AN ABSTRACT OF THE THESIS OF

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Title: DEVELOPMENT OF VESICULAR-ARBUSCULAR
MYCORRHIZAE AND PINK ROOT (PYRENOCHAETA TERRESTRIS) IN COMMERCIAL ONION FIELDS IN
RELATION TO NUTRITION, PH, AND SOIL FUMIGATION

Abstract approved: Redacted for privacy
Dr. Robert G. Linderman

Eighteen commercial onion fields in E. Oregon and S. W. Idaho
were sampled in 1976 for the occurrence of vesicular-arbuscular (VA)
ymycorrhizae (VAM) and pink root disease (Pyrenoachaeta terrestris
(Hansen) Gorenz, Walker and Larson). VAM were ubiquitous in all
sampled fields. Pot cultures that were established using onion root
inoculum from the sampled fields revealed that Glomus mosseae
(Nicol. & Gerd.) Gerdemann & Trappe, comb. nov., was the pre-
dominant VA fungal species infecting onions in the sampled area.

Three fields which had been under cultivation for five years
or less (new fields) and had never been fumigated, had significantly
lower levels of phosphorus, higher pH's, and higher numbers of VA
fungal spores than 15 other fields which had been under cultivation
20 years or longer (old fields). Onions in the new fields had greater
VAM incidence per field and greater average infection per field than old fields. VAM incidence per field and average infection per field were greatest in plants from soils having less than 25 ppm phosphorus, less than 25 ppm nitrogen, and a pH of 8.0 or higher.

Pink root was present in the majority of fields sampled, although old fields had greater incidence, greater disease severity and higher numbers of *P. terrestris* propagules than new fields. Disease severity did not seem strongly influenced by differing concentrations of either phosphorus and nitrogen, or pH. Pink root incidence or severity either early or late in the season, did not seem to be reduced by soil fumigation, in that similar levels of pink root incidence and severity were found in both fumigated and non-fumigated fields. However, onions grown in fumigated fields exhibited a lower incidence and lower average infection of VAM than onions grown in non-fumigated fields.

The increase in pink root incidence during the season was greater in the old fields than in the new fields, and it was postulated that the new fields contain a qualitative suppressive factor which limits development of pink root. One possibility is that the higher levels of VAM in the new fields may be involved in the suppression of pink root in some way.
Development of Vesicular-Arbuscular Mycorrhizae and Pink Root (Pyrenochaeta terrestris) in Commercial Onion Fields in Relation to Nutrition, pH, and Soil Fumigation

by

Anne-Cressey McGraw

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Redacted for privacy
Professor of Botany and Plant Pathology
in charge of major

Redacted for privacy
Head of Department of Botany and Plant Pathology

Redacted for privacy
Dean of Graduate School

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Typed by Opal Grossnicklaus for Anne-Cressey McGraw
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This thesis is dedicated to my mother, Cressey T. McDermott, who obviously helped in a very direct and special way.
# TABLE OF CONTENTS

## INTRODUCTION

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

## LITERATURE REVIEW

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors Influencing VA Mycorrhizal Infection</td>
<td>4</td>
</tr>
<tr>
<td>VA Mycorrhizae-Disease Interactions</td>
<td>10</td>
</tr>
<tr>
<td>Assessment of VA Mycorrhizal Infection</td>
<td>12</td>
</tr>
<tr>
<td>Pink Root Disease</td>
<td>13</td>
</tr>
</tbody>
</table>

## MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field Locations and Plant Sampling</td>
<td>16</td>
</tr>
<tr>
<td>Soil Sampling</td>
<td>16</td>
</tr>
<tr>
<td>Grower's Questionnaire</td>
<td>17</td>
</tr>
<tr>
<td>Identification of VA Fungi Infecting Commercially-Grown Onions</td>
<td>17</td>
</tr>
<tr>
<td>Quantification of VA Mycorrhizae in Onion Roots</td>
<td>19</td>
</tr>
<tr>
<td>Quantification of Pink Root in Onion Roots</td>
<td>22</td>
</tr>
<tr>
<td>VA Fungal Inoculum Densities</td>
<td>24</td>
</tr>
<tr>
<td>P. terrestris Inoculum Densities</td>
<td>25</td>
</tr>
<tr>
<td>Rate of VA Mycorrhizae and Pink Root Increase in Onion Fields</td>
<td>25</td>
</tr>
</tbody>
</table>

## RESULTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of VA Fungi Infecting Commercially-Grown Onions</td>
<td>27</td>
</tr>
<tr>
<td>Effect of Age of Field on VA Mycorrhizae and Pink Root Infection</td>
<td>27</td>
</tr>
<tr>
<td>Effects of Cultural Practices on VA Mycorrhizae and Pink Root Infection</td>
<td>30</td>
</tr>
<tr>
<td>Soil Fertility</td>
<td>30</td>
</tr>
<tr>
<td>Soil pH</td>
<td>33</td>
</tr>
<tr>
<td>Soil Fumigation</td>
<td>36</td>
</tr>
<tr>
<td>Relationship of Inoculum Density of VA Fungi and P. terrestris to Levels of VA Mycorrhizae and Pink Root</td>
<td>40</td>
</tr>
<tr>
<td>Rate of Pink Root and VA Mycorrhizae Increase with the Advancing Season</td>
<td>41</td>
</tr>
<tr>
<td>Seasonal Effects on VA Mycorrhizae and Pink Root Incidence and Average Infection</td>
<td>44</td>
</tr>
</tbody>
</table>
DISCUSSION

LITERATURE CITED
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Summary of cultural histories of fields sampled in 1976.</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Effect of age of commercial onion fields (new and old) on either VA Mycorrhizae (VAM) and pink root incidence or on average VAM infection per field and average pink root severity per field at two sampling times (June and September) during the 1976 growing season.</td>
<td>29</td>
</tr>
<tr>
<td>3.</td>
<td>Nutrients (P, NO$_3$-N), pH, inoculum density (both VA fungal and <em>Pyrenoachaeta terrestris</em>), average VAM infection per field and average pink root severity per field in new and old fields.</td>
<td>34</td>
</tr>
<tr>
<td>4.</td>
<td>Effect of fumigation vs. non-fumigation on either levels of VA mycorrhizae (VAM) incidence and average infection or levels of pink root incidence and severity.</td>
<td>37</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Irregular-shaped vesicles and hyphae in the cortex of a mycorrhizal onion root taken from a commercial onion field.</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Selective medium assay for <em>P. terrestris</em> in infected onion roots (lower right-hand plate contains non-infected roots).</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td><em>Glomus mosseae</em> chlamydospore and mycelium attached to onion root.</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Effect of age of commercial onion fields on VA mycorrhizae (VAM) and pink root incidence at two sampling times (June and September) during the 1976 growing season.</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>Effect of age of commercial onion fields on VA mycorrhizae (VAM) average infection and pink root severity at two sampling times (June and September) during the 1976 growing season.</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>VA mycorrhizae (VAM) infection and concentrations of phosphorus (ppm) in commercial onion fields.</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>VA mycorrhizae (VAM) infection and concentrations of nitrate-nitrogen (ppm) in commercial onion fields.</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>Pink root severity and concentrations of nitrate-nitrogen (ppm) in commercial onion fields.</td>
<td>35</td>
</tr>
<tr>
<td>9</td>
<td>VA mycorrhizae (VAM) infection and soil pH in commercial onion fields.</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>VA mycorrhizae (VAM) and pink root incidence in non-fumigated and fumigated commercial onion fields.</td>
<td>38</td>
</tr>
<tr>
<td>11</td>
<td>VA mycorrhizae (VAM) infection in fumigated and non-fumigated commercial onion fields.</td>
<td>38</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>12.</td>
<td>Pink root severity in fumigated and non-fumigated commercial onion fields</td>
<td>39</td>
</tr>
<tr>
<td>13.</td>
<td>Rate of increase (QR) for pink root in &quot;new&quot; and &quot;old&quot; commercial onion fields (1976).</td>
<td>42</td>
</tr>
<tr>
<td>14.</td>
<td>Rate of increase (QR) for VA mycorrhizae (VAM) in &quot;new&quot; and &quot;old&quot; commercial onion fields (1976).</td>
<td>42</td>
</tr>
<tr>
<td>15.</td>
<td>VA mycorrhizae (VAM) and pink root incidence in a commercial onion field (15 years old, never in onions) at successive times during the growing season (1977) (1= May, 2=June, 3=July, 4=Aug., 5=Sept.).</td>
<td>43</td>
</tr>
<tr>
<td>16.</td>
<td>VA mycorrhizae (VAM) and pink root infection in a commercial onion field (15 years old, never in onions) at successive times during the growing season (1977) (1= May, 2=June, 3=July, 4=Aug., 5=Sept.).</td>
<td>43</td>
</tr>
</tbody>
</table>
DEVELOPMENT OF VESICULAR-ARBUSCULAR MYCORRHIZAЕ AND PINK ROOT (PYRENOCHAETA TERRESTRIS) IN COMMERCIAL ONION FIELDS IN RELATION TO NUTRITION, PH, AND SOIL FUMIGATION

INTRODUCTION

Vesicular-arbuscular (VA) mycorrhizal fungi are beneficial endophytes in the roots of many agricultural crops, including onions (30, 70, 84). Some of the potential benefits of VA mycorrhizae (VAM) include increased uptake of nutrients and water by plants, and possible protection of plants from root pathogens.

Mosse (66) suggested that VA mycorrhizal infections are labile and can be affected by agricultural practices such as soil fertilization and pesticide usage. High soil fertility levels, especially of nitrogen and phosphorus, can reduce VAM infection (70); low levels of fertilizers might promote higher levels of VAM and still allow growth of plants comparable to plants in high-fertility-low VAM soils.

Although soil fumigation can effectively control certain soil-borne diseases, it also reduces VA fungal inoculum (58). Soil fumigation followed by inoculation with an effective VA fungal species could result in greater host growth than in non-fumigated, non-inoculated soils (71). Re-evaluation of soil fumigation practices and the effectiveness of indigenous and introduced VA fungal species is important to insure maximum VAM levels while providing possible
protection from root pathogens (9).

A mycorrhizal association may be particularly beneficial for nutrient and water uptake by onions which have a coarse root system characterized by minimal branching and few root hairs. In addition, VAM may protect onions from soil-borne plant pathogens. Mycorrhizal onion roots challenged with *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson (causal agent of the pink root disease of onion) (38) showed less colonization and disease severity than challenged non-mycorrhizal roots (6). Since onion roots are killed by *P. terrestris*, the disease reduces available absorptive root surface area. Establishment and maintenance of VAM in onion roots might reduce disease severity and yield loss by maintaining sufficient absorptive surface area. Although mycorrhizal development may not completely protect plants from infection by a pathogen, VAM might protect plants by inducing resistance not found in non-mycorrhizal roots, or by reducing the pathogenicity of the disease-causing organism (85).

Preliminary observations indicated that low levels of VAM existed in onion roots from commercial onion fields in E. Oregon and S. W. Idaho. Pink root is an important disease in this area (45). The low levels of VAM and the widespread prevalence of pink root suggested a relationship might exist between VAM and pink root. Field surveys and a field study were conducted to help elucidate this relationship. The incidence per field and average infection
per field of VAM and pink root disease were determined along with the effects of cultural practices on VAM and pink root in onions. In addition, a field study was conducted to follow the development of VAM and pink root infections as the growing season progressed.
LITERATURE REVIEW

Factors Influencing VA Mycorrhizal Infection

Many factors may influence mycorrhizal infection in cultivated plants. Soil fertility may greatly affect VAM (41). High levels of phosphorus, nitrogen or complete fertilizers reduce mycorrhizal infection under field conditions (39, 55, 61, 97, 113, 115). Mosse (70) suggested that high phosphorus concentrations in the soil, resulting in higher plant concentrations of phosphorus, make the plant resistant to mycorrhizal infection. In low phosphorus soils, plant roots grew slower which resulted in a reduction in the growth rate of the plant and allowed for greater establishment and development of mycorrhizal infection (51). In high nitrogen soils, the host used more of its carbohydrates to produce rapid root growth which the mycorrhizal fungus was unable to completely colonize (39, 68, 95, 113).

Since phosphate application may lead to an increase in nitrogen content of the soil (22), the nitrogen-phosphorus balance is also important to VA mycorrhizal infection (41). Although high levels of phosphorus and nitrogen may reduce VAM infection, other researchers have suggested that in high phosphorus soils, additional nitrogen application helped diminish the reduction in VAM infection (16, 70, 113), presumably through a dilution of phosphorus
concentration in the plant tissue (70).

Soil pH affects VA mycorrhizal infection, probably indirectly by influencing spore germination and subsequent infection (36, 61, 62, 94, 117, 118). VA fungal spores were commonly reported to be more abundant in high pH soils (61, 94).

The effects of pesticides on VAM vary, but in general pesticides inhibit mycorrhizal infection even at low concentrations, probably by reducing VAM propagule numbers. Nesheim and Linn (81) found that arasan, botran, lanstan, terrachlor, captan, mylone, vapam and vorlex each restricted VAM formation in corn. Bertoldi (9) found that the systemic fungicide benomyl, inhibited VAM formation in onions, but that captan, a non-systemic fungicide, did not affect VAM infection. Jalali and Domsch (50) showed that wheat seed treatment with three systemic fungitoxicants, thiabendazole, benomyl and ethirimol had an adverse effect on the formation of VAM in roots. Foliar sprays of fungitoxicants including triforine, chloramform ethane, tridemorph, thiophanate, triadernifon, benomyl, maneb, dichlofluanid, and captan each reduced VAM formation and chlamydospore production. Chlamydospore production was also inhibited by methyl bromide and PCNB (58, 63). In contrast, Bird (11) reported that fumigating soil with DBCP, 1, 3-dichloropropene, or related C3-hydrocarbons increased VAM infection in cotton. Bertoldi (9) concluded that the use of soil fungitoxicants to protect plants from
soil-borne pathogens should be re-evaluated to determine whether "net gains" of protection from pathogens outweighs damage attributable to the loss of mycorrhizae and rhizosphere microbes.

Environmental conditions influence VA mycorrhizal infection. High soil moisture and low soil temperatures were detrimental to VA mycorrhizal infection (27, 30, 40, 56, 62, 73, 94, 95, 106). Schenck et al. (105) reported that a temperature of 34°C was also inhibitory. High light intensity stimulated infection, presumably by inducing greater photosynthetic rates which increased carbohydrate production by the host and increased available carbohydrates for the endophyte (27, 40, 93).

Soil type also influences VA mycorrhizal infection (30, 42, 54, 61, 68, 70, 73, 84, 102). Plants inoculated with VA fungi showed varying growth responses in different soil types which had equivalent additions of phosphate (70). Mosse (70) observed phosphorus-toxicity in mycorrhizal plants in several of the soil types; the amount of added phosphate causing toxicity depended on the soil type. Kruckelmann (61) found greater numbers of VA fungal chlamydo-spores in loamy soils than in sandy soils.

Agricultural usage, soil moisture, soil pH and possibly other factors will determine the number of VA fungal species in a field (30, 70, 71, 73). The intermittent root growth of plants in agricultural soils seems to stimulate spore production, spore diversity,
and VA mycorrhizal infection (5, 30, 73). Usually field soils contain three or four species (21, 28, 39, 104), occasionally one or two species (39, 54, 73, 103), and rarely five species (54, 73).

Since VA fungal species differ in their ability to improve plant growth, and cultural practices may change the species composition (10, 16, 17, 64, 69, 70, 74, 76), agricultural land may lack the most effective VA endophytes for a beneficial host growth response (5, 30, 73). Because of this we should consider inoculating seeds, seedlings or transplants with more effective VA fungal species than the indigenous species (55, 68, 71, 74).

Effectiveness of a VA fungal species is not always related to the percentage of host roots infected or to the intensity of infection (33, 37, 51, 74, 76, 92). Host specificity, crop susceptibility to VA mycorrhizal infection, host dependency on mycorrhizae, root morphology, and environment may affect the degree of stimulation of plant growth by VAM (5, 10, 19, 25, 37, 64, 68, 71, 89, 122).

The form of inoculum can affect the extent of VA mycorrhizal infection. Germinated spores rather than infected root segments are generally the initial infective propagules (17, 19, 60). Spores survived freezing and lyophilization better than infected roots (15), and are thought to remain viable for at least one year (41, 42). Although spores are probably responsible for long-term survival and initial infections, the major form of inoculum during a growing
Another important consideration is the initial density and distribution of VAM inoculum (10, 41, 49, 72, 73, 77, 92). Hayman (39) found the greatest VA mycorrhizal infection in fields with the highest spore numbers, although Daft and Nicolson (19) reported in one experiment, that the final level of infection was not related to the initial spore concentration. Mosse (72) suggested that the inoculum density rather than the soil phosphate status seemed to determine responses to VA fungal inoculum. Higher spore numbers are found at the end of a growing season compared to early in the season, since the rate of spore production is maximum as infection nears completion (20, 27, 56, 57, 80, 84, 112, 118).

Several researchers showed that hyphae from germinated spores are usually not attracted to host roots, but that once contact was made, some hyphal growth stimulation occurred (65, 92). Since the growth of the VA fungal germ tube is very limited, the site of the first infection is probably determined almost entirely by the positioning of the inoculum (67). Immobility of inoculum places importance on the crop's root morphology; an extensive, finely-branched root system might become infected more rapidly than a coarse root system. Depending on specific host root morphology, inoculum placement should be an integral factor in establishing VA mycorrhizal infection. Host species deficient in root hairs tend to have a greater
dependency on mycorrhizae, or added phosphorus in phosphorus-deficient soils, than plants with finely-branched root systems and abundant root hairs (4).

In culture once an infection was established in a root, the root became more prone to further infection from new entry points (77). Mycelial spread within a root is limited; multiple infections are established by external hyphal growth on the outer surface of the host's epidermis, and subsequent penetration at different sites along the root (117). Recently-developed lateral roots usually became mycorrhizal, though infection of actively growing root tips rarely occurred (77). Though there did not seem to be a critical seedling age after which infection would not occur (3), mycorrhizal roots rarely contained new infection points after a certain stage in development (64). Daft and Nicolson (17) reported a similar parallel where VA mycorrhizal infection in natural conditions frequently reached a level potential for host stimulation which was not exceeded.

Mycorrhizal infection is not always beneficial to the host. Under certain conditions of phosphorus concentration, temperature, and light intensity, specific VA fungi were detrimental to the plant (13, 27, 51, 55, 57, 70, 97).
Over 25 studies on mycorrhizae-disease interactions have been conducted showing varying results depending on the particular host-pathogen combination considered. Most studies have been in combination with pathogenic fungi or parasitic nematodes, although several studies include interactions with viruses, and one with bacteria. In most instances, a decrease in disease severity was observed, although a simultaneous reduction in VAM incidence and beneficial effects also occurred.

Extractable virus was higher and viral lesions were greater in mycorrhizal plants as compared to non-mycorrhizal plants (22, 108).

Fewer nematode larvae developed into adults in mycorrhizal tobacco, oat and tomato plants, and nematode populations decreased in mycorrhizal carrot plants as compared to non-mycorrhizal plants (112). Schenck et al. (105) found that high nematode populations were associated with low levels of endomycorrhizal fungi in soybean, and that some species of VA mycorrhizal fungi reduced nematode populations; the fungi having this effect varied with the soybean cultivar. Bird et al. (11) reported a negative correlation between endoparasitic nematodes and endomycorrhizae in cotton roots, and suggested that nematodes might limit the mycorrhizal potentials of
nematode-infested soils. Fox and Spasoff (26) found that *Endogone gigantea* and *Heterodera solanacearum* mutually suppressed reproduction of one another on tobacco.

Mycorrhizal cotton plants were less severely damaged than non-mycorrhizal plants by *Thielaviopsis basicola* (107). Fewer *T. basicola* chlamydospores were formed in mycorrhizal plants than in non-mycorrhizal plants; a negative correlation existed between the numbers of chlamydospores formed and the degree of mycorrhizal colonization (1, 107). Chou et al. (12) found that fewer mycorrhizal than non-mycorrhizal plants were killed by *Phytophthora megasperma* var *sojae* race 3. In conflict with Chou et al. (12) results, Ross (98) reported mycorrhizal soybean plants showed more severe *Phytophthora* root rot symptoms than non-mycorrhizal plants. Mycorrhizal tomato plants had significantly less wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* as compared to non-mycorrhizal plants (24). Mycorrhizae of strawberry plants apparently reduced the pathogenicity of *Cylindrocarpon destructans*, and caused *Cylindrocarpon* to have a stimulatory effect on plant growth along with the beneficial effects of the VA fungi (85). Simultaneous inoculation of VA fungi, *Pythium ultimum* and *Rhizoctonia solani* caused stunting in poinsettias; however, a 20-day delay before adding the two pathogens after first establishing VA mycorrhizal infection, gave shoot growth equivalent
to plants without pathogens (114).

Becker (6) found that localized sites on mycorrhizal onion roots appeared to have resistance to *Pyrenochaeta terrestris*. He observed that mycorrhizal onion roots prevented the internal development of *P. terrestris* by cell wall thickenings (callosities or lignitubers). Safir (100) suggested that the larger amounts of reducing sugars he found in mycorrhizal onions could explain the reduced root infection by *P. terrestris*.

**Assessment of VA Mycorrhizal Infection**

VAM cause little or no morphological modification in roots (30) and are usually quantitatively assessed by microscopic observation of stained material. The most common method for estimating infection is by counting infected root segments (14, 17, 18, 19, 20, 21, 27, 39, 40, 43, 52, 55, 59, 64, 75, 78, 81, 83, 87, 88, 91, 94, 96, 99, 109, 117). Other methods include: measuring the length of infected roots (39, 52, 76, 88); recording the number or relative abundance of internal or attached mycelium, arbuscules, vesicles or chlamydo- spores (8, 11, 19, 27, 39, 55, 59, 73, 78, 83, 94, 98); recording the intensity of infection or intracellular fungal development (52, 64, 99, 111); estimating the dry weight of mycorrhizal roots from a stained sample (117); calculating the ratio of arbuscules to hyphae (52); visually estimating intensity of infection based on the production
of yellow pigment common to many mycorrhizal roots (19, 20, 29, 70); extracting and colorimetrically determining the intensity of the yellow pigment to estimate infection (7); and colorimetrically measuring the conversion of fungal chitin to glucosamine to estimate the intensity of infection (44).

**Pink Root Disease**

Hansen (38) first described the causal agent of onion pink root as *Phoma terrestris*, but this was later changed to *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson (35). The first disease symptoms are pink pigmentation of roots, the shade varying with severity and age of infection, followed by a water-soaked appearance of roots with loss of turgidity, and finally, shriveling and death of roots (120). After death of infected roots, onions expend energy producing new roots (121), impairing normal bulb development and resulting in undersized or split bulbs (86, 119). The fungus may attack onion roots at any time during the growing season (23). The optimum pH range for disease development is 4-8, and the optimum temperature range is 24-28 C (34). The highest inoculum levels usually occur at the end of the onion growing season (47, 119), and high inoculum levels are found in fields cropped consecutively with onions (90, 119).

After penetration of root epidermal cells by *P. terrestris*,
cortical ramification occurs primarily by enzymatic dissolution rather than by pressure (46). Induced synthesis of endopolygalacturonase, cellulase and pectinesterase by the fungus is implicated in fungal ramification of host tissue. The high sugar content of onions represses the synthesis of endopolygalacturonase and cellulase by *P. terrestris* (47). Safir (100) found higher root sugar content in mycorrhizal roots and suggested that mycorrhizal onions may have greater resistance to pink root development.

Moderately pathogenic isolates of *P. terrestris* were able to penetrate the cortex of a susceptible onion variety, and enter the xylem, destroying the internal structure of roots more effectively than in the resistant varieties. In the resistant varieties, callosities and thickened cell walls hindered hyphal penetration in the cortex (116). Resistant varieties showed no resistance when very high levels of inoculum were present (82). Resistance to pink root has been identified and is controlled by a single recessive gene (53, 82). However, resistance is variable and many so-called resistant varieties are still invaded (47).

Some control of pink root is also afforded through crop rotation, although many other crops are also susceptible to pink root (119). Siemer (110) studied the effects of various soil fumigants on pink root control, including telone, vorlex, vapam and DD, and found that fumigation did not decrease disease severity, but did increase
yield. No current studies have been published on disease loss as a result of *P. terrestris*. 
MATERIALS AND METHODS

Field Locations and Plant Sampling

Onions (*Allium cepa* L. var. Yellow Sweet Spanish) were collected from fields located in the Treasure Valley area of E. Oregon and S. W. Idaho and examined for VAM and pink root infection. In one study, three "new" fields (cropped with a variety of plants for five years or less), and 15 "old" fields (cropped with a variety of plants, including onions, for 20 years or more) were sampled in June and September of 1976. In 1977, an onion field which had not previously been cropped with onions but which had been cropped 15 years to other crops, was sampled five times during the growing season (May, June, July, August, September) to follow the development of VAM and pink root infection with the advance of the season. In both studies, samples consisted of 50 plants collected along two diagonal transects across each field. Root basal plates and root systems were excised and placed in plastic bags for later examination for VA mycorrhizal and *P. terrestris* infection.

Soil Sampling

Composite soil samples were taken in September from each of 17 fields sampled in 1976 to determine nutrient levels, pH, and inoculum densities of the VA fungi and *P. terrestris* (soil analysis and inoculum density data were missing for one field). Each sample
consisted of 25 15-cm soil cores each collected near the plants along two diagonal transects across the field. Samples were stored in plastic bags for several days, air-dried for five days, and thoroughly mixed in a Twin-Shell dry blender. A 200 cc sub-sample from each composite was analyzed for nutrients and pH by the Soil Testing Laboratory, Soils Dept., Oregon State University. Although a specific soil series name was not determined for each soil sample, the soils in the sampled area are typically considered to be silty loams.

Grower's Questionnaire

In August 1976, a questionnaire was sent to the owners of the sampled fields to obtain additional information on the agricultural history of each field. A summary of that information is shown in Table 1.

Identification of VA Fungi Infecting Commercially-Grown Onions

Indigenous VA fungal species that infect onions in commercial fields in E. Oregon and S. W. Idaho were determined by establishing pot cultures (65) with onions. Air-dried mycorrhizal onion root inoculum from two of the sampled fields (previously determined to have high levels of endomycorrhizal infection) was used to inoculate
Table 1. Summary of cultural histories of fields sampled in 1976.

<table>
<thead>
<tr>
<th>Field Age (YR)</th>
<th>Total YRS</th>
<th>1st, 2nd Consecutive (YR) in Onions</th>
<th>Planting Date</th>
<th>Previous Crops (1975, 1974,...)</th>
<th>Fumigated Fall 1975</th>
<th>Available P (ppm)</th>
<th>NO₃-N (ppm)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NEW FIELDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1st</td>
<td>3/20/76</td>
<td>corn (2YR); sugarbeets</td>
<td>no</td>
<td>5</td>
<td>22.34</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1st</td>
<td>3/27/76</td>
<td>corn (2YR); sugarbeets</td>
<td>no</td>
<td>25</td>
<td>12.99</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1st</td>
<td>3/15/76</td>
<td>sugarbeets; potatoes; onions</td>
<td>yes</td>
<td>27</td>
<td>32.10</td>
</tr>
<tr>
<td><strong>OLD FIELDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>5</td>
<td>1st</td>
<td>3/15/76</td>
<td>sugarbeets; potatoes; onions</td>
<td>yes</td>
<td>48</td>
<td>7.58</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4</td>
<td>1st</td>
<td>4/76</td>
<td>grain; potatoes, corn</td>
<td>yes</td>
<td>28</td>
<td>5.71</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>3</td>
<td>1st</td>
<td>4/1/76</td>
<td>beans; alfalfa (5YRS)</td>
<td>no</td>
<td>67</td>
<td>30.65</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>12-15</td>
<td>1st</td>
<td>3/27/76</td>
<td>potatoes</td>
<td>no</td>
<td>41</td>
<td>54.96</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>6-7</td>
<td>2nd</td>
<td>4/6/76</td>
<td>sugarbeets; corn; onions</td>
<td>yes</td>
<td>42</td>
<td>19.22</td>
</tr>
<tr>
<td><strong>NEW FIELDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1st</td>
<td>3/20/76</td>
<td>corn (2YR); sugarbeets</td>
<td>no</td>
<td>19</td>
<td>37.51</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1st</td>
<td>3/27/76</td>
<td>corn (2YR); sugarbeets</td>
<td>no</td>
<td>46</td>
<td>8.73</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1st</td>
<td>3/8/76</td>
<td>grain; sugarbeets; onions</td>
<td>yes</td>
<td>39</td>
<td>58.70</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td></td>
<td>1st</td>
<td>3/24/76</td>
<td>sugarbeets; corn</td>
<td>yes</td>
<td>52</td>
<td>49.77</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>6-10</td>
<td>1st</td>
<td>3/30/76</td>
<td>sugarbeets; corn</td>
<td>yes</td>
<td>57</td>
<td>79.79</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>6-10</td>
<td>1st</td>
<td>3/20/76</td>
<td>grain; sugarbeets; onions</td>
<td>yes</td>
<td>54</td>
<td>19.84</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>9</td>
<td>1st</td>
<td>4/1/76</td>
<td>grain; potatoes; corn</td>
<td>no</td>
<td>57</td>
<td>34.18</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>1</td>
<td>1st</td>
<td>4/1/76</td>
<td>grain; potatoes; corn</td>
<td>no</td>
<td>19</td>
<td>90.70</td>
</tr>
</tbody>
</table>
Allium cepa L. var. Yellow Sweet Spanish. After 14 wk, onion roots were carefully removed from soil, lightly rinsed in tap water, and observed with a dissecting microscope (30X). Identification of VA fungal species was based on the presence of characteristic spores attached to the onion roots (32).

Quantification of VA Mycorrhizae in Onion Roots

Roots were cleared and stained using a modified technique of Phillips and Hayman (88). Each root system was gently washed in running tap water, divided into equal halves, and cut into one (for 1977 data) or two cm segments (for 1976 data). One half was retained for later determination of VA mycorrhizal infection. Root segments were placed in test tubes with 10% KOH and kept at room temperature for 24 hr (94). After acidifying in HCl and staining in trypan blue, ten randomly selected segments from each root system were scanned with a dissecting microscope (30X) for the presence or absence of VA fungi. Inter- and intracellular hyphae, vesicles, extramatrical mycelia and/or spores were apparent in infected segments. The relative abundance (low, medium, high) of these VA fungal structures in each root was also recorded. Frequent checks with a compound light microscope (400X) were made to verify the identity of VA fungal structures observed under the dissecting microscope.

Infected roots showed irregular-shaped vesicles and hyphae in
the cortex (Fig. 1). Vesicles and hyphae were observed in root tissue at both sampling dates in the 1976 survey, however, arbuscules were rarely observed, possibly because their senescence had already occurred (27).

Percent VAM infection per plant, average percent VAM infection per plant per field, and incidence of VAM infection per field were calculated as follows (94):

(1) VAM infection per plant

\[ \frac{\text{No. of infected root segments}}{\text{Total no. of segments}} \times 100\% \]

(2) Average VAM infection per field

\[ \frac{\sum \% \text{ VA mycorrhizal infection/plant}}{\text{Total no. of plants}} \times 100\% \]

(3) Incidence of VAM infection per field

\[ \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100\% \]

Because data was often collected using an interval scale (e.g., 10%, 20%, ..., 100%, average VAM infection per field), median analysis was thought to be more appropriate in expressing results in the bar graphs, however, mean analysis is the more typical manner of expressing bar graphs. Both mean and medians were expressed in many instances and similar trends occurred regardless of which method was applied.
Fig. 1. Irregular-shaped vesicles and hyphae in the cortex of a mycorrhizal onion root taken from a commercial onion field.
Quantification of Pink Root in Onion Roots

Individual plant root systems were gently washed in running tap water to loosen adhering soil and were visually rated for pink root infection using a modified disease rating index of Gorenz et al. (34), where 1 = 0% diseased, 2 = 1-25% of root system diseased, 3 = 26-50% of root system diseased, 4 = 51-75% of root system diseased, and 5 = 76-100% of root system diseased.

An alternative method of assessing pink root disease was to surface sterilize ten randomly selected 1-cm root segments in 0.5% sodium hypochlorite, plate segments on sodium polygalacturonic acid (NPA) medium (Flota and Zalewski, personal communication), incubate the plates 7-10 days at 30°C, and count the number of segments giving rise to pink _P. terrestris_ colonies (Fig. 2).

Measurements of pink root by the disease rating index and by the percent of colonized segments on selective medium showed a high correlation (r = .88; significant at P = .01).

Pink root severity per plant, average pink root severity per plant per field, and pink root incidence per field were calculated as follows:

(1a) Pink root severity per plant = \( \frac{\text{No. infected root segments}}{\text{Total no. of segments}} \times 100\% \)

(based on selective medium assay)
Fig. 2. Selective medium assay for *P. terrestris* in infected onion roots (lower right-hand plate contains noninfected roots).
(1b) Average pink root severity per field = \[\frac{\sum \text{Pink root severity/plant}}{\text{Total no. of plants}} \times 100\%\]
(based on selective medium assay)

(2a) Pink root severity per plant = disease severity index per plant
(based on disease severity index)

(2b) Average pink root severity per field = \[\frac{\sum \text{Pink root severity/plant}}{\text{Total no. of plants}}\]
(based on disease severity index)

(3) Incidence of pink root per field = \[\frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100\%\]

Means or medians were both expressed in many of results for the same reasons stated in the preceding section.

**VA Fungal Inoculum Densities**

A wet-sieving and decanting method (31) was used to assay soil from each of 17 fields for VA fungal spores. A 100 g subsample from each soil composite was suspended in one liter of water and the supernatant was decanted through 589 μm, 175 μm and 88 μm mesh sieves. Sievings from the 175 μm and 88 μm sieves were each suspended in 50 ml of water. A 20-ml aliquot from each 50-ml suspension was pipetted into a petri dish, and 20 randomly selected microscope fields (30X) were observed for spores. Counts were made, and calculations performed to express the results as
propagules per g soil.

\[ P. \text{terrestris} \text{ Inoculum Densities} \]

NPA medium was used to assay soil from each of 17 fields for \( P. \text{terrestris} \) inoculum densities. A 100 g subsample from each of the soil composites was sieved through a 589 \( \mu \)m sieve and ground in a gem rock tumbler apparatus for 20 min. Four ten g sub-samples per field were diluted in sterile 1% water agar and added to NPA medium to a final 1:20 dilution. Four plates were poured for each of four replicates for a total of 16 plates per field. Plates were incubated at 30 C for 6-7 days, \( P. \text{terrestris} \) colony counts made, and results expressed as propagules per g of soil.

\textbf{Rate of VA Mycorrhizae and Pink Root Increase in Onion Fields}

Van der Plank's equation (123) for determining rate of disease increase was used to determine pink root and VAM rate of infection increase in sampled onion fields in 1976:

\[ QR = \frac{1}{t_2 - t_1} \left( \log_e \frac{1}{1 - x_2} - \log_e \frac{1}{1 - x_1} \right) \]

where, \( QR = \) daily increase

\( t = \) time

\( 1 - x = \) portion of susceptible tissue

\( (x \text{ is based on VAM and pink root incidence in the fields}) \)
Rates were determined for the period of June to Sept. 1976, in new and old fields.
RESULTS

Identification of VA Fungi Infecting Commerciy-Grown Onions

Onion roots from established pot cultures exhibited numerous attached VA fungal chlamydospores. Spores were identified as belonging to the species *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, comb. nov., (32). Fig. 3 shows a *G. mosseae* chlamydospore and mycelium attached to an onion root. *G. mosseae* was the only species found in the pot cultures. Results from the soil inoculum assays support the conclusion that *G. mosseae* may be the predominant VA fungal species infecting onions in the commercial onion fields of E. Oregon and S. W. Idaho.

Effect of Age of Field on VA Mycorrhizae and Pink Root Infection

New fields had significantly greater incidence of VAM than old fields at both sampling dates (June and Sept., 1976) (Table 2). Average VAM infection was significantly greater in the new fields than in the old fields only early in the season. Possibly more infections occurred and became established in the new fields earlier in the season than in the old fields, but as the season progressed, development of infection was as great in the old fields as in the new fields. VAM incidence and average infection in the new fields and in the old did not
Fig. 3. *Glomus mosseae* chlamydospore and mycelium attached to an onion root.
Table 2. Effect of age of commercial onion fields (new and old) on either VA mycorrhizae (VAM) and pink root incidence or on average VAM infection per field and average pink root severity per field\(^1,2\) at two sampling times (June and September) during the 1976 growing season.

<table>
<thead>
<tr>
<th>Field Type</th>
<th>VAM Incidence Per Field</th>
<th>VAM Average Infection Per Field</th>
<th>Pink Root Incidence Per Field</th>
<th>Average Pink Root Severity Per Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;New&quot;</td>
<td>88a</td>
<td>24a</td>
<td>57a</td>
<td>32a</td>
</tr>
<tr>
<td>&quot;Old&quot;</td>
<td>24b</td>
<td>43b</td>
<td>8b</td>
<td>12a</td>
</tr>
</tbody>
</table>

\(^1\)means in the same vertical columns followed by the same letter do not differ significantly (P=.05) by t-tests.

\(^2\)means in adjacent columns within boxes followed by (*) are significantly different (P=.05) by t-tests.

\(^3\)pink root disease severity is based on a disease severity index (scale of 1-5) of Gorenz et al. (34).
increase significantly between the early and later sampling dates.

At both sampling dates, old fields had significantly greater pink root incidence than new fields (Table 2). Pink root severity was significantly greater in old fields as compared to new fields at the first sampling date but not at the second sampling. As the season progressed, pink root incidence increased significantly in the old fields, but not in the new fields.

Fig. 4 and 5 show either VAM and pink root incidence, or average VAM infection per field and average pink root severity per field during the 1976 growing season.

Effects of Cultural Practices on VA Mycorrhizae and Pink Root Infection

Because the results could be similarly interpreted whether fields were treated as a composite or whether they were separated into new vs. old fields, results were expressed using a field composite.

Soil Fertility

When new and old fields were treated as a composite, the level of VAM infection was apparently negatively related to available phosphorus (Fig. 6). Fields with 25 ppm P or less generally showed greater levels of VAM infection as compared to fields with greater than 25 ppm P. Data expressed as medians or means yielded similar results. At levels of P greater than 25 ppm, less than 50 percent of
Fig. 4. Effect of age of commercial onion fields (new and old) on VA mycorrhizae (VAM) and pink root incidence at two sampling times (1 = June, 2 = Sept.) during the 1976 growing season. Bars represent the mean of fungal incidence in either three new fields or in 15 old fields.

Fig. 5. Effect of age of commercial onion fields (new and old) on VA mycorrhizae (VAM) average infection and pink root severity at two sampling times (1 = June, 2 = Sept.) during the 1976 growing season. Pink root severity is based on a disease severity index (scale of 1-5) of Gorenz et al. (34). Bars represent either the means of VAM infection or the mean of pink root severity in either three new fields or in 15 old fields.
Fig. 6. VA mycorrhizae (VAM) infection and concentrations of phosphorus (ppm) in commercial onion fields. Bars represent means or medians of the average % VAM infection in fields grouped at each concentration of phosphorus. New fields are included in bars with (*).

Fig. 7. VA mycorrhizae (VAM) infection and concentrations of nitrate-nitrogen (ppm) in commercial onion fields. Bars represent means or medians of the average % VAM infection in fields grouped at each concentration of nitrogen. New fields are included in bars with (*).
the plants were infected, and the infected plants had less than ten percent average infection. New fields had significantly less available phosphorus than old fields (Table 3). Increasing concentrations of P in the commercial onion fields apparently reduce VAM infection.

When new and old fields were treated as a composite, the level of VAM infection was negatively associated with NO$_3$-N (Fig. 7). Fields with 25 ppm NO$_3$-N or less generally showed higher levels of VAM as compared to fields which had greater than 25 ppm. At levels of NO$_3$-N greater than 25 ppm, less than 50 percent of the plants were infected, and the infected plants generally had less than ten percent average infection. There was no significant difference in the levels of nitrate-nitrogen (NO$_3$-N) in the new fields compared to the old fields (Table 3). An apparent reduction in VAM infection in the commercial onion fields was found with increasing concentrations of NO$_3$-N. Although old fields had significantly greater levels of phosphorus along with higher levels of pink root incidence and severity, pink root severity did not seem to be related to differing concentrations of available P or NO$_3$-N (Table 3); high levels of pink root severity were found at both low and high concentrations of NO$_3$-N (Fig. 8).

Soil pH

When new and old fields were treated as a composite, the level of VAM infection apparently increased with increased pH (Fig. 9). At
Table 3. Nutrients (P, NO₃-N), pH, inoculum density (both VA fungal and Pyrenochaeta terrestris), average VAM infection per field¹ and average pink root severity per field² in new and old fields.³

<table>
<thead>
<tr>
<th>Field Type</th>
<th>P (ppm)</th>
<th>NO₃-N (ppm)</th>
<th>pH</th>
<th>Inoculum Density</th>
<th>Average VAM Infection Per Field</th>
<th>Average Pink Root Severity Per Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;New&quot;</td>
<td>13a</td>
<td>43a</td>
<td>8.0a</td>
<td>34a</td>
<td>95a</td>
<td>32a</td>
</tr>
<tr>
<td>&quot;Old&quot;</td>
<td>43b</td>
<td>37a</td>
<td>7.5b</td>
<td>13b</td>
<td>300b</td>
<td>12b</td>
</tr>
</tbody>
</table>

¹ average VA mycorrhizal infection per field or average pink root severity per field is based on either means from three new fields or means from 15 old fields.

² pink root severity is based on a disease severity index (scale of 1-5) of Gorenz et al. (34).

³ means in vertical columns followed by same letter do not differ significantly (P=.05) by t-tests.
Fig. 8. Pink root severity and concentrations of nitrate-nitrogen (ppm) in commercial onion fields. Bars represent means or medians of the average pink root severity (based on disease severity index (scale of 1-5) of Gorenz et al. (34)) of fields grouped at each concentration of nitrogen. New fields are included in bars with (*).

Fig. 9. VA mycorrhizae (VAM) infection and soil pH in commercial onion fields. Bars represent means or medians of the average % VAM infection in fields grouped at each pH level. New fields are included in bars with (*).
pH values less than 7.5, less than 50 percent of the plants were infected, and the infected plants had less than ten percent average infection. Soils from the new fields were significantly more alkaline than soils from the old fields (Table 3). VAM infection apparently increases in the commercial onion fields with increasing soil pH.

Pink root severity did not seem to be related to variations in pH.

Soil Fumigation

Based on the information from the 1976 Grower's Questionnaire (Table 1), fields were classified as non-fumigated or as fumigated. Although data is not complete, the majority of fumigated fields were fumigated with telone, telone 2, telone 2-C or terracide. VAM and pink root disease data from the early season sampling date were used for the following information, although data from the second sampling showed the same trends. Non-fumigated fields showed greater incidence and greater average infection of VAM as compared to fumigated fields early in the season (Table 4, Fig. 10 and Fig. 11). Fumigation apparently reduces VAM infection in commercial onion fields.

Pink root incidence and severity were not significantly different in fumigated versus non-fumigated fields (Table 4, Fig. 10 and Fig. 12). The slight differences observed may have resulted from errors
Table 4. Effect of fumigation vs. non-fumigation on either levels of VA mycorrhizae (VAM) incidence and average infection or levels of pink root incidence and severity. \(^2, 3\)

<table>
<thead>
<tr>
<th>Fields</th>
<th>VAM Incidence</th>
<th>VAM Average Infection per Field</th>
<th>Pink Root Incidence per Field</th>
<th>Average Pink Root Severity per Field(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumigated</td>
<td>26.9a</td>
<td>9.5a</td>
<td>64.9a</td>
<td>1.67a</td>
</tr>
<tr>
<td>Non-fumigated</td>
<td>40.6a</td>
<td>21.2a</td>
<td>46.8a</td>
<td>1.93a</td>
</tr>
</tbody>
</table>

\(^1\) pink root severity based on disease severity index (scale of 1-5) of Gorenz et al. (34).

\(^2\) values represent means of fields grouped as non-fumigated or fumigated.

\(^3\) means on the same vertical columns followed by the same letter do not differ significantly \((P = .01)\) by t-tests.
Fig. 10. VA mycorrhizae (VAM) and pink root incidence in non-fumigated and fumigated commercial onion fields. Bars represent means of fungal incidence in grouped fields. No new fields were fumigated.

Fig. 11. VA mycorrhizae (VAM) infection in fumigated and non-fumigated commercial onion fields. Bars represent field means or medians of the average VAM infection. No new fields were fumigated.
Fig. 12. Pink root severity in fumigated and non-fumigated commercial onion fields. Bars represent field means or medians of the average pink root severity (based on a disease severity index (scale of 1-5) of Gorenz et al. (34). No new fields were fumigated.
during sampling.

Relationship of Inoculum Density of VA Fungi and
P. terrestris to Levels of VA
Mycorrhizae and Pink Root

When fields were treated as a composite, VAM infection was
quite variable and seemed not to be strongly related to VA fungal
inoculum density in commercial onion fields. Inoculum density
ranged from 4-48 spores per g soil. New fields had significantly
higher numbers of VA fungal spores compared to old fields (Table 3).
This may account for the greater VAM incidence and average infec-
tion in new fields compared to old fields.

When fields were treated as a composite, P. terrestris
inoculum density did not seem to be strongly related to pink root
severity. Insufficient data was present to draw any conclusions
about the relationship of P. terrestris inoculum density to disease
incidence. Generally high levels of incidence occurred. If any rela-
tion were to be found it would probably occur at low levels of
P. terrestris inoculum (less than 15 propagules per g soil). Inocu-
lum density ranged from 15 to 610 propagules per g soil. Old fields
had significantly higher numbers of P. terrestris propagules than
new fields (Table 3). This may account for the greater pink root
incidence and severity found in old fields as compared to new fields,
although even the new fields may have sufficiently high numbers of
propagules to cause the same levels of disease occurring in the
old fields.

Because pink root incidence values were all high, it would be difficult to determine a correlation between pink root incidence and disease severity. It would seem that at inoculum numbers lower than 15 propagules per g of soil, a threshold for disease might occur.

Rate of Pink Root and VA Mycorrhizae Increase with the Advancing Season

New fields had a slower rate of pink root increase than old fields (Fig. 16). In new fields, the QR value equaled .0072 units (of increase)/day, while in old fields the QR value was .0294 units/day. New fields appeared to suppress pink root development as was indicated by the lower QR value.

The same equation for rate of disease increase was used to compare the rate of VA mycorrhizal infection increase in new and old fields with the advance of the season. A small decrease in the infection rate was found in new fields (QR=-.0048 units/day), as compared to old fields where the rate was increasing (QR=.0048 units/day) (Fig. 17). Although the incidence and average infection of VAM was less in the old fields compared to new fields, the actual rate of infection was increasing in old fields.
Fig. 13. Rate of increase (QR) for pink root in "new" and "old" commercial onion fields (1976). (X) equals the amount of disease. Plotted points are based on three means (new fields) or 15 means (old fields); each mean is composed of 50 observations.

Fig. 14. Rate of increase (QR) for VA mycorrhizae (VAM) in "new" and "old" commercial onion fields (1976). (X) equals the amount of disease. Plotted points are based on three means (new areas) or 15 means (old fields); each mean is composed of 50 observations.
Fig. 15. VA mycorrhizae (VAM) and pink root incidence in a commercial onion field (15 yrs old, never in onions) at successive times during the 1977 growing season (1 = May, 2 = June, 3 = July, 4 = Aug., 5 = Sept.). Mean of 50 observations at each sample date was plotted.

Fig. 16. VA mycorrhizae (VAM) infection and pink root severity (based on a selective medium assay) in a commercial onion field (15 yrs old, never in onions) at successive times during 1977 growing season (1 = May, 2 = June, 3 = July, 4 = Aug., 5 = Sept.). Mean of 50 observations at each sample date was plotted.
Seasonal Effects on VA Mycorrhizae and Pink Root Incidence and Average Infection

The incidence and average infection of VAM was greater throughout the growing season than that of pink root incidence and severity (Fig. 18 and 19). Over the season, VAM incidence and average infection and pink root incidence and severity did not increase significantly. During early onion seedling growth, the root growth rate is sufficiently slow to allow extensive fungal colonization (both VA fungal and P. terrestris), but as the root growth rate increased, fungal growth may have been unable to keep up with new root tissue, and with a constant root volume being sampled, results would effectively be expressed as a decline in the percent of fungal infection and incidence. It should be stressed that although VAM or pink root infection decreased on a basis of percent diseased root to percent healthy, it is unlikely either VAM or pink root infection actually decreased. If one assumes uniform spacial distribution of inoculum and susceptibility of host to VAM infection, the rate of VAM and pink root development may not have changed.
DISCUSSION

To determine the ecological and physiological significance of VA mycorrhizae in agricultural soils, quantitative data on their occurrence is essential. Although VA fungi may be present in most soils, they may not exist in sufficient numbers or be distributed in an optimum manner to promote maximum infection of host roots (49). The presence and distribution of VAM are extremely pertinent to studies of VA fungi in agricultural soils, especially since by manipulating the nutrient regimes or the prevalent VA endophytic species it may be possible to substantially increase plant growth in response to VAM.

The ubiquitous nature of VAM (70, 84) was demonstrated when every field sampled in 1976 contained VAM, although considerable variability in levels of infection existed.

Although other VA fungal species were observed in lesser proportions, *Glomus mosseae* was the predominant species found in the commercial onion fields. The occurrence of *G. mosseae* is favored by neutral to alkaline conditions (32), and similarly the germination of *G. mosseae* is favored by high pH (36). It is possible that high pH favors *G. mosseae* more than other species of VA fungi (70); this may explain why *G. mosseae* was the only species found infecting onions in the commercial fields, and why VAM infection and VAM fungal inoculum density were greatest in those fields with
pH's above 7.5.

In the field survey of 1976, fields which had not been cultivated more than five years had significantly greater VAM incidence, greater average infection and higher numbers of VA fungal propagules than fields under cultivation for longer than 20 years. The high levels of VAM in the new fields was probably due in large part to the absence of such VAM-inhibitory soil factors as high levels of phosphorus (72) and fumigation (58, 81).

Pink root was present in the majority of fields sampled, although old fields had significantly more *P. terrestris* inoculum, and greater pink root incidence and severity than new fields.

The differences in either levels of VAM and pink root infection in commercial onion fields may be explained by differences in soil fertility (especially phosphorus and nitrogen), soil pH, soil fumigation, and either VA fungal or *P. terrestris* inoculum density. The maximum observed VAM development was characterized as occurring in low phosphorus and nitrogen soils. VAM levels were greatest in soils with less than 25 ppm phosphorus and less than 25 ppm nitrate-nitrogen. Other workers have found this same trend of increased VAM infection in low phosphorus and low nitrogen soils under field conditions (39, 55, 61, 68, 95, 97, 113). New fields had lower levels of P indicating that nutrient build-up commonly found in old fields, had not occurred. This emphasizes the fact that the fertility levels
(especially N and P) which inhibit VA mycorrhizal infection must be determined for each crop and VA species combination used.

Huber (48) discussed the form of nitrogen as it affects many plant diseases, either by inducing greater disease severity, or by reducing disease severity, depending on the crop and pathogen considered. In the present study, differing concentrations of NO$_3^-$-N or P apparently did not affect the level of pink root in the commercial onion fields.

Soil pH has been shown to influence VAM infection by favoring the build-up of certain VA species (70). Generally, the highest VAM levels and the highest number of VA fungal propagules commonly occurred in higher pH soils (61, 94). In E. Oregon and S.W. Idaho, higher levels of VAM occurred in the more alkaline soils characteristic of the new fields. Fields with pH's of above 8.0 had the highest levels of VAM infection. In contrast, pink root infection did not seem to be affected by variation in soil pH.

VAM infection and VA fungal inoculum can be detrimentally affected by soil fumigation. Studies of the effects of methyl bromide plus chloropicrin (58), vapam, and vorlex (31) on VAM and VA fungal spores revealed that soil fumigation, even at low concentrations, inhibits mycorrhizal infection probably by reducing VA fungal inoculum. Soil fumigation had not yet been practiced in the new fields presumably because _P. terrestris_ incidence, disease
severity and yield loss as a result of increased pink root severity were minimal. Soil fumigation practiced in many of the old fields combined with low levels of VAM infection and inoculum density in the old fields support the currently accepted belief that fumigation practices do not significantly reduce pink root incidence or severity as measured by the indicated methods. Siemer (110) conducted soil fumigation trials for control of pink root in commercial onion fields in E. Oregon and S.W. Idaho, and similarly concluded that telone, vorlex, lanstan, and chloropicrin did not reduce disease severity, and in several instances even increased disease severity. However, yields were generally improved when soil fumigants were used. Siemer suggested that the variety of onion used (Yellow Sweet Spanish) was tolerant to pink root. Soil fumigation may induce high host vigor and allow tolerant onion varieties to regenerate roots faster than _P. terrestris_ destroys them. Perhaps VAM infection induces a similar phenomenon.

Even though old fields had significantly greater pink root incidence, greater disease severity and higher levels of inoculum than new fields, pink root incidence or severity did not seem closely related to _P. terrestris_ inoculum density. Perhaps a threshold _P. terrestris_ inoculum level exists above which little additional disease will occur. If a relationship were to be found between _P. terrestris_ inoculum density and pink root incidence it would probably be found at low levels of inoculum (less than 15 propagules per g soil). The same
thing may be occurring with the VA fungal inoculum in the commercial onion fields.

The rate of pink root increase in new vs. old fields suggests that other factors besides inoculum density are involved. Although data was only collected at two times during the growing season, there is a strong suggestion that between June and Sept., 1976, a slower rate of pink root increase occurred in the new fields as compared to old fields. Van der Plank (123) suggests several factors which may influence the rate of disease increase: host tissue susceptibility, pathogenicity of the pathogen, environmental conditions and the presence of organisms antagonistic to the pathogen. Some qualitative factor in the new fields appears to be limiting the rate of pink root increase. One possibility is that presence of greater VAM levels in the new fields might in some way limit the rate of pink root development, perhaps as antagonists.

With regard to the possible suppressiveness of VAM on pink root disease, Becker (6) stated that mycorrhizal sites on onion roots protect the plant from _P. terrestris_ by limiting the extent of pathogen invasion. Resistance was attributable to epidermal cell wall thickening and callosities preventing extensive invasion of the cortical layers. It then seems likely, that the greater the proportion of VAM-infected root cortex, the less likely _P. terrestris_ will successfully penetrate, colonize and damage the root. A mycorrhizal
root would have less available infection courts for \textit{P. terrestris}, and this might effectively reduce pink root severity. In an attempt to establish and maintain high levels of VAM for possible protection from \textit{P. terrestris}, pre-inoculation of the host with VA fungi becomes especially relevant.

If sufficient VAM fungal inoculum is available for field inoculations, the following factors should be considered in an inoculation program: form of inoculum, method of inoculation, initial field inoculum density, quantity of inoculum added, persistence of inoculum and spread of infection, previous field cultural practices (fertilizer applications, fumigation practices), and nutrient requirements for host and VA endophyte.

No reports were found in the literature suggesting that the rate of increase of VAM infection in the field has been recorded. Although Van der Plank's equations are currently applied to plant diseases, they also seem useful to determine the rate of increase of VA fungal incidence or fungal infection under field conditions. In this respect, factors limiting VAM infection in the field may be characterized by information from the equations. If these equations are employed, data should include at least three sampling times, and preferably sampling times that would cover planting date to harvest date, and span several years.

If one uses Van der Plank's equation for a simple interest
infection (one which does not spread from plant to plant during the growing season) to compute the rate of VAM increase, the rate values calculated suggest that in the new fields a VAM level potential for host stimulation may have already been reached at the first sampling date; hence, results were expressed as a negative slope, since further increase did not occur. The negative slope may also be explained by the possibility of decreased availability of non-mycorrhizal roots restricting colonization of the roots, thus leading to a net cessation of mycorrhizal infection (117). In the old fields, the level potential for host stimulation may not have been reached, thus, a continued rate of increase was observed. It should be stressed that, although new fields had a smaller rate of VAM increase than the old fields, new fields still had greater VAM incidence and average infection than the old fields.

During the growing season mycorrhizal infection lags behind root elongation (4). Infection may lag for one of several reasons; the host tissue may not be uniformly susceptible to VA fungal infection, spacial distribution of inoculum may be non-uniform or the host root growth may exceed the ability of the VA fungus to colonize the new tissue. Neill (79) suggested that differences in degree of infection associated with fertility were related to the growth rate of roots. Thus, as the 1977 season advanced and a more extensive
root system developed, VAM and pink root incidence and average infection in mid-season appeared to be below early (May) and late (Sept.) season maxima. Only when the root growth began to slow down did any increase in either VA fungal or *P. terrestris* colonization occur. The relative rates of VAM fungal and *P. terrestris* colonization were different. VAM showed a shallower slope between June and July, and a steeper slope between July and August, than pink root did during these same periods. The differing slopes indicate that VA fungal colonization may not be affected as strongly by root growth as *P. terrestris* is; VA fungi may be better adapted to rapid root colonization than *P. terrestris*. Additionally, physiological changes may have occurred in the plants, creating less susceptible tissue to *P. terrestris* during this time of rapid root elongation.

Levels of VAM infection were always greater than pink root infection and VAM reached a higher level of infection at the end of the season than at the beginning of the season. By contrast, pink root infection in the field did not increase substantially from early to late season, and it is interesting to speculate on whether the VAM in association with pink root was related to this phenomenon. Again, perhaps a root system with more of its cortex colonized by VA fungi would offer the plant some protection either from invading soil-borne plant pathogens, or from further plant pathogenic development and damage after previous invasion of the cortex. Here
pre-inoculation of the host with VA fungi and early establishment of VAM in the field before the onset of pink root during the warmer temperature accompanying the end of the season, would be essential. Thus, a field with high levels of VAM early in the season would increase plant growth and might serve to deter pink root severity which generally is maximum at the end of the season.

VAM might alter the physiology of the host; i.e., altered amounts of amino acids, changes of sugar content in roots (2, 100, 124); or, VAM could directly affect the plant pathogen. Paget (85) suggested that *Cylindrocarpon destructans* produced a phytotoxin at the non-mycorrhizal host root surface which stunted and inhibited strawberry seedlings, but the phytotoxin may not have been produced at the root surface of a mycorrhizal strawberry plant. Paget proposed that VAM reduced the pathogenicity of *Cylindrocarpon*.

Experiments under controlled environmental conditions should be conducted to determine whether VAM offer onion plants protection from *P. terrestris*, or in any way reduce the severity of pink root.

It is conceivable that the cropped soils in E. Oregon and S.W. Idaho may develop increasing salinity levels, nutrient imbalances, and improper soil structures as time progresses, and that yields are being produced from "tired" soils (personal communication with Hugh Gardner, Oregon State Univ., Soils Dept.). After a plateau of apparent maximum yields with high levels of fertilizer applications
and continued soil fumigation practices, yields are slowly declining. At this time it becomes extremely important to elucidate any additional factors which might maintain the high yields; for this reason, the aspect of VAM as a beneficial plant growth factor should continue to gain support for applied research.


