

## Seed and Root Rots of Ginseng (*Panax quinquefolius* L) Caused by *Cylindrocarpon destructans* and *Fusarium* spp.

Reeleder, R.D.<sup>#</sup>, Roy, R. and Capell, B.

Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada,  
1391 Sandford St, London, ON, Canada N5V 4T3

(Received April 14, 2002)

**Abstract :** Ginseng (*Panax quinquefolius* L.) has become one of the most valuable herb crops grown in North America. However, traditional cropping practices are favourable to disease and significant losses due to root disease are common, despite frequent use of fungicides. Seedlots are often contaminated with pathogens, however, little is known about the causes of seed decay and the role of seed pathogens as incitants of root rots. It was shown that both *Fusarium* spp. and *Cylindrocarpon destructans* were able to rot seeds and that *C. destructans* was more virulent than *Fusarium* spp. on seedling roots. A modified rose bengal agar (MRBA) medium (1 g KH<sub>2</sub>PO<sub>4</sub>; 0.5 g MgSO<sub>4</sub> · 7H<sub>2</sub>O; 50 mg rose bengal; 10 g dextrose; 5 g Bacto peptone; 15 g Bacto agar; 30 mg streptomycin sulfate; 250 mg ampicillin; 10 mg rifampicin; 500 mg pentachloronitrobenzene; 500 mg dicloran; and 1 L distilled water) was superior to potato dextrose agar in detecting *C. destructans* in diseased roots. Isolation of *C. destructans* from diseased seedlings arising from seeds sown in replant soil supported the hypothesis that this pathogen is a cause of ginseng replant failure in North America.

**Key words :** replant, *Panax quinquefolius*, disappearing root rot, replant decline, replant failure, *Cylindrocarpon*, *Fusarium*, Canada, North America, selective media

### INTRODUCTION

Over the past two decades, ginseng (*Panax quinquefolius* L.) has become one of the most valuable herb crops grown in North America.<sup>1)</sup> Although regarded as a difficult and high-risk crop to cultivate, relatively high profit margins have attracted growers to ginseng. Traditional systems of cultivation result in environments favourable to disease and severe losses are not uncommon.<sup>2-4)</sup> Commercial seedlots are often contaminated with pathogens. This is in part a consequence of the procedure used to stratify ginseng seeds. During stratification, seed is held in containers that are buried in the ground for up to 10 months. Containers are often located adjacent to existing ginseng plantations. Thus seeds are exposed to soilborne pathogens for a considerable period of time. Use of rotted or contaminated seed may introduce pathogens such as *Cylindrocarpon destructans*, *Botrytis cinerea* or *Rhizoctonia solani* into ginseng plantation sites during seeding operations.<sup>5,6)</sup> Soil carried by strong winds may also dis-

tribute pathogens from field to field. *Fusarium* spp. are reported as ginseng root pathogens<sup>7)</sup> although they appear to be less virulent on roots than isolates of *C. destructans*.<sup>5)</sup> However, little is known with regard to the ability of root-rotting pathogens of ginseng to cause seed decay. This report describes results of trials to compare *Fusarium* spp. and *C. destructans* as seed and root pathogens of ginseng. A useful isolation medium for *C. destructans* is described and a procedure for obtaining healthy seedlings for inoculation trials is outlined.

Grower experience suggests that cultivation of ginseng on sites previously used for ginseng production often results in failure of the second crop, a phenomenon known as replant disease or replant decline. In Asia, ginseng replant disease is attributed to *C. destructans*.<sup>8)</sup> *Cylindrocarpon* spp. are also associated with replant disorders of other crops.<sup>9)</sup> Here, we report results from trials designed to determine the cause of ginseng replant disease in Canada.

### MATERIALS AND METHODS

#### 1. Sources of isolates

Isolates used in these trials were obtained by plating

<sup>#</sup> To whom correspondence should be addressed.  
(Tel) +1-519-457-1470; (Fax) +1-519-457-3997  
(E-mail) reelederr@agr.gc.ca

sections of surface-disinfested partially-rotted seeds from commercial ginseng seedlots or diseased seedlings collected from commercial ginseng farms onto potato dextrose agar (PDA; Difco, Detroit, MI) amended with 300 mg novobiocin per L (PDAN) or onto a modified rose bengal agar (MRBA) containing: 1 g  $\text{KH}_2\text{PO}_4$ ; 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 50 mg rose bengal; 10 g dextrose; 5 g Bacto peptone (Difco); 15 g Bacto agar (Difco); 30 mg streptomycin sulfate; 250 mg ampicillin; 10 mg rifampicin (dissolved in 1 ml ethanol); 500 mg pentachloronitrobenzene; 500 mg dicloran; and 1 L distilled water. Antibiotics and fungicides were added after autoclaving and cooling of agar to 55°C. MRBA plates were held at 8°C in the dark until use. After adding diseased seed pieces, MRBA plates were held at room temperature ( $22 \pm 2^\circ\text{C}$ ) in the dark for two days then exposed to ambient light conditions. PDAN plates were held at room temperature under ambient light conditions. Fungi growing from diseased seeds were transferred to fresh PDA plates. Pure cultures to be used in studies were maintained by placing small blocks of agar cultures in sterile saline solution and storing at room temperature.<sup>5)</sup>

## 2. Production of inoculum

Conidia and mycelial macerates used for inoculations were obtained from 5-day-old cultures grown in dilute V-8 broth<sup>10)</sup> or potato dextrose broth (Difco). Mycelial mats from broth cultures were macerated with a blender, then the macerate was poured through two layers of sterile cheesecloth to separate conidia from mycelial fragments. Conidia were washed twice by centrifugation and adjusted to  $1 \times 10^6$  conidia/mL with sterile distilled water (SDW).

## 3. Pathogenicity of isolates to seed

To assess pathogenicity to seeds, ripe ginseng berries were collected from plots at the Delhi (Ontario, Canada) research farm of the Southern Crop Protection and Food Research Centre and mechanically depulped. Seeds were kept cool (6°C) and moist until use. Approx 100 mL of autoclaved silica sand was added to sterile polypropylene cups (capacity: 227 mL) (Fisher Scientific, Nepean, ON). SDW (5 mL) was added to each cup. Four cups were prepared for each fungal isolate. Thirty ginseng seeds were placed on the sand surface of each cup then 15 mL of conidial suspension was pipetted over the seeds. Four control cups were treated with sterile distilled water. Approximately 0.5 cm (30 g) of sand was placed over seeds, and moistened lightly with sterile water. Caps were placed loosely on the cups. Four cups of each treatment were

stored at  $10 \pm 1^\circ\text{C}$ . After 6 months, seeds were recovered from cups by washing sand onto a sieve. Seeds were surface-disinfested with 0.5% sodium hypochlorite then seedcoats were removed. Interiors of seeds were recorded as being rotted or healthy and sections of seeds were placed on PDAN. Percentage of seed rotted in each treatment replicate was determined; then data were subjected to arc-sine transformation prior to analysis with general linear model software.<sup>11)</sup>

## 4. Pathogenicity of isolates to seedling roots

Seedling production: As the conventional practice for seed stratification was observed to have a high rate of contamination with fungal pathogens, pathogenicity to ginseng seedlings was assessed using plants derived from seed stratified with an incubator procedure. Depulped ginseng seed was held at  $6 \pm 1^\circ\text{C}$  for approx 2 months after harvest then frozen in moist sand at  $1 \pm 1^\circ\text{C}$  for 6 months. The temperature then was increased to  $15 \pm 1^\circ\text{C}$  for 6 months. Distilled water was added as required to keep the sand moist. After a second cold treatment of 6 months, the seed was separated from sand by sieving and then held in a refrigerator at  $8 \pm 2^\circ\text{C}$ . Ginseng seed was observed to germinate within 2 weeks and seedlings were transferred to plastic cell trays (3×3×5 cm) (Kord Products Ltd, Bramalea ON) containing Pro Mix BX (Premier Horticulture, Rivière du Loup QC). Trays were placed in a growth chamber operating at  $20 \pm 1^\circ\text{C}$  with a 12 h photoperiod (light intensities of  $44 \mu\text{mol/s/m}^2$ ) and 95% RH. Seedlings were used in pathogenicity tests after 1-3 months of growth.

Pathogenicity test: Prior to inoculation, seedlings were transferred to 10-cm-diam. plastic pots (three replicate-pots per isolate) containing autoclaved silica sand (3 seedlings per pot). For each fungal isolate, 10 mL of suspension ( $1 \times 10^6$  conidia/mL) was evenly distributed across the pot surface. Then a 1 cm layer of autoclaved vermiculite was placed on the surface of the silica sand and the pots returned to the growth chamber. Pots were watered as required to keep the sand moist. After 4 weeks, plants were removed from pots and assigned to a disease severity class using a scale of 1 to 8, where 1 indicated a symptomless plant and 8 indicated a dead plant. A disease severity index for each replicate was then determined<sup>12)</sup> and treatments were compared using a general linear model.<sup>11)</sup>

## 5. Replant field trials (tile microplots)

The replant phenomenon was investigated in the following manner. In the Fall of 1994 soil samples were col-

**Table 1.** Crop history of soils used in this study

| Soil | Soil location <sup>a</sup>      | Soil type      | Year Ginseng Harvested | Previous Crop (grown in 1994) |
|------|---------------------------------|----------------|------------------------|-------------------------------|
| 1    | Delhi <sup>b</sup> : check soil | Fox loamy sand | never grown            | Winter Rye                    |
| 2    | Delhi: check soil fumigated     | Fox loamy sand | never grown            | Winter Rye                    |
| 3    | Delhi                           | Fox loamy sand | 1992                   | Winter Rye                    |
| 4    | Langton                         | Fox loamy sand | 1993                   | Winter Rye                    |
| 5    | Waterford                       | sandy loam     | 1993                   | Winter Rye                    |
| 6    | Teeterville                     | sandy loam     | 1986                   | Winter Rye                    |
| 7    | Waterford                       | loam           | 1992                   | Winter Rye                    |
| 8    | Waterford                       | loam           | 1985                   | pepper                        |
| 9    | La Salette                      | sandy loam     | 1993                   | Rye/Red Clover Mixture        |
| 10   | Windham Centre                  | sandy loam     | 1990                   | Winter Rye                    |

<sup>a</sup>All soils were located in Norfolk and Brant counties in southwestern Ontario, Canada, and collected in September and October 1994.

<sup>b</sup>Delhi: Delhi research farm of Agriculture and Agri-Food Canada.

lected from fields located throughout the ginseng production area in Ontario, Canada, (Table 1) that had previously (1-10 years) been cropped with ginseng. Soil samples from locations at the Delhi research farm that had not previously been cropped with ginseng were also collected and used as control (non-ginseng) soils. The area sampled from each field was about 0.20 ha in size. A 5×15 cm soil corer was used to grid-sample the field in order to obtain 0.6 cubic meters of soil. Upon return to the experimental site at Delhi, the soils were subdivided into four sub samples. Each sub sample was used to fill microplots prepared from plastic irrigation tiles. Tiles (58 cm in diam.) had previously been cut into 29 cm long sections and placed upright in raised beds in a wood-lath-shaded ginseng garden established at the Delhi research farm. At each tile location, bed soil was excavated to provide an opening for the tile. Four replicate tiles (one for each soil sub sample) were randomly assigned to each soil (Table 1) using a randomized complete block design. Tiles were then filled with the specified soil sub sample to a depth of 20 cm. Each tile was thus used as a microplot and served as a treatment replicate.

Fumigation of the soils in the microplots was achieved by using a hand-held injector to deliver a chloropicrin-containing fumigant (Vorlex CP) at 337 L of product/ha. The soil from the Delhi farm not previously cropped with ginseng (soil 1 in Table 1) was, however, not fumigated. Soil 2 (Table 1) had been fumigated with the same product before collection and thus was not fumigated again. Two weeks after fumigation, ginseng seeds were placed 1-cm deep in the soil of each tile (69 seeds per tile). Plots were then mulched with oat (*Avena sativa* L.) straw to a

depth of 5 cm.

Plant emergence and disease development in each microplot were followed during the 1995 growing season (May-Sep). Soil samples were collected from each microplot and compared with aliquots collected prior to fumigation with respect to populations of *Pythium* spp. A soil dilution plate procedure, in combination with a semi-selective medium (PARP), was used to estimate *Pythium* populations.<sup>13,14</sup> Fungal isolations from samples of diseased roots were made using media described above<sup>5</sup>. Resulting fungal identification data were used to compare the efficacy of PDAN and MRBA in isolating *C. destructans* from diseased roots.

In August 1995, all remaining plants (if any) were removed from the microplot tiles and all soils (including the previously nonfumigated soils) were again fumigated as described above. Root lengths and root dry weights were determined on surviving plants. Dry weight was determined by drying roots at 65°C for 3 days prior to weighing. Mean weight per plant was then determined.

In October 1995, microplots were re-seeded and mulched as before. Plant development was observed during the 1996 growing season and fumigation was carried out for a third time in the Fall of 1996. Microplots were again re-seeded in October 1996. Observations on plant growth were recorded during the 1997 growing season (May-Sep). At the end of the 1997 growing season, the experiment was terminated.

## 6. Replant greenhouse trial

A composite "replant" soil was prepared by removing samples from each replant soil (Table 1, soils 3-10). After

mixing, the composite soil was divided into three sub samples. One sub sample was pasteurised with pressurised steam (Lindig Manufacturing Co., St Paul MN) at 75°C for 30 min, one was autoclaved (121°C; 103 kPa) for 1 h each day on two successive days, and one sub sample was left untreated. Each of the three treatments was then further subdivided into 6 portions, with each portion added to a 12-cm-diam pot. Pots had previously been filled to a depth of 5 cm with perlite. Six stratified 2-yr-old greenhouse-grown ginseng roots were transplanted into each pot. Pots were held in a greenhouse at 20°C under plastic shade. Pots were arranged in a randomized complete block design, with 3 soil treatments and 6 replicate pots of each treatment. After 8 weeks, surviving plants were removed from each pot and rated for disease severity, as described above. Diseased roots tissue was observed under a microscope to determine the causal agent. Roots from the pasteurised and untreated soils were separated from tops, then roots were dried at 65°C for 3 days. Mean root weight was determined for each pot. Data for disease severity and root dry weight were compared for treatment effects.<sup>15)</sup>

## RESULTS AND DISCUSSION

### 1. Seed and root rot pathogenicity trials

All isolates of *Fusarium* spp. and *Cylindrocarpon destructans* caused seed rot (Fig. 1) and all isolates used in inoculation tests were recovered from rotted seed. Both genera thus appear to be able to cause rots of seeds during the stratification process used by most growers in North America. In seedling tests, *C. destructans* isolates generally were more virulent than *Fusarium* isolates (Fig. 2). Damage caused by *Fusarium* isolates was mainly restricted to rotting of secondary and tertiary roots. Although *Fusarium* can be isolated without difficulty from diseased roots, it appears that they are relatively weak pathogens of vigorously growing roots. They may act as secondary invaders of root tissue colonized by *C. destructans* and other pathogens and, possibly, damage caused to roots by *Fusarium* may provide wounds that promote infection by *C. destructans*. More intensive testing of a wider range of *Fusarium* isolates is suggested to confirm its role in root rots of *P. quinquefolius*. *C. destructans* has also been shown to cause root rots of *P. ginseng* in Korea and China.<sup>8,16-18)</sup> The incubator stratification procedure used here produced seedlings that were healthier than those derived from conventional stratification procedures. This procedure may be useful in studies where healthy seedlings are desired.

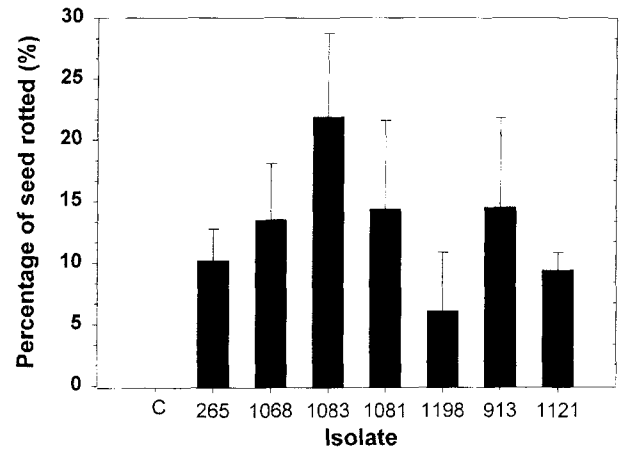


Fig. 1. Rotting of ginseng seed by isolates of *Fusarium* spp. and *Cylindrocarpon destructans*. Isolates 265, 1068 and 1083 are *Cylindrocarpon destructans*; isolates 1081 and 1198 are *Fusarium roseum*; isolates 913 and 1121 are *F. solani*. C=control treatment (seeds not inoculated). Mean percentage of seed rot was calculated by examining each seed in each replicate container and judging the seed to be either healthy or rotted. Error bars represent standard deviations from the mean of treatment replicates (n=4). Absence of error bars indicates that there was no variation among replicates of that treatment.

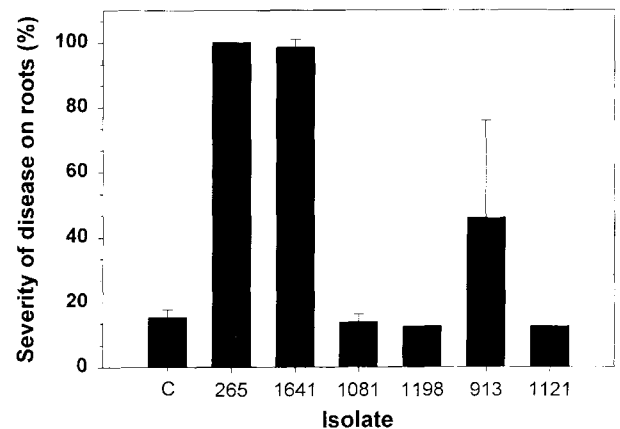
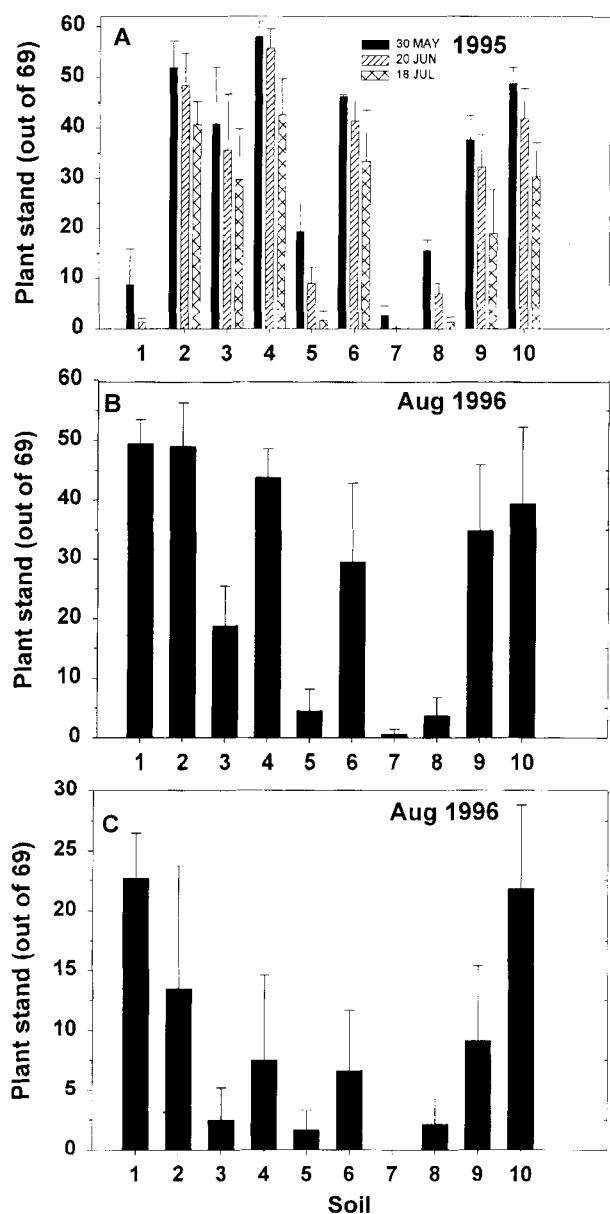


Fig. 2. Severity of root rot caused by isolates of *Fusarium* spp. and *Cylindrocarpon destructans*. Isolates 265 and 1641 are *Cylindrocarpon destructans*; isolates 1081 and 1198 are *Fusarium roseum*; isolates 913 and 1121 are *F. solani*. C=control treatment (plants not inoculated). Error bars represent standard deviations from the mean of treatment replicates (n=3). Absence of error bars indicates that there was no variation among replicates of that treatment. Plants were rated on a disease severity scale of 1-8 where 1=healthy plant and 8=dead plant. Disease indices were calculated by using the formula  $(SS_i/n/8N) \times 100$  ( $i=1,2,3$ ), where  $S$  is the appropriate disease scale,  $n$  = number of diseased plants with the same disease severity, and  $N$  = number of plants rated.

## 2. Field replant trial (tile microplots)

The work reported here supports the hypothesis that *C. destructans* is a major contributor to replant disease. In



**Fig. 3.** Plant stands over three years in tile microplots containing replant soils. Seeds were added to each microplot in October of 1994, 1995 and 1996, two weeks after fumigation of soils with a chloropicrin-containing fumigant. Soil 1 was not fumigated in 1994, but was fumigated in 1995 and 1996. Stand counts were recorded as number of plants observed out of 69 seeds planted per microplot. **A:** Stand counts recorded in May, June and July 1995. **B** and **C:** Stand counts recorded in Aug 1996 and 1997, respectively. Error bars represent standard deviations from the mean of treatment replicates (n=6).

1995, several differences in plant response to the different soils were noted. First, most emerging plants in soil 1 were, by mid-May, killed by *Pythium* spp. This fungus was also associated with damping-off in other soils, although, with the exception of soils 5, 7 and 8, the amount of damping-off during emergence in May 1995 was limited (Fig. 3A). In soil 7, where early damping-off was as prevalent as in soil 1, emerged plants that survived this period were yellowed in appearance. Populations of *Pythium* spp., as determined on selective agar media, were quite variable within a given soil (Table 2). However, population data (colony-forming-units (CFU) per g dry soil) suggest that fumigation reduced populations by approx. 66% (range 40-86%) in replant soils 3-10. As fumigation appears to reduce *Pythium* populations, and as these fungi are known to be virulent to ginseng seedlings,<sup>5)</sup> the differences in plant stand in 1995 between soils 1 and 2 are likely due mainly to the lack of fumigation in soil 1 and the subsequent higher populations of *Pythium* in this soil.

As the season progressed, surviving plants in soils 3-9 became more yellowed than those in soils 2 and 10 where plants remained green and vigorous. Some of these yellowed plants in soils 3-9 died, leading to further reductions in stand by July (Fig. 3). Plating onto agar media of root tissue from plants that damped-off in July and August indicated that most plant death at this time was

**Table 2.** Effect of soil fumigation on *Pythium* populations in replant soils.<sup>a</sup>

| Soil <sup>b</sup> | Population prior to fumigation |                 | Population after fumigation |       |
|-------------------|--------------------------------|-----------------|-----------------------------|-------|
|                   | CFU <sup>c</sup>               | SD <sup>d</sup> | CFU                         | SD    |
| 1                 | 137.5                          | 74.4            | ND                          | ND    |
| 2                 | ND <sup>e</sup>                | ND              | 29.2                        | 29.2  |
| 3                 | 425.0                          | 88.6            | 62.5                        | 30.6  |
| 4                 | 312.5                          | 172.7           | 87.5                        | 37.9  |
| 5                 | 87.5                           | 64.1            | 45.8                        | 33.2  |
| 6                 | 250.0                          | 92.6            | 66.7                        | 30.3  |
| 7                 | 437.5                          | 118.8           | 83.3                        | 58.4  |
| 8                 | 850.0                          | 130.9           | 300.0                       | 117.3 |
| 9                 | 512.5                          | 99.1            | 250.0                       | 122.5 |
| 10                | 762.5                          | 159.8           | 470.8                       | 87.2  |

<sup>a</sup>Data represent populations in soil samples collected before and after fumigation of soil in the Fall 1994.

<sup>b</sup>Refer to Table 1 for soil descriptions.

<sup>c</sup>CFU colony-forming-units per g dry soil, as determined with PARP agar medium.

<sup>d</sup>SD standard deviation.

<sup>e</sup>ND not done.

due to *C. destructans*, particularly in soils 3, 4, 5, 7, 8, 9. *Pythium* was less frequently isolated from damped-off plants at this time. When roots of surviving plants were plated onto agar media, *C. destructans* was the pathogen most commonly isolated from the roots. The root symptoms were similar to those reported previously.<sup>5,8,16</sup> *Chaetomium* sp., previously shown to be common on ginseng seed<sup>19</sup> was frequently isolated from ginseng roots in 1995 but not in subsequent years. Disease ratings were variable

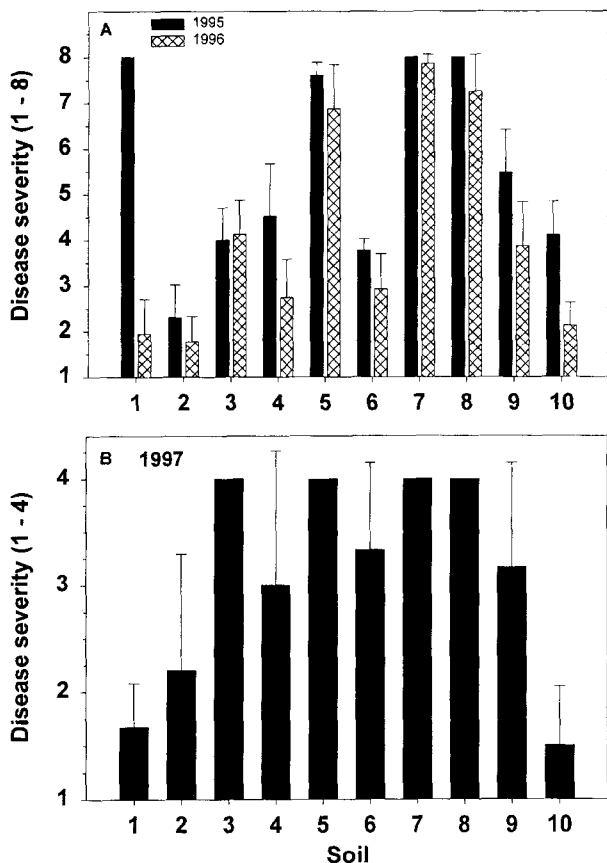
from soil to soil (Fig. 4) with soils where damping-off occurred having the higher disease ratings.

The yellowing symptoms observed in 1995 were noted again in 1996 and 1997, despite the additional fumigation treatments. These additional fumigations likely caused further reductions in populations of *Pythium* spp. For example, soil 1, which was not fumigated in 1994, but which was fumigated in the Fall of 1995 and 1996, exhibited no *Pythium* damping-off in 1996 or 1997. Fumigation appears to have reduced the *Pythium* populations sufficiently to allow good emergence in those years (Fig. 3). While fumigation reduced the effects of *Pythium*, symptoms of *C. destructans* and the associated yellowing of plants developed to varying degrees in replant soils 3-10 in both 1996 and 1997. Plants in soils 5, 7 and 8 had the plants with the most developed yellowing symptoms in 1996 and 1997. Disease severity in soil 3 appeared to be more severe in 1997 than in previous years, relative to disease severity observed on plants growing in other soils. *C. destructans* was readily isolated from the roots of plants growing in these four soils in 1996 and 1997. Apart from *Fusarium* spp., no other fungus was recovered commonly in 1996 or 1997 from symptomatic roots. The overall reduced emergence in 1997 was likely the result of poor seed quality, but relative differences between treatments remained similar to those observed in 1996.

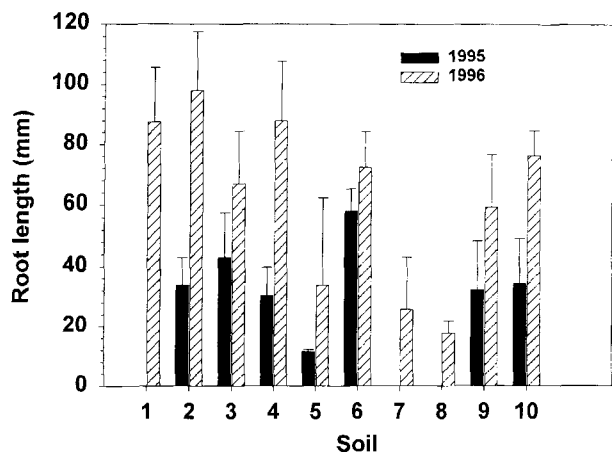
Mean root length varied markedly from soil to soil in 1995 but less so in 1996 (Fig. 5). This may be an effect of the second fumigation in the Fall of 1995 and consequent reductions in populations of *Pythium* and perhaps other organisms. Root dry weights in 1995 and plant dry weights in 1996 (Fig. 6) generally reflected disease severities.

The use of MRBA was found to be helpful in isolation of *C. destructans* from diseased seed and roots. When MRBA and PDAN were used to isolate pathogens from yellowed, diseased seedlings (collected from the replant field trial) with symptoms of *Cylindrocarpon* root rot, only 11.8% of 144 isolates obtained from PDAN were identified as *C. destructans*. The remainder were mostly *Fusarium* or *Chaetomium* spp. In contrast, 64.4% of 73 isolates obtained from MRBA were *C. destructans*. This fungus has a characteristic appearance on MRBA and the medium is more suitable for the isolation of *C. destructans* than PDA-based media, particularly when *Fusarium* is present.

*C. destructans* was the pathogen most frequently isolated from diseased roots of stunted, yellowed plants. Root symptoms were similar to those found in pathogenicity trials reported here and in other studies.<sup>5,17,20</sup> The



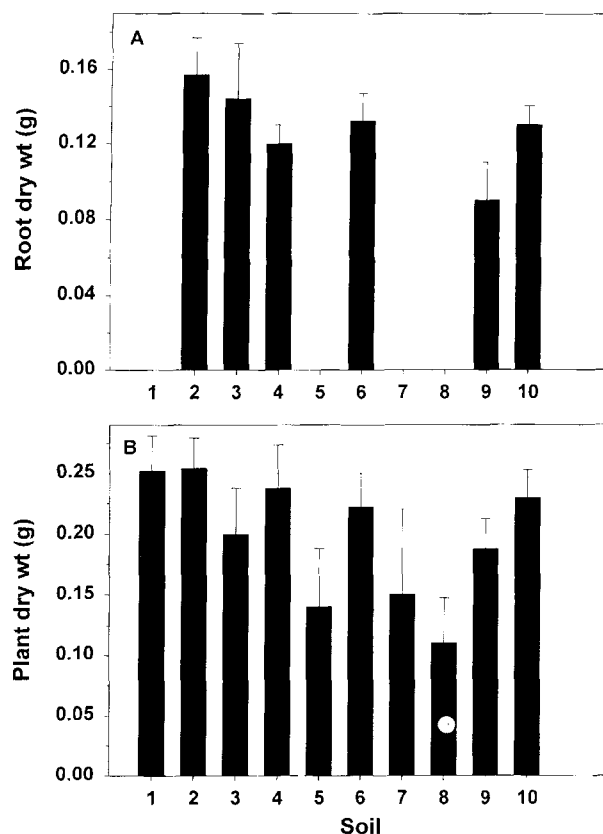
**Fig. 4.** Disease severity of ginseng seedlings in tile microplots containing the different replant soils. See Table 1 for soil descriptions. **A.** Disease severities in August of 1995 and 1996. Severities are the means of 6 replicate microplots of each soil. For each microplot, 10 plants were collected and rated on a 1-8 disease severity scale, where 1=healthy root system and 8=dead plant. The average rating represented the value for that microplot. **B.** Disease severity in Aug 1997. A 1-4 scale was used, where each microplot was assigned to a severity class; 1=plants with healthy root systems and 4=plants yellowed and with severely rotted roots. Error bars represent standard deviations from the mean disease severity for that treatment (n=6). Absence of error bars indicates that there was no variation among replicates of that treatment.



**Fig. 5.** Root lengths of ginseng seedlings in tile microplots containing the different replant soils (1995, 1996). See Table 1 for soil descriptions. Data for each soil are means of maximum root length of 10 plants per microplot collected in August of each year. Error bars represent standard deviations from the mean ( $n=6$ ).

extent of disease, however, varied from soil to soil and there was no obvious relationship between years since last harvest and the degree of symptoms. The lack of efficacy of the repeated fumigation treatments is puzzling and needs more investigation. This pathogen readily produces thick-walled chlamyospores<sup>17)</sup> and these structures may be more resistant to fumigants than those of organisms such as *Pythium*. Alternatively, *C. destructans* propagules may remain immersed in root residue for longer periods than *Pythium* and this residue may shield the pathogen from the fumigant. Loamy soils tended to have more severe replant symptoms. If *C. destructans* is a major contributor to replant failure, then the ability of this fungus to survive in certain soils long after the first crop has been harvested may be an important factor in replant problems. Practices that reduce development of this pathogen in the first garden may be critical to the use of the site for a second planting. Crop rotations, organic amendments, composts, fungicides and fumigants may all have roles to play in suppressing *C. destructans*. Gardens that have been essentially free of *C. destructans* during the first planting may be better candidates as replant sites than gardens where *C. destructans* rots are evident. *Fusarium* spp. were also recovered commonly from diseased roots in this study. However, based on results of pathogenicity trials reported here and elsewhere,<sup>7)</sup> they appear to be weakly virulent and unable to cause the symptoms found in replant soils.

Use of assays such as the tile microplots used here



**Fig. 6.** Root dry weights of ginseng seedlings in tile microplots containing the different replant soils (1995, 1996). See Table 1 for soil descriptions. **A.** Mean root dry weights (g) of plants collected in August 1995. **B.** Mean plant dry weights (roots and foliage) of plants collected in August 1996. Plant tissue was dried at 65 °C for 3 days prior to weighing. Error bars represent standard deviations from the mean ( $n=6$ ).

might be helpful in predicting whether replant failure will occur in a given site. However, more direct comparisons of assay results with replant gardens is required to validate this approach. The use of molecular techniques may also be valuable in estimating populations of *C. destructans* in soils.<sup>20)</sup> Population estimates might provide a guide as to whether or not a given site is free of *C. destructans*.

### 3. Greenhouse replant trial

In the greenhouse trial, aliquots of a composite replant soil were subjected to pasteurisation or autoclaving and compared to untreated soil for effects on plant growth. Both autoclaving and pasteurisation significantly decreased root disease severity when compared to the untreated check ( $P=0.012$ ). Respective mean root disease severity indices of plants grown in the autoclaved, pasteurised, and untreated soils were 26.3, 23.2, and 51.4%. Mean root

weights for the pasteurised and untreated soils were 0.58 g and 0.36 g, respectively, and were significantly different ( $P=0.006$ ). Dry weights were not determined for roots from the autoclaved soil, although the roots appeared to be of a size similar to those of the pasteurised soil. Disease symptoms observed in the untreated soil were typical of disappearing root rot,<sup>2)</sup> the disease most often associated with *C. destructans* in North America. This fungus was observed in diseased tissue. These results provide confirmation that *C. destructans* is associated with the replant disease in North America. Replant disease of *P. ginseng* in Korea is also associated with this pathogen.<sup>8)</sup>

In summary, the fungi *Fusarium* spp. and *C. destructans* were shown to cause rots of ginseng seed when seed is held at low temperatures similar to those used during stratification of seed. Partially rotted seed may be one mechanism by which these pathogens are introduced into ginseng plantings. *C. destructans*, however, is more virulent than isolates of *Fusarium* spp. with respect to seedling root disease. *C. destructans* is also associated with ginseng replant disease and typical symptoms of disease caused by this fungus are readily produced on seedlings growing in replant soils as long as 10 years after an initial ginseng crop. *C. destructans* is a virulent, long-lived pathogen and new management strategies are required to control this fungus.

## ACKNOWLEDGEMENTS

This work was supported by the Ginseng Growers Association of Canada through the Matching Investment Initiative of Agriculture and Agri-Food Canada. The assistance of R. Grohs is gratefully acknowledged. For the Department of Agriculture and Agri-Food, Government of Canada, ©Minister of Public Works and Government Services Canada (2002).

## REFERENCES

1. Proctor, J. T. A. and Bailey, W. G. : Ginseng: Industry, botany and culture. *Horticultural Reviews* **9**, 187-236 (1987).
2. Hildebrand, A. A. : Root rot of ginseng in Ontario caused by members of the genus *Ramularia*. *Canadian Journal of Research* **12**, 82-114 (1935).
3. Parke, J. L. and Shotwell, K. M. : *Diseases of Cultivated Ginseng*. University of Wisconsin-Extension, Madison (1989).
4. Whetzel, H. H., Rosenbaum, J., Brann, J. W. and McClintock, J. A. : *Ginseng Diseases and Their Control*. U. S. Dept. of Agriculture-Bureau of Plant Industry, Washington (1916).
5. Reeleder, R. D. and Brammall, R. A.: Pathogenicity of *Pythium* species. *Cylindrocarpon destructans*, and *Rhizoctonia solani* to ginseng seedlings in Ontario. *Canadian Journal of Plant Pathology* **16**, 311-16 (1994).
6. Reeleder, R. D. and Fisher, P. : *Diseases of Ginseng*. Ontario Ministry of Agriculture, Food, and Rural Affairs, Toronto, Canada 4 pp. (1995).
7. Punja, Z. K.: Fungal pathogens of American ginseng (*Panax quinquefolium*) in British Columbia. *Canadian Journal of Plant Pathology* **19**, 301-306 (1997).
8. Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H. and Lee, H. S.: Root-rot development of 2-year old ginseng (*Panax ginseng* C.A. Meyer) caused by *Cylindrocarpon destructans* (Zinssm.) Scholten in the continuous cultivation field. *Korean Journal of Ginseng Science* **19**, 175-180 (1995).
9. Braun, P. G. : The combination of *Cylindrocarpon lucidum* and *Pythium irregulare* as a possible cause of apple replant disease in Nova Scotia. *Canadian Journal of Plant Pathology* **13**, 291-297 (1991).
10. Tuite, J.: *Plant Pathological Methods: Fungi and Bacteria*. Burgess Publishing. Co., Minneapolis MN. 239 pp. (1969).
11. Statistical Analysis System (SAS), Version 6.12. Carey NC (1996).
12. Zhou Ting and Reeleder, R. D. : Application of *Epicoccum purpurascens* spores to control white mold of bean. *Plant Disease* **73**, 639-642 (1989).
13. Jeffers, S. N. and Martin, S. B. : Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* **70**, 1038-1043 (1986).
14. Reeleder, R. D. and Hagedorn, D. J. : *Pythium* populations in Wisconsin bean fields. *Canadian Journal of Plant Pathology* **3**, 90-93 (1981).
15. SigmaStat 2.03. SPSS Inc., Chicago IL (1995).
16. Cho, D. H., Ahn, I. P., Yu, Y. H., Ohh, S. H. and Lee, H. S.: Effect of incubation period, temperature and pH on mycelial growth of *Cylindrocarpon destructans* (Zinssm.) Scholten causing root-rot of ginseng. *Korean Journal of Ginseng Science* **19**, 181-187 (1995).
17. Cho, D. H., Yu, Y. H., Ohh, S. H. and Parke, J. L. : Production and isolation of chlamydospores in *Cylindrocarpon destructans* causing root rot of *Panax quinquefolium*. *Journal of Ginseng Research*. **22**, 304-309 (1998).
18. Gao Shuyan: Preliminary study on the biological characteristics of *Cylindrocarpon destructans* of ginseng. *Acta Agriculturae Universitatis Jilinensis* **11**, 28-32 (1989).
19. Ziezold, M., Hall, R., Reeleder, R. D. and Proctor, J. T. A.: Seedborne fungi and fungicide seed treatment of ginseng. *Journal of Ginseng Research*. **22**, 229-236 (1998).
20. McMullen, C., Yee, D., Reeleder, R., Seifert, K. and Dobinson, K. F. : Molecular diagnostics and genetic diversity of *Cylindrocarpon destructans*. *Canadian Journal of Plant Pathology* **21**, 203 (Abstract) (1999).