ABSTRACT

Two apple rootstock clones, MM.111 (field resistant to Phytophthora crown rot under New York orchard conditions) and MM.106 (field susceptible), were inoculated on the first of each month, except August, for 25 mo (January 1983 through January 1985). Previous growing season's shoots were cut into 65-mm lengths and inoculated in the laboratory with two isolates of Phytophthora cactorum, three isolates of P. megasperma, two isolates of P. cryptogea (A1), one isolate of P. cambivora (A1), and one isolate of an unidentified Phytophthora sp. (A1). All isolates had previously been recovered from diseased apple trees in New York. Necrosis values, calculated from the arcsin [square root] transformation of the proportion of twig length that was necrotic, were plotted over time to produce patterns of seasonal variation in the extent of colonization by Phytophthora spp. Over all 25 mo, MM.111 was colonized as much as or to a greater extent than MM.106 by all isolates except one of P. cactorum. There was a significant isolate × rootstock interaction, which indicated a difference in virulence of these nine isolates to the two rootstocks. Monthly changes in the extent of colonization of both rootstocks by each isolate were highly significant; seasonal patterns between years were similar. The five species of Phytophthora fell into two groups depending on when relative peaks of colonization of the two rootstocks occurred: P. cactorum and P. cambivora had one peak during late spring and summer; P. megasperma, P. cryptogea, and Phytophthora sp. had two peaks, one during summer and one during winter.

Phytophthora crown rot is the most serious disease affecting the crown and roots of apple (Malus pumila Mill.) in New York and probably worldwide. Various species of Phytophthora are associated with this disease, but P. cactorum (Leb. & Cohn) Schroeter has been reported most often (6; S. N. Jeffers and H. S. Aldwinckle, unpublished). In New York, P. cactorum, P. megasperma Drechs., and two unidentified Phytophthora isolates (Phytophthora sp. I = NY.001 and Phytophthora sp. II = NY.082) were reported previously as causal agents of crown rot (6). Both of these unidentified isolates have since been tentatively identified as P. cryptogea Pethyb & Laff., mating type A1 (S. N. Jeffers, unpublished). In addition, P. cambivora (Petri) Buism. (A1) has been recovered from apple orchard soil, apple nursery stock, and one apple tree in New York (S. N. Jeffers and H. S. Aldwinckle, unpublished). Another heterothallic isolate of Phytophthora, NY.180, has been isolated from a 2-yr-old symptomatic apple tree in a western New York orchard. This isolate is morphologically and culturally unlike any of the above species but is similar to the unidentified species isolated from cherry in California (15) and New York (14).

The extent to which apple scion and rootstock cultivars were colonized by P. cactorum and P. syringae fluctuated depending on the time of year inoculations were made (1,4,11,13). Time of peak colonization differed for the two species; colonization by P. cactorum was associated with active growth, and that by P. syringae was associated with dormancy. Seasonal variation in the extent of colonization of woody apple tissues by other Phytophthora spp. has been reported only once (9). Identifying periods of maximum colonization by Phytophthora crown rot pathogens is necessary for effective timing of both fungicide applications in the orchard and test inoculations of selections in rootstock breeding programs.

Our goals in this research were 1) to compare, over the entire year, the extent of colonization of two apple rootstocks, Malling-Merton (M.M.) 106 (field susceptible) and MM.111 (field resistant), by all Phytophthora spp. recovered from apple in New York; 2) to compare the periods when colonization by P. cactorum is greatest in New York with those reported elsewhere; and 3) to determine if these two rootstocks exhibit seasonal changes in the extent of colonization by species of Phytophthora other than P. cactorum and, if so, when the periods of greatest colonization occur.

MATERIALS AND METHODS
Phytophthora isolates. All isolates tested were recovered from apple trees with Phytophthora crown rot in New York and are maintained in our culture collection. The only exception was P. megasperma NY.125, which was recovered from apple orchard soil collected around an affected tree. At least two isolates of each species were used when available: P. cactorum NY.097 and NY.188; P. megasperma NY.055, NY.125, and NY.176; P. cryptogea (A1) NY.001 and NY.082; P. cambivora (A1) NY.113; and Phytophthora sp. (A1) NY.180. During this investigation, active cultures of each isolate were maintained on a selective medium containing cornmeal agar, 10 mg/L pimaricin, 200 mg/L ampicillin, and 10 mg/L rifampicin (PAR) (S. N. Jeffers and S. B. Martin, unpublished) at 22 C in the dark and were transferred monthly.

Collection of rootstock material. Woody shoots were collected from the rootstock clones MM.106 and MM.111 growing in a hedgerow planting at the New York State Agricultural Experiment Station, Geneva. The hedgerow was subjected to normal cultural management but had not been pruned regularly.

The most recent growing season's terminal shoots were collected at monthly intervals for 25 mo, from January 1983 through January 1985. No shoots were collected in August when the previous growing season's shoots were difficult to locate and current season's shoots were too succulent for inoculation. Shoots collected between June and November were carefully stripped of their leaves before inoculation. Dates of shoot collection and inoculation varied between days 1 and 11 of each month but usually were between days 1 and 6 (in 18 of 23 mo). Periods between collection dates ranged from 27 to 36 days except for July to September, when it was 63 days; the average period was 30 days. Inoculations. All inoculations were performed in vitro using the excised twig assay of Jeffers et al. (5) with slight modifications. Before each inoculation date, isolates were grown on cornmeal agar amended with 5 g/L additional agar.
and 20 mg/L pimaricin (PCMAA) in Pyrex storage jars for 12–14 days at 22°C in darkness. One jar of each isolate was used for each rootstock on each inoculation date.

Excised apple shoots from the orchard were immediately disinfested in 1% NaOCl for 10 min, thoroughly rinsed in tap water, blotted dry, and cut to 65-mm lengths ("twigs"). Fifteen twigs were selected at random and the proximal ends were pared, as described previously (5), before they were pushed vertically into PCMAA at the colony periphery. Each month, 15 twigs of each rootstock clone were pushed similarly into PCMAA without fungus and served as controls. The lids on storage jars were sealed with Parafilm, and jars were incubated at 22°C in the dark. After 14 days, each twig was measured for total length of necrosis either with or without the periderm removed to expose the phloem-cambium region.

Data analysis. The proportion of twig length above the agar surface that was necrotic was calculated for each twig, and proportions were transformed by an arcsin [square root] calculation (=necrosis value) to stabilize variances (12). Before transforming data, 0.0 proportions were converted to 1/4n (= 0.01667) and 1.0 proportions were converted to (n − 1/4)/n (= 0.98333) as recommended by Snedecor and Cochran (12). Data analyses on the transformed proportions were done by analysis of variance. Mean monthly necrosis values and 95% confidence intervals for MM.106 and MM.111 were plotted over time to produce seasonal patterns of the extent of colonization by individual isolates of Phytophthora spp. Inoculations made in mid-May 1984 are not included on the plots.

RESULTS

General observations. Disinfestation in 1% NaOCl for 10 min did not eliminate all contaminants. After 2 wk at 22°C, mycelium of saprophytic fungi occasionally grew on twig and agar surfaces, particularly on twigs with much necrosis. These contaminants did not interfere with the assay.

Uninoculated control twigs remained green and without necrosis except in June, when several twigs of each clone became infected and discolored. Infection was most often associated with a bud or lenticel or the cut distal end of the twig but was not associated with the pared basal end pushed into the agar. Isolations from these twigs onto PAR selective medium (S. N. Jeffers and S. B. Martin, unpublished) yielded no pythiaceous fungi.

Lesions on twigs did not always show the distinctive orange-brown coloration of phloem tissues that was observed previously (5). Frequently, they appeared as a light discoloration of the outer bark and a faint yellow-orange coloration of the normally white phloem tissue. Such lesion lengths were most easily measured without removing the periderm. At the end of the incubation periods for the first two inoculations, periderm strips were removed from inoculated twigs and placed on PAR or PARH medium (S. N. Jeffers and S. B. Martin, unpublished) to ensure that length of colonization and length of necrosis were similar. Typical Phytophthora mycelium almost always emerged from periderm strips up to the end of visible necrosis.

For a given isolate/rootstock combination, variability among twigs was considerable and was not consistent from month to month. The arcsin [square root] transformation of proportion of necrotic twig length did not completely achieve a stabilization of variances. The overall range in magnitude of standard deviations of mean monthly necrosis values for each isolate/rootstock combination was reduced, but for many isolates, standard deviations were still not entirely independent of their means. At times, when rootstocks were colonized most extensively, necrosis frequently extended to the ends of twigs regardless of isolate and rootstock clone. After most inoculations, one to several twigs per isolate had no necrosis above the agar surface.

Inoculations over 25 mo. There was a definite effect of season for each isolate on both MM.106 and MM.111. Each isolate/rootstock combination had a highly significant F statistic (P = 0.01), calculated from an analysis of variance with 22 and 322 degrees of freedom, for its 23 monthly necrosis values. The seasonal effect was most pronounced (i.e., had the greatest F statistic) when rootstocks were inoculated with P. cactorum or P. cambivora.

Necrosis values tended to be greater in 1984 than in 1983. On MM.106, seven of nine isolates had yearly mean necrosis values that were greater in 1984 than in 1983; four of these were significant (P = 0.05). On MM.111, eight of nine isolates had yearly mean necrosis values that were greater in 1984 than in 1983, and five of these were significant (P = 0.05).

Colonization of MM.106 vs. MM.111. When the mean necrosis values over all months for each isolate on each rootstock (means ± standard deviations in Table 1) were compared in a two-way analysis of variance (6 and 6,192 degrees of freedom), a highly significant (P = 0.01) isolate × rootstock interaction occurred. Independent analyses of isolates within a species produced similar significant interactions for P. cactorum (P = 0.01) and P. megasperma (P = 0.05) but not for P. cryptogea. However, the main effects of rootstock and isolate for this latter species were highly significant (P = 0.01); NY.001 was more virulent than NY.082, and MM.111 was colonized more extensively than MM.106.

Mean necrosis values over all months for MM.106 and MM.111 are compared by isolate in Table 1. MM.106 was colonized more extensively than MM.111 by three isolates, but the difference was significant (P = 0.01) in only one instance. MM.111 was colonized more extensively than MM.106 by six isolates, and two of these differences were highly significant (P = 0.01). Necrosis on MM.111 and MM.106 was comparable for most isolates.

Seasonal colonization by P. cactorum. Colonization of both rootstocks by P. cactorum increased to a maximum in June and then declined to a minimum between October and December (Fig. 1A). Both isolates of P. cactorum, NY.097 and NY.188, produced similar seasonal patterns. Necrosis values from NY.097 often were less on MM.111 than on MM.106 (Fig. 1A); over all months, this difference was significant (Table 1). Correlation between mean monthly necrosis values in 1983 and 1984 was high for both P. cactorum isolates (Table 2). Inoculations conducted in mid-May yielded a necrosis value intermediate to the May and June necrosis values for MM.111 and a necrosis value equivalent to or less than the May value on MM.106.

| Table 1. Necrosis values on excised shoots of apple rootstocks MM.106 and MM.111 inoculated with species of Phytophthora* |
|-----------------|----------------|----------------|
| Species          | Isolate | MM.106 | MM.111 |
| P. cactorum      | NY.097  | 38.5 ± 26.1** | 33.3 ± 22.2 |
| P. megasperma    | NY.188  | 34.5 ± 24.9 | 37.5 ± 23.3 |
|                  | NY.055  | 34.8 ± 24.5 | 33.3 ± 24.3 |
| P. cryptogea     | NY.125  | 28.4 ± 21.6 | 34.0 ± 23.6** |
| P. cambivora     | NY.176  | 31.6 ± 21.0 | 34.6 ± 23.2 |
| Phytophthora sp. | NY.001  | 36.5 ± 18.6 | 39.2 ± 18.3 |
|                  | NY.082  | 27.4 ± 20.0 | 33.1 ± 19.0** |
|                  | NY.113  | 32.5 ± 26.6 | 32.3 ± 25.8 |
|                  | NY.180  | 40.3 ± 27.3 | 43.5 ± 28.3 |

*Previous season's shoots were collected at monthly intervals, except August; 65-mm twigs were cut and inoculated in vitro with each isolate. The necrotic proportion of each shoot length was transformed by an arcsin [square root] calculation (=necrosis value) before analysis.

**A significantly greater necrosis value (P < 0.01) on one rootstock by an analysis of variance with 1 and 688 degrees of freedom.

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Seasonal colonization by *P. megasperma*. Seasonal colonization patterns of MM.106 and MM.111 by three isolates of *P. megasperma* were variable between rootstocks and among isolates (Fig. 1B); periods when the extent of colonization was relatively great did not always coincide. A seasonal effect was the least pronounced with this species, although it was still highly significant (*P* = 0.01). Both rootstocks appear to have two peaks in the extent of colonization, one between June and July and one between December and January (Fig. 1B). In summer 1983, maximum colonization of MM.111 occurred later and lasted longer than that of MM.106. Over all months, NY.125 colonized MM.111 more extensively (*P* = 0.01) than MM.106 (Table 1). Mean monthly necrosis values in 1983 and 1984 were moderately to well correlated for all isolate/rootstock combinations except for NY.055/MM.106, which was not correlated (Table 2). Necrosis occurring in mid-May 1984 was slightly greater than that recorded at the beginning of May on MM.106 and was equal to or less than that recorded at the beginning of May on MM.111.

![Graphs](image-url)

**Fig. 1.** Patterns of variation in mean monthly necrosis values and 95% confidence intervals on excised shoots of MM.106 (*) and MM.111 (○) apple rootstocks inoculated with nine isolates of five *Phytophthora* spp. At the beginning of each month, except August, for 25 mo, previous season's shoots were collected, and 15 65-mm lengths were inoculated in vitro with each isolate. The necrotic proportion of each twig length was transformed by an arcsin [square root] calculation (= necrosis value). Monthly means for MM.106 and MM.111 are shown slightly offset to avoid overlapping confidence intervals. Triangles indicate time of full bloom (FB). (A) *P. cactorum* isolates NY.097 and NY.188; (B) *P. megasperma* isolates NY.055, NY.125, and NY.176; (C) *P. cryptogea* isolates NY.001 and NY.082; and (D) *P. cambivora* isolate NY.113 and *Phytophthora* sp. isolate NY.180.

**Seasonal colonization by *P. cryptogea***. Seasonal colonization of the two rootstocks by *P. cryptogea* NY.001 was fairly similar, but that by NY.082 was less consistent (Fig. 1C); MM.111 was colonized more extensively by NY.082 than was MM.106 (Table 1). Maximum colonization of both rootstocks occurred between June and July, and another peak in colonization occurred between December and January. Monthly necrosis values in 1983 and 1984 were well correlated for both isolates on each rootstock (Table 2). On MM.106 and MM.111, necrosis in mid-May 1984 was
only slightly greater than that measured at the beginning of May for both isolates.

**Seasonal colonization by P. cambivora.** The greatest seasonal effect was exhibited by *P. cambivora* NY.113. Patterns of colonization for the two rootstocks were nearly identical (Fig. 1D). Both MM.111 and MM.106 were colonized most extensively in June and July and very little in all other months; monthly necrosis values between 1983 and 1984 were highly correlated (Table 2). Inoculation in mid-May 1984 yielded a necrosis value equal to that at the beginning of May on MM.111 and intermediate between May and June values on MM.106.

**Seasonal colonization by Phytophthora sp. NY.180.** Considerable variability in necrosis (i.e., large 95% confidence interval in Fig. 1D) was associated with twigs inoculated with *Phytophthora* sp. NY.180. Colonization of MM.106 compared with MM.111 varied over the 25-on-the-first-period (Fig. 1D). Correlation between monthly necrosis values in 1983 and 1984 was moderate for MM.111 but very poor for MM.106 (Table 2). Both rootstocks had two periods when the extent of colonization was relatively great, one in June and July and the other around December and January (Fig. 1D). The duration of the two periods varied between 1983 and 1984 for both rootstocks. The mid-May 1984 mean necrosis value was equal to that recorded in the beginning of May for MM.111 and was intermediate between May and June values for MM.106.

**DISCUSSION**

By using an excised twig assay, we made inoculations year-round under uniform conditions. Others inoculated stems of trees in the orchard (7,11,13), but colonization was then dependent on ambient environmental conditions. Air temperatures during winter months are normally too cold in New York (<0 C) for growth of *P. cactorum* and may limit year-round orchard inoculations elsewhere (1).

Results from inoculating stems with *P. cactorum* in situ and in vitro have not been consistent (1,7), but the method of inoculation that is more meaningful has not been determined. Stem inoculations, regardless of method, are measuring not absolute resistance that would be expressed in the orchard (5,11) but rather a relative estimate of susceptibility of the phloem-cambium tissues to colonization by *Phytophthora* spp. (termed "inherent susceptibility" by Sewell and Wilson [11]). Artificial inoculation circumvents the infection process altogether, and resistance mechanisms that normally function at this level are not expressed. Consequently, until evidence is available to correlate susceptibility as it occurs in the orchard with results of artificial inoculations, we will refrain from using the terms "susceptible" or "resistant" to refer to such results.

Incubation of jars at 22 C for 2 wk, compared with 25 C for 7 days (5), allowed more necrosis to develop on inoculated twigs and provided a better spread between isolates during periods of little colonization. However, it also produced increased variability in necrosis between twigs; standard deviations for 15 inoculated twigs were less when the incubation time was 7 days (5). In almost any month, colonization of some twigs did not progress at all or progressed only slightly above the agar surface. Consequently, the variability in necrosis values for an isolate/rootstock combination, especially during periods when colonization extent was intermediate, was often large (95% confidence interval in Fig. 1A-D).

The excised twig assay we have used was originally developed to allow easy, uniform inoculation of numerous twigs for each isolate and with a variety of *Phytophthora* spp. (5). All twigs inoculated with one isolate are incubated in the same jar and therefore may not be replicates in the true sense, i.e., they may not be independent of each other. We have treated them as replicates because in our experience, there was much greater variability among twigs within a jar than between jars. Growing conditions among jars were considered to be identical.

Under New York orchard conditions, incidence of *Phytophthora* crown rot is common on MM.106 but rare on MM.111 (personal observation). This difference in resistance was not associated with the extent of colonization in our investigation; MM.111 was colonized as much as or more extensively than MM.106 by all isolates except *P. cactorum* NY.097. Schwinn (10) reported results similar to ours; after artificial inoculation, a resistant scion cultivar was colonized as much as a susceptible scion cultivar. In contrast, Mirchett et al (9), who also used excised shoots, reported that MM.111 was colonized less extensively than MM.106 by *P. cambivora* in California.

Our data suggest that the resistance of MM.111 in the field may be related to the inability of the fungus to gain ingress and is not a result of its ability to retard colonization after infection has occurred. If environmental and physiological stresses are important in the incidence of *Phytophthora* crown rot as we have suggested (6, S. N. Jeffers and H. S. Aldwinkle, unpublished), MM.111 may be more tolerant than MM.106 to such perturbations. MM.111 is more cold-hardy than MM.106 (8), and we observed that it began growth and bloomed later than MM.106 in both 1983 and 1984.

There was a significant isolate × rootstock interaction for all isolates on the two rootstocks and for *P. cactorum* and *P. megasperma* when isolates of each species were analyzed separately. This is further evidence that isolates of *Phytophthora* spp. are differentially virulent on apple cultivars (2,6,13).

Both rootstocks inoculated with all isolates exhibited seasonal patterns in the extent of colonization. Of the *Phytophthora* spp. tested, two groups were identified depending on when relative peaks in colonization occurred for both MM.106 and MM.111. *P. cactorum* and *P. cambivora* colonized apple rootstocks most extensively only during late spring and summer, but *P. megasperma*, *P. cripoidea*, and *Phytophthora* sp. NY.180 colonized rootstocks extensively during summer and winter. Maximum or near-maximum necrosis occurred on both rootstocks in the first week of June, regardless of the isolate tested. This is apparently a time of year when apple shoots are very prone to colonization; even some of the uninoculated control twigs developed lesions from saprophytic fungi at this time of year. After breaking dormancy, trees go through major physiological changes in late May and early June as they bloom and initiate active vegetative growth.

Seasonal variation in the extent of colonization by *P. cactorum* has been demonstrated previously. Some reports indicate that maximum necrosis on inoculated shoots occurred during bloom (3,4,11); in some of these reports, a reduction in necrosis (a decrease in the extent of colonization) occurred at the time of shoot extension (3,11). We found that maximum necrosis by *P. cactorum* did not occur until the first week of June—well after full bloom and when vegetative growth was beginning. Both MM.111 and MM.106 were still colonized rather extensively by *P. cactorum* in the first week of July, when shoot elongation was actively under way. Similar results

Table 2. Correlation between two years of monthly necrosis values on excised shoots of two apple rootstocks inoculated with species of *Phytophthora*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>MM.106</th>
<th>MM.111</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. cactorum</em></td>
<td>NY.097</td>
<td>0.86</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>NY.188</td>
<td>0.89</td>
<td>0.86</td>
</tr>
<tr>
<td><em>P. megasperma</em></td>
<td>NY.055</td>
<td>0.05</td>
<td>0.54</td>
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<tr>
<td></td>
<td>NY.125</td>
<td>0.67</td>
<td>0.79</td>
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<tr>
<td></td>
<td>NY.176</td>
<td>0.66</td>
<td>0.57</td>
</tr>
<tr>
<td><em>P. cripoidea</em></td>
<td>NY.001</td>
<td>0.77</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>NY.082</td>
<td>0.62</td>
<td>0.80</td>
</tr>
<tr>
<td><em>Phytophthora</em> sp. NY.180</td>
<td>0.07</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

a Previous season’s shoots were collected and inoculated at monthly intervals, except August for 24 mo; 15-65-mm lengths of each rootstock were inoculated in vitro with each isolate. The necrotic proportion of each twig length was transformed by an arcsin [square root] calculation (= necrosis value) before analysis.

b Correlation coefficient between months in 1983 and 1984.
have been reported (2,13). Bielenin (1) inoculated stems of apple with *P. cactorum* in the orchard, in the Phytotron, and in vitro and found that the time of maximum necrosis varied with inoculation technique from bloom to when shoots were growing vigorously. In 1984, we inoculated twigs in mid-May, 7–10 days before full bloom, when MM.106 was at tight cluster and MM.111 was at 1-cm green. The resulting necroses were similar to those recorded earlier in May or were intermediate to the measurements recorded in May and June, which showed that the real peak in colonization had not been missed. In California, six rootstock cultivars were all colonized most extensively by *P. cambivora* in May (9), which on the basis of tree phenology may correspond to June in New York and would be consistent with our data.

On the basis of our data, applications of fungicides for *Phytophthora* crown rot in apple orchards may be most effective if made in the spring around budbreak. This would protect the trees before woody tissues become more likely to be colonized extensively by *Phytophthora* spp. A second application in the fall would protect them against species that are capable of colonizing extensively during winter months. The second application may be more critical in geographical areas where winter soil temperatures are not low enough to inactivate or limit the activity of crown rot pathogens. Inoculations of new or potential rootstock selections should be done in June or July with all species and again around January with *P. megasperma, P. cryptogea*, and isolates of *Phytophthora* similar to NY.180.

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LITERATURE CITED