host. Only isolates from carrot are included. Trace infections were considered negative. Results were: cabbage *(Brassicae oleracea* L. cv. Viking extra early strain) 0/21; celery *(Apium graveolens* L. var. *dulca* D.C. cv. Utah Green) 7/16; coriander *(Coriandrum sativum* L.) 6/11; dill *(Anethum graveolens* L. cv. Long Island Mammoth) 10/13; lettuce (*Lactuca sativa* L. cv. Great Lakes) 17/21; oats *(Avena sativa* cv. Clintland 60) 0/12; onion (*Allium cepa* L. cv. Early Yellow Globe) 2/13; parsley (*Petroselinum crispum* Nym. cv. Moss Curled) 5/13; spinach (*Spinacia oleracea* L. cv. America) 8/16; tomato (*Lycopersicum esculentum* Mill. cv. Gardener) 5/15; wheat *(Triticum aestivum* L. cv. Kent) 4/12. Some common weeds were also tested: lamb's quarters *(Chenopodium album* L.) 7/14; broadleaf plantain *(Plantago major* L.) 9/17; purslane *(Portulaca oleracea* L.) 3/12; groundsel *(Senecio vulgaris* L.) 1/8; yellow foxtail grass (*Setaria glauca* (L.) Beauv.) 10/17; dandelion (*Taraxacum officinale* Weber) 1/11.

Tests on commercial and wild carrots indicated there were no differences in susceptibility. Additional tests were done on cv. Spartan Fancy and a new carrot cultivar under trial, both of which have shown some resistance to rusty root in field tests; however, these cultivars were not resistant to O. *brassicae.*

Circumstantial evidence for strains of Pythium sulcatum

During tests with O. *brassicae* it was observed that differences occurred in host range and virulence of *P. sulcatum* frequently associated with carrot root necrosis in some carrot soils. For example *P. sulcatum* on carrot roots originating from a number of different soils generally did not cause necrosis of celery roots. However *P. sulcatum* in a Grandbend soil (No. 42) in which carrots were grown in 1973 and previously in 1964 did not infect carrot roots but was associated with severe root necrosis and stunting of celery plants in laboratory tests.

Pythium irregulare was also isolated from necrotic as well as from healthy carrot roots on a number of occasions and is probably an important cause of root necrosis in pot-grown carrots. However *P. sulcatum* may also have been present in these necrotic roots and not isolated because of its slower growth rate. *P. sulcatum* often occurs in roots without producing oospores and, therefore, can be easily overlooked in direct microscopic examination of roots.

Discussion

The presence of O. *brassicae* in carrot roots and muck soils is not correlated with the rusty root disease syndrome. The fungus occurs in both rusty-root problem and non-problem soils throughout Ontario and Quebec. O. *brassicae* is certainly a primary invader of carrot roots; it is an obligate parasite but does not induce necrosis or multiply in necrotic roots. There appears to be nothing unique about O. *brassicae* isolates from the various problem soils as compared with those from non-problem areas. Some isolates grow abundantly on carrot and other plants, and some are confined to carrot; however both types occur in commercial carrot growing areas where the disease has not been found and also on wild carrot. Whether the populations with affinity for carrot originate in the marsh areas or from a natural reservoir of "carrot strains" from wild carrot, has not been resolved in this study.

With one exception, TNV was detected in carrot roots only in rusty root problem soils. If the virus does occur in non-problem soils it was present in the samples examined at concentrations below the levels detectable by the bioassay method used. Whenever TNV was detected in carrot roots, *Olpidium* sporangia and/or resting spores were easily found.

There is no doubt from this study and others (McElroy et al. 1971, Mildenhall et al. 1971, and Sutton 1975) that *Pythium* is associated with root necrosis. However *Pyrhium* root necrosis in our pot experiments should not necessarily be equated with rusty root symptoms in the field. One significant question needs answering: if *Pyrhium* spp. are solely responsible for rusty root then why doesn't the disease occur in other carrot growing areas outside of the Holland-Bradford and Keswick marshes? Virulent strains of *Pythium* spp., particularly *P. sulcatum* implicated by others with rusty-root-like diseases of carrots, occur in many carrot soils from fields in which the disease has not been found. The theory that *Pyrhium* spp. alone are responsible for rusty root disease does not seem to be conclusive from our observations; the possibility cannot be overlooked that O. *brassicae,* TNV, or O. *brassicae* plus TNV predisposes carrots to rusty root and are implicated with *Pythium.* Nor, however, can certain unique conditions, such as soil type or irrigation system, that might contribute to the disease in the Holland-Bradford and Keswick marshes, be ignored.

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