AN ABSTRACT OF THE THESIS OF

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Title: RESISTANCE MECHANISMS AGAINST ONION PINK ROOT (PYRENOCHAETA TERRESTRIS), BIOASSAY OF INOCULUM POTENTIAL IN SOIL AND CHEMICAL CONTROL

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Eight onion varieties showed four host-pathogen relationships when artificially inoculated with Pyrenochaeta terrestris (Hansen) Gorenz, Larson and Walker. Allium fistulosum roots did not support any mycelial growth on their surfaces and were not penetrated by the fungal mycelium. The presence of an inhibitory substance was indicated. Onion breeding lines #223, #36 and Ia2997B supported fungal colonization on their root surfaces and mycelium was observed to penetrate their roots but at differing levels. Line #223 stopped fungal growth almost immediately after root entry; #36 stopped fungal growth after entry but not as rapidly as #223; Ia2997B only slowed fungal growth after root entry. The commercial Yellow Sweet Spanish variety showed tolerance to the pathogen, demonstrated by near complete mycelial ramification of all root tissue except the xylem vessels, yet the plants remained in vigorous growing condition. The variety

Southport White Globe and onion breeding lines #248 and #249 showed complete susceptibility; their roots were permeated with mycelium and all plants ultimately wilted and died.

The polyphenol oxidase (PPO) levels of nine onion lines and of common mushroom and of potato peelings were compared. All onion root extracts showed comparable levels of activity but the enzyme required the catechol substrate to be between pH 7.3 and 8.0 for maximum activity. The PPO enzyme activity in the onion roots was about one-half that of common mushroom and about one-fifteenth that of potato peelings.

Phenolic constituents of roots of A. fistulosum as determined by thin-layer chromatography were different from those of Yellow Sweet Spanish and B5546B. Constituents of Yellow Sweet Spanish were different from those of B5546B. Comparison of extracts from non-diseased, slightly, moderately, and severely diseased Yellow Sweet Spanish roots showed a changing phenolic pattern with an increase in disease level. Extracts of the B5546B line did not show a definite phenolic pattern.

A procedure was developed to assay the soil for P. terrestris propagules pathogenic on onion roots. Sieved soil was diluted with quartz sand and planted with Southport White Globe onion seed. Disease readings made after six weeks gave the highest infection percentages, while readings made less than five weeks after test

initiation were erroneously low. Use of this method showed infective P. terrestris propagules were most strongly associated with the 0.5mm to 1.0mm soil particle size fraction and survive to a depth of 18 inches in the soil. The method supported field readings that showed Telone (85% 1,3-dichloropropene - 15% related chloropropenes-chloropropanes) at 40 gal/A has little effect on inoculum levels of this fungus and that Vorlex (80% dichloropropenes-dichloropropanes and 20% methyl isothiocyanate) at 30 gal/A does reduce the inoculum level. It was shown that considerable variation exists in the level of pathogenic inoculum in the same field and in different fields. There was significant variation between the inoculum level in the soil of the east and west halves of the Malheur Branch Experiment Station's field for selecting pink root resistant onion hybrids.

Fumigant materials were evaluated for their ability to control pink root disease. Two materials were selected as best because of their performance, availability, cost and other factors. Vorlex at 30 gal/A gave the best biological response but the result was not predictable and the cost was excessive. Additional work testing at lower rates was initiated and appeared promising. Telone at 40 gal/A gave slightly less effective biological response but because of increased early growth, dependable results, and an acceptable cost figure, was the most beneficial compound studied.

Three methods of soil fumigant application were studied.

Broadcast treatment in the fall is the most practical, though high cost is a major drawback. Fumigation of preformed beds in the fall reduces the material cost and the biological effect was the same as that obtained with broadcast applications, but there is a risk of losing efficacy of the treatment if excessive ground preparation is required in the spring before planting. Mid-season under-the-row application of fumigants showed promise with several materials, notably Vapam (32.7% sodium N-methyldithiocarbamate dihydrate), but the phytotoxicity potential is great and much additional testing would be needed to perfect this practice.

Resistance Mechanisms Against Onion Pink Root (*Pyrenochaeta terrestris*), Bioassay of Inoculum Potential in Soil and Chemical Control

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INTRODUCTION

Onions form a portion of man's diet throughout the world. Their production is less restricted by environment than most other agronomic crops. Egypt, Turkey and Japan devote greater land area to onion production, but the United States is the world's leading onion producer because of higher per acre yields (27). More than a million tons are harvested annually in the United States from about 100,000 acres (46, 74). Nearly every state of the nation produces onions, but more than two-thirds of the commercial acreage is in the northern states, from the Atlantic to the Pacific (74).

Onion production in Oregon accounts for about 11 percent of the annual U. S. production, ranking Oregon as the sixth state in over-all production. Oregon acreage is split between two areas, Western Oregon at Labish, Sherwood and Gaston, and Eastern Oregon, primarily in Malheur County. In the former area about 2,000 acres of Danver Onions are grown on muck soil; in the latter about 3,000 acres of Yellow Sweet Spanish onions are grown on irrigated mineral soil. Farm value of the onions grown in the eastern area is approximately 3 million dollars annually.

Pink root disease, caused by Pyrenochaeta terrestris (Hansen) Gorenz, Walker and Larson, causes insidious losses and affects this crop on nearly a world-wide basis. Losses have been as high as 100% (60) with fields being removed from profitable production, causing a shrinkage of available acreage. Only in those regions of the world where onions are grown with low soil temperatures is pink root disease of reduced importance.

Pink root disease is a problem in both eastern and western Oregon but it is of greater economic concern in the eastern growing region because soil temperatures generally average higher and because the market is based on onions exceeding 3 inches in diameter. Energy used for root regeneration is lost to the bulbs reducing their ultimate size. Infestations in the Eastern Oregon area have become so severe in some fields as to remove them from profitable onion production. Rotation of onions with potatoes and sugar beets has been used to minimize disease loss but this is more in deference to economics than to disease control. Some growers have resorted to growing the variety Southport White Globe (SWG) which is more susceptible to pink root disease than the Yellow Sweet Spanish variety because the market for the White Globe variety is based on a smaller onion. This is not a satisfactory solution for the problem, however, since the market for SWG is limited.

Onion breeding for pink root resistance has been conducted at

least since 1929 (54). Progress has been made but commercially acceptable varieties highly resistant to pink root disease and adapted to Oregon conditions have not yet been obtained. The national program is complicated by market requirements and environmental factors peculiar to each area. Short day varieties grow poorly under long day conditions and vice versa. Onion varieties selected and grown for resistance in one area often are susceptible when grown in other regions. The same variety grown in successive years in the same area may be either resistant or susceptible. This phenomenon has been reported frequently but no data are available to explain why.

Research effort has been directed mainly to the taxonomy of the organism, etiology of the disease, and breeding for resistance. More recently research has been conducted on methods of greenhouse evaluation of potentially resistant hybrids, fungus physiology and methods of promoting sporulation for taxonomic purposes. Various workers have tested numerous chemical treatments for control of the disease but without clear-cut success.

Four areas of research were pursued in these studies. The first was a series of studies to determine if differences exist in the mode or level of fungal penetration into roots of onion lines grown in Oregon. The second series of studies was to determine if there is a functional polyphenol oxidase system in onion roots and, if so, do

sufficient differences exist between resistant and susceptible onions to offer an explanation for resistance. A parallel study was to determine if differences between endogenous phenolic constituents would aid in presenting a basis for biochemical disease resistance.

The third was an attempt to devise a quantitative means of determining the inoculum level of P. terrestris in soil, and the fourth was an evaluation of chemicals and methods of application for relief from the pink root disease problem. Methods of judging chemical efficacy also were evaluated to determine those methods most applicable to this crop-disease situation.

LITERATURE REVIEW

Disease Occurrence

In 1917 Taubenhaus and Johnson (66) published a note on a newly discovered disease affecting onions in Texas. They used the descriptive name "Pink Root," indicating the most obvious plant symptom. Taubenhaus (65, p. 217) in 1919 described the disease as follows:

> The disease is confined to the roots only and not to the bulb. As fast as the old roots are affected new ones are produced, these in turn becoming diseased. In the end, the bulb spends all its energies in producing new roots which in turn become affected, thus failing to attain the commercial standard. Diseased bulbs remain dwarfed and small to the end of the season, although apparently sound in every other way.

Fusarium malli n. sp. was proposed as the causal organism.

Edgerton (10, p. 9) in 1921 indicated that pink root disease had been recognized in Louisiana since 1909. He made the first statement distinguishing between pink root disease and another disorder "apparently caused by a species of Fusarium. . . . This fungus causes a rotting of the roots and sometimes even causes a rot of the base of the stalk."

After the initial report the disease was discovered in numerous other areas. Its occurrence has been reported from the United States, Bermuda, Canada, Japan, Egypt, Spain, Union of South

Africa, Argentina, Brazil, Australia and the Netherlands (5, 9, 30, 51). Without doubt pink root is a problem wherever onions are grown.

Determination of Causal Agent

Sideris (59), in 1924 indicated many fusaria were associated with onion roots but surface sterilization was not used in this work and little was added to determination of the causal agent. Hansen (19, p. 64) in 1926 was the first to apply rigid surface sterilization procedures. He observed that

> The extent and diversity of the cryptogamic flora obtained when diseased onion roots are cultured depend largely on the condition and treatment of the material used. If the roots cultured are partly decayed and shriveled they yield a large number of Fusaria and several other members of the soil flora; if, on the other hand, roots are cultured that, though distinctly pink, are still turgid and firm, the number of fungi obtained is greatly reduced, and if roots in the same condition are immersed in mercuric chloride 1:500 for three minutes and then cultured only one fungus is obtained which, according to the following description, belongs in the genus Phoma: . . . ."

Subsequent work by Hansen (18) showed that artificial inoculation with the Phoma sp. isolated would incite the disease and the fungus could be reisolated. He was unable to produce pink root with any of the Fusarium spp. previously reported as inciting the disease, and concluded that the fusaria were acting only as secondary invaders. At this time (1929) Hansen named the causal agent Phoma terrestris.

A critical morphological study conducted by Gorenz, Walker,

and Larson (17) showed that the causal agent of pink root in fact was not a member of the genus Phoma but rather the genus Pyrenochaeta. On the basis of their studies they renamed the organism Pyrenochaeta terrestris which is the accepted name.

Even though it seems apparent that pink root of onions is caused by P. terrestris the disease is still sometimes attributed to Fusarium spp. (78).

Variability of Pyrenochaeta terrestris

Hansen (20, p. 424) was first to report variation in isolates of P. terrestris. He observed that

> The differences, both macro and microscopic are so obvious and of such magnitude that, were it not for the fact that all these organisms are able to cause the same specific disease, one would be justified (according to precedents set in the taxonomic treatment of other genera in fungi imperfecti) in naming three new species and several new varieties.

Hansen (20) considered the spores of P. terrestris to be binucleate, but Struckmeyer et al. (63) referred to uninucleate spores. Gorenz, Walker and Larson (17) made no mention of the number of nuclei per spore, but they were confronted with the same level of variation. They were impressed, as was Hansen, with the unique property of all isolates being capable of causing pink root of onions. Thus, their species description was made sufficiently broad to encompass the variation encountered in isolates examined by them.

Others have been impressed by variation. Gasiorkiewicz (13) studied cultural and pathogenic variability induced by nitrogen mustard. All of the induced mutants fell within the range of natural variability and he concluded that natural mutants arise by spontaneous mutation from existing strains.

Kulik (34) and Kulik and Tims (35) collected 91 isolates of P. terrestris from a single field. The level of cultural and pathogenic variability found in this study supported the findings of Gasiorkiewicz (13). After 57 days in culture, eight of the 91 isolates produced spore bearing pycnidia, ten produced pycnidia-like bodies void of spores and setae and all other isolates were void of pycnidia. Pathogenically the isolates were distributed fairly evenly in each of four disease categories used. An apparent correlation between production of pycnidia or pycnidia-like bodies and pathogenicity was found.

Host-Parasite Relationships

Taubenhaus (65) reported that onion and its near relatives--chive, shallot, garlic and leek--were susceptible to infection by P. terrestris. He also reported that other members of the family Liliaceae were not susceptible. Those examined were narcissus, lily, tulip, funkia, iris, freesia and calla lily. Hansen (18) isolated P. terrestris from cowpeas, lima beans, and potatoes. Kreutzer (32), using inoculated soil, found that barley, cane sorghum,

canteloupe, carrot, cauliflower, corn, cucumber, eggplant, millet, muskmelon, oats, pea, pepper, soy bean, spinach, squash and wheat were susceptible. At the same time he found resistant lines in many of these crops.

Sugar cane and sweet clover were shown by Caravajal (4) to be hosts of P. terrestris. Tims (70) added pigweed (Amaranthus retroflexus), crabgrass (Digitaria sanguinalis), crowfoot grass (Eleusine indica), and jungle rice (Echinochloa colona) to the long list of hosts. Wilhelm (76) found the organism attacking hairy nightshade (Solanum sarachoides) and strawberry. Sprague (62) found this fungus to be widespread as a minor parasite of cereals, many grasses and various other plants of North Dakota. He concluded that P. terrestris should be recognized as a widespread but minor parasite and saprophyte on the underground parts of these crops. Pink root has been reported to be a serious root disease of tomato (68). Hess (22) added several other hosts to the list. It is interesting that only onion and some of its very close relatives suffer recurring major economic damage.

Recent work in Germany has shown that P. terrestris occurs on several crops in that country and that it causes the corky root disease of tomato (14). Schneider followed the original work by confirming that in all important details the organism identified as P. terrestris in Germany was the same as isolates obtained from

the United States, Brazil and South Africa (58).

Interaction of P. terrestris with Other Organisms

Sideris (59) showed that numerous fusaria are associated with pink root-affected onion roots. Hansen (19) subsequently concluded from his work that fusaria act only as secondary invaders. Davis and Henderson (8) showed that Fusarium vasinfectum var. zonatum could attack roots or bulbs of onion only after invasion by P. terrestris or another pathogen or after mechanical injury.

Kehr, O'Brian and Davis (29) showed Fusarium oxysporum f. sp. cepae could act as a primary pathogen of onion roots. They made no mention however of pink root in their description of symptoms caused by this organism. In their breeding experiments, no cross protection was detected. Lorbeer and Stone (41) showed that ten onion varieties had differential susceptibility to Fusarium basal rot caused by F. oxysporum f. cepae but all were equally susceptible to P. terrestris.

Hess (22), comparing pathogenicity of P. terrestris and Fusarium sp., showed that all isolates tested were capable of significantly reducing plant stands. He concluded that while both organisms caused root disease on onions "the P. terrestris treatments and the P. terrestris and Fusarium treatments caused typical

pink root symptoms, but the isolates of Fusarium alone did not cause typical symptoms" (22, p. 43). No positive proof has been presented that fusaria are involved directly in the pink root disease.

Breeding for Pink Root Resistance

Porter and Jones (54) in 1933 reported on one of the early trials for selection of pink root resistant onions. Of six species tested Allium fistulosum, A. porrum (leek, Giant Musselberg variety) and A. schoenoprasum (chives) were either immune or highly resistant. A. sativum (garlic) A. ascalonicum (shallot) and most varieties of A. cepa (common onion) were susceptible to P. terrestris. The onion variety Sweet Spanish was classed as moderately susceptible.

In 1939 a cooperative breeding program was established between the USDA and the Texas Agricultural Experiment Station (52, 53). This program has since been expanded to other states (7, 48, 69). Pink root resistant short day varieties have resulted from these programs through field selection and breeding (53). Nichols, Larson and Gabelman (48) studying the relative resistance of many commercial onion varieties and hybrids, found that the southern onion types had the greatest resistance.

Pink root disease resistance has been found to vary with environmental conditions, inoculum potential and isolates of the

fungus (16). Davis (7) and Hess (22) found that some varieties resistant to P. terrestris when grown in Texas are susceptible when grown in Oregon. Other varieties have shown resistance during certain years, but when grown in the same soil during subsequent years have not shown the same level of resistance.

There is evidence that pink root resistance is due to a single gene character. Jones and Perry (26) working with resistant varieties crossed with very susceptible varieties obtained a 3:1 ratio of susceptible to resistants in the F_2 generation. Nichols (47, 49) reported production of several inbred lines of onions which demonstrated pink root resistance governed by a single recessive gene. At the same time, however, he reported evidence of a multigenic resistance mechanism.

Mechanism of Resistance

Kreutzer (32) working with Yellow Globe Danver onions grown in artificially infested soil found the fungus first appeared on the surface of young roots as small, irregular colonies. The same result was observed when onion roots were placed adjacent to P. terrestris cultures. Primary invasion hyphae were observed, which after entry proceeded in all directions from the point of entry, ramifying throughout the cortex, intra- and intercellularly. Hyphal constrictions were observed where walls of the cortical cells were penetrated.

In artificial inoculations, the fungus generally appeared to sweep across the cortex in a hyphal mass. Under such conditions the root soon collapsed.

Kreutzer (32) observed that pigment usually was confined to hyphae of the pathogen, although some diffusion from the hyphae into the invaded cell occurred. Pigment was not observed in non-invaded cells. He did not find the extensive diffusion of pigment reported by Hansen (18).

Kreutzer (31) found invaded root-cap cells soon collapsed. Cells into which fewer hyphae penetrated were plasmolysed and the nuclei distorted. Cells adjacent to those invaded, but apparently not parasitized, showed slight plasmolysis. He found no invasion of the promeristematic region.

Struckmeyer et al. (63) working with one susceptible and two resistant onion varieties in sand culture showed differences in root penetration. Roots of Texas Grano 502, susceptible, were decomposed after penetration of fungus hyphae into all tissues of the root. Hybrid 28 and Excel, resistant, showed hyphal penetration only into the epidermis and outer cortex. The fungus hyphae apparently progressed in identical fashion through walls of the outer cells in susceptible and resistant varieties but did not invade inner cells of the cortex in resistant varieties. These workers observed formation of pegs where hyphae failed to penetrate cell walls and stated

that these pegs were composed of a hyphal tip surrounded by the stretched or otherwise altered host cell wall. Resistance of Hybrid 28 and Excel was overcome by a particularly virulent isolate, though the relative resistance of Hybrid 28, Excel and Texas Grano 502 was still maintained. The susceptible line succumbed in 15 days; the two resistant varieties in 20 days. Gorenz, Larson and Walker (16) have shown that resistance can be overcome by increased pathogen virulence or by increased inoculum concentration of a pathogen of lower virulence. Kreutzer (32) had previously shown this phenomenon.

Hess (22) found evidence of a biochemical resistance mechanism. Excised roots of leek (American Flag), Evergreen variety of bunching onion, both resistant to P. terrestris, and five susceptible bulbing onion varieties were propylene oxide sterilized and placed in low-nutrient medium to which mass mycelial transfers were made. All roots were colonized by P. terrestris, as indicated by appearance of red pigment. Non-propylene treated roots did not show any red coloration.

Disease Control

Taubenhaus (65) tried several means of disease control. Steam sterilization of the soil was effective, as was treatment with formaldehyde at one pint per 20 gallons of water per 20 square feet.

He showed, however, that proper management and fertilization gave high vigor and brought yields back to normal. This he thought was due to the plant's ability to regenerate roots faster than the fungus destroyed them. At this time Taubenhaus also reported that short term rotations did not reduce pink root disease. Liming the soil had no apparent effect on the disease. Taubenhaus and Mally (67) later published a bulletin expanding on the above control procedures as they applied to Texas.

Kreutzer and Mantagne (33) reported that a 65% formulation of chlorobromo-propene when applied to soil by injection at 25 gallons per acre three weeks prior to planting gave economic control of P. terrestris.

Tims (69) reported studies with numerous chemical compounds, but none was of commercial value. When disease control was obtained plant injury was excessive.

Tims (71) reported briefly on trials conducted with Vapam 4.0 E.C. (4 pounds per gallon, emulsifiable concentrate) and an 85% Mylone formulation. Vapam gave almost complete control of the disease in naturally infested soils at all rates tested. Mylone at high rates gave good control, but some disease developed with use of lower rates. In artificially inoculated sand or soil, these chemical treatments were not effective.

Kulik and Tims (36) published results of their fungicide

screening trials for pink root control. They examined in the laboratory 54 fungicides and 29 chemo-theraputants and found 25 and 8 respectively were effective at 1000 ppm. Only 9 fungicides were effective at 200 ppm, and none of these gave conclusive results in the field.

Hess (22) reported on control studies in eastern Oregon with chloropicrin, Vorlex, Mylone and Vapam. All treatments significantly increased onion yield and decreased pink root in the field. Chloropicrin covered with a polyethylene tarp increased onion yield more than fifty percent. However, none of the treatments were commercially feasible due to high treatment costs. Vaughan and Fisher (72) followed up Hess' work using chloropicrin in an unsuccessful attempt to reproduce the previously obtained results.

Pack (51) reported studies conducted on pink root control in eastern and western Oregon. Ten chemicals, all but one having fumigant properties, were tested. Most compounds increased onion yields 30 to 75 percent but did not noticeably reduce pink root disease. No estimate of commercial feasibility was made in this paper.

MATERIALS AND METHODS

Standard Materials

Soil Characteristics

Soil fumigation experiments were conducted on soils characterized in Table 1. The soils in the Ontario, Oregon area have not been classified, therefore a specific soil series name cannot be applied. However, consultation with the Soil Scientist in the Soil Conservation District adjacent to the Ontario area indicated all soils in these studies would be included in one of the following series:

Greenleaf Silt Loam
Nyssa Silt Loam
Nyssa Ton Silt Loam
Feltham Loamy Fine Sand
Malheur Silt Loam

Most soils used will probably be in the first two series.

The whole area is comprised of aluvial deposits of the Snake River, and soils are generally quite deep, in excess of 20 feet. This has led to the practice of making deep cuts and fills to level land for furrow type irrigation. As a result many irregularities occur in nearly all fields. These irregularities are not reflected in Table 1 since it was not possible to sample each field sufficiently to categorize it completely. Therefore, the data presented are only indicative of the total condition.

Table 1. Chemical characterization of fumigated soils. All soils were collected from fields near Ontario, Oregon.

Growers Field	Soil pH	Milliequivalents/100g				% Base Saturation	% Organic Matter	%		
		CEC*	K	Ca	Mg			Sand	Silt	Clay
Teramura	7.20	20.9	2.80	16.1	8.2	130	1.1	30.3	40.9	28.8
Wakasugi	6.80	20.2	1.74	12.8	7.5	109	1.6	24.0	43.3	32.7
Nakada	7.65	22.2	1.78	13.9	7.0	102	1.0	42.9	28.6	28.5
Saito	8.20	16.5	2.18	10.3	7.0	118	1.4	52.3	25.6	22.1
Murata	8.30	15.3	1.37	13.0	5.6	131	1.0	47.5	34.2	18.3
Yano	6.70	19.8	3.00	12.0	8.6	119	1.7	27.4	41.4	31.2

*Cation Exchange Capacity

Nutrient Solution and Media

Hoagland's Solution

Hoagland's solution one (25) was used in all onion cultures. Stock solutions (10M) were made up of KH_2PO_4, KNO_3, $Ca(NO_3)_2$ and $MgSO_4 \cdot 7H_2O$ and diluted to proper concentrations when needed. A minor element stock solution and an iron tartrate stock solution were made separately to prescribed concentrations. The diluted solution was used for watering the onions.

Mannitol Media

Wright and LeTourneau (79) showed that the main storage component of P. terrestris was the sugar alcohol, mannitol. They further showed that mannitol allowed maximum growth in culture. This work was repeated using mannose, galactose, mannitol and xylose as carbon sources for four eastern Oregon isolates of P. terrestris. Good growth occurred in all media but most rapid growth occurred in media containing mannitol. A study of pH in conjunction with the carbon source study showed that no pH adjustment of the prepared media was required.

As a result of these studies the following components were used for all studies requiring use of liquid culture and in some solid media studies: mannitol - 27.1 g /1; and the basal media used by Wright

and LeTourneau, i.e.: $MgSO_4 \cdot 7H_2O$-0.5g; KH_2PO_4-1.5g; $NaNO_3$-1.0g; 0.5ml of a $Fe(NO_3)_3$ solution 0.2mg; 0.5ml of a minor elements solution containing such concentration that the final diluted concentration was Cu and Zn-0.1mg; Mo and Mn-0.02mg.

Shake cultures were prepared initially by transferring mycelial plugs to a shake culture flask, but growth was slow and yield was low. A more satisfactory method was found to be washing the plate surface with five ml of sterile distilled water and scrubbing lightly with a transfer loop. The washings from one or more plates were transferred to about 100ml of sterile distilled water and thoroughly agitated. This inoculum suspension was then pipetted (ca. 5ml) into the desired shake culture flasks. In three days at room temperature abundant inoculum was available.

Potato Dextrose Agar (PDA)

In a few studies standard PDA was used to increase P. terrestris inoculum.

Genetic Lineage of Onion Plant Materials

Plant materials were obtained from the test plots of the Malheur Experiment Station at Ontario, Oregon or from onion breeders. Seed of commercial lines of onion were obtained from seed houses. All seed was stored at 5°C until used.

Table 2 shows the pedigree of all onion materials used in these studies if the pedigree was available. In several cases onion varieties were supplied by commercial seed companies and the pedigrees were not publically available. The Identification Numbers are those assigned each seed lot in the 1966 breeding trial. Pink Root Susceptibility readings are those obtained from rating the material in 1966, except for B5546B, Ia2997B, YSS and SWG which have been rated in past tests conducted at the Malheur Experiment Station.

Pyrenochaeta terrestris Cultures

Several newly isolated P. terrestris cultures and several from the collection established by Hess (22) were used in preliminary studies. The isolates obtained from fresh root tissue did not produce pycnidia, even after extended exposure to ultra-violet light. Hess' isolates C-53-3 and D-4-5 were both used initially but in later work the latter isolate was used exclusively because it sporulated readily and its identity could be positively established at all times. It was also highly pathogenic causing severe symptoms in all susceptible onion stocks. The isolate was transferred asceptically from Hess' soil stored culture to a new soil culture so that samplings for new inoculum would not contaminate the original culture.

Table 2. Genetic identification of onions studied showing their identification number, pedigree if known, and pink root susceptibility.

I. D. Number[1]	Pedigree	Pink Root Susceptibility[2]
2	A. fistulosum	VR
36	PI 223853 ms X PI 261639	R
223	Crookham Co.	SS
248	P Grano 1 X P52-448-65-2M prr 0?	S
249	P6334 X P53-350 prr 0 (2)	VS
	B5546B	VS
	Ia 2997B	SS
	Yellow Sweet Spanish (YSS) Utah Strain K x L Selection Langer Seed Co.	MS
	Southport White Globe (SWG)	VS

[1] Numbers refer to those listed in the Pink Root Onion Breeding trial plot plan for the 1966 Malheur Experiment Station.

[2] VR = very resistant; R = resistant; SS = slightly susceptible; MS = moderately susceptible; VS = very susceptible.

Disease and Bulb Grading System

In several of the laboratory studies and all field studies, onions were graded for disease and/or for bulb size. Root systems were visually rated for disease during the growing season using the scale first proposed by Gorenz, Larson and Walker (16), i.e.:

1 = 0 disease
2 = 1 -25% of root system diseased
3 = 26-50% of root system diseased
4 = 51-75% of root system diseased
5 = 76-100% of root system diseased

Bulbs were rated for size using the following scale:

1 = diameter < 1/2"
2 = diameter 1/2 - 1"
3 = diameter 1" - 1 1/2"
4 = diameter 1 1/2" - 2"
5 = diameter 2" - 3"
6 = diameter 3" - 4"
7 = diameter > 4"

In all fumigation trials, the following method of evaluation was used. Each fumigation plot, always at least six rows (10 ft.) wide, was sampled by establishing a randomly selected sampling station on the four inside rows, near the middle of each plot, thus leaving a treated buffer area at least one row wide on each side of the area sampled. Each row was sampled individually for disease, bulb size and harvest data. The root systems were visually rated for disease during the growing season by digging ten onion plants from each row. In 1966 the 10 onions were given an average disease rating. In 1967

each of the 10 onions was read individually. A disease index was computed by multiplying the number in each disease category by the rating number and the product was divided by the total number of onions examined. In each individual plot four sub-sample readings were made. Bulb index ratings were made on the same plants rated for disease. The bulb size index was calculated in the same way as the disease index.

Two disease and size readings were made in all but one test. Samples to be rated were taken by digging plants growing adjacent to the previous samplings to minimize differences due to variation in disease occurrence within a field.

Harvest Evaluation

Harvest evaluations were made by obtaining the bulb weights in five grade categories. Each plot was sampled at the previously established sampling site, by lifting and topping all onions in a ten foot row segment. Four separate samples were taken from each site but care was taken to collect samples from undisturbed portions of the rows.

The grade criteria used were those set forth in the Oregon Standards for Onions and Onion Sets (50) effective March 31, 1958. Two grading tables were constructed for these studies. Each table had two 3-inch holes and two 2-inch holes (1 1/2 inch for White

White onions). Onions not passing through the three inch hole were jumbo onions, if they conformed to type they were judged grade one, if they varied from type they were judged grade two's. Onions passing through the three inch hole but not the two or one and a half inch hole were "medium" one's or two's according to conformity to type. Onions passing through the smallest hole were culls as were all scallions, onions showing rot or other gross tissue deterioration, bruises or severe growth defects. After each sample was graded, the onions in each category were weighed. These weights were statistically analyzed by computer analysis of variance. They were also used to obtain the total weight and the percentage of the yield in each grade, which was then calculated on a per acre basis. From the grade data per acre, yield comparisons were made and dollar returns determined. For economic comparisons market prices were obtained for two days in each of four years. These values and their respective dates are presented in Tables 3 and 4.

Statistical Analysis

Soil: sand dilution assay data were analyzed for analysis of variance using the Link-Wallace method (39). In all cases three or more degrees of freedom were used. The 1:1 and 1:10,000 dilution levels were not analyzed because of factors discussed later.

Field data were analyzed by computer for analysis of variance.

Table 3. F. O. B. prices for Yellow Sweet Spanish onions from Malheur County on the indicated dates (all prices based on the 50 pound bag unit).

Selected Market Date	Yellow Jumbos		Yellow Mediums	
	U. S. #1	U. S. #2	U. S. #1	U. S. #2
Sept. 1, 1963	2.00	0.95	1.00	0.45
Dec. 31, 1963	2.65	0.00	2.50	0.00
Sept. 1, 1964	1.40	0.65	0.85	0.25
Dec. 31, 1964	2.35	1.15	1.30	0.60
Sept. 1, 1965	1.25	0.60	1.05	0.50
Dec. 31, 1965	1.00	0.55	0.70	0.25
Sept. 1, 1966	1.75	0.85	1.55	0.75
Oct. 4, 1966	1.80	0.90	1.60	0.75

Table 4. F. O. B. prices for White onions from Malheur County on the indicated dates (all prices based on the 50 pound bag unit).

Selected Market Date	White Jumbos	White Mediums
Sept. 1, 1964	1.70	1.60
Dec. 31, 1964	2.55	2.40
Sept. 1, 1965	2.45	2.25
Dec. 31, 1965	2.50	2.50
Sept. 1, 1966	1.95	1.95
Dec. 31, 1966	3.00	3.00
Sept. 1, 1967	2.45	2.60

With these data 11 degrees of freedom were the minimum used while 24 and 33 degrees of freedom were used with most studies. Correlation coefficients were determined, by computer analysis, for interactions between disease index and bulb index, disease index and yield and bulb index and yield. Interactions were sought for using all obvious combinations.

Fumigant Material Characterization

The chemical composition of the various fumigant formulations used in the field research are shown in Table 5. Trade names are used for convenience only and do not reflect any recommendation of the compound. All listed compositions are those given to the author by the respective manufacturers. Prices used for several of the materials are only approximations obtained in the field from distributors. Prices will vary though, according to area and the volume purchased.

Table 5. Chemical composition of fumigants used.

Material Name (Trade or Common)	Percent of Constituents[1/]						
	Propargyl Bromide	Trichloromethane	MIT	DD	1, 3-D	1-chloro, 2-nitro-propane	Telone
Telone PBC	5	15					80
Chloropicrin		99					
EP201		15	20	65			
Vapam[2/]							
EP248			10	90			
Vorlex			20	80			
EP297			5	95			
Telone				10-15	85-90		
DD				40	60		
Lanstan						44	

[1/] MIT methyl isothiocyanate
DD dichloropropanes - dichloropropenes
1, 3-D 1, 3-dichloropropene
Lanstan has 56% inactive ingredients.

[2/] Vapam is 32. 7% Sodium N-methyldithiocarbamate dihydrate, with 67. 3% inactive ingredients.

RESULTS

Laboratory and Greenhouse Research

Root Penetration

Kreutzer (32) working with Yellow Globe Danvers showed P. terrestris was able to penetrate host roots directly. Struckmeyer et al. (63) showed hyphae in the epidermis and outer cortex only of resistant onions, but roots of susceptible lines were disintegrated. It was of interest in these studies to determine if there were differences in root penetration of onions selected for demonstrated resistance or susceptibility. For these studies eight onion lines were selected (Table 2).

Plastic crisper trays were filled to a depth of four inches with non-sterile vermiculite. Fresh seed of each variety were planted in four rows in each crisper, and covered with about one quarter inch of vermiculite. The vermiculite was then saturated with Hoagland's solution and covered with kraft paper to hold surface moisture. Four 40 watt Gro-lux florescent tubes were suspended about two feet above the vermiculite surface. Using an electric timer, the photoperiod was set at 16 hours light and 8 hours dark. No attempt was made to regulate room temperature but it never went below 70°F nor above 80°F.

After five days the paper cover was removed. Distilled water was applied when needed, and every seven days Hoagland's solution was added. Liquid culture flasks were prepared by placing ten ml of Hoagland's solution in a glass vial 25mm I. D X 83mm. Polypropylene caps (Bacti Capall 25mm) which had had two holes melted through them were placed over each vial. The vials and their caps were covered with aluminum foil and autoclaved 15 minutes @ 121°C and 15psi. After the liquid in the vials had cooled, 10ml of P. terrestris liquid shake culture was added aseptically to each vial. The shake culture was prepared by using mannitol liquid media to which was added 5ml of sterile distilled water washed across a PDA plate upon which P. terrestris (Hess' isolate D-4-5) was growing.

Five plants of uniform size of each variety were taken from the vermiculite plantings, exercising care to minimize root damage. Any plants that were obviously injured were discarded. The roots were washed in sterile distilled water and the tops were passed through one hole in the vial cap. The roots were suspended at a uniform distance from the cap and sterile cotton was wrapped around the tops and forced into the cap hole to support the onions. A small amount of cotton was sufficient and the tops were shaded only slightly. When five vials were thus prepared for each onion variety they were placed in a metal rack with a plastic manifold suspended over them. Air that had been passed through an autoclaved fiberglass trap was

bubbled through the solution in the vials, using a centrifugal air pump. With equal liquid volume in all vials there was an equal hydraulic head so that a constant, near equal air flow was passed through each culture. The air served to agitate the inoculum and to aerate the suspended roots. The light and photoperiod were the same as used for growing the plants.

Twenty four hours after inoculation free-hand longitudinal sections were made of all plants in one vial from each variety. The roots were cut in a drop of distilled water using a dissecting microscope and placed in lacto phenol-cotton blue or distilled water mounting medium with the cut surface placed against the slide. In a few instances root segments were prepared without longitudinal sectioning. Glass cover slips were placed over the root sections and two applications of clear fingernail polish were applied after the space between the slide and cover slip was filled with mounting medium. These slides were then examined with a compound microscope for hyphal penetration. Massing of mycelium on some root sections was observed but hyphal penetration was not observed at this time.

McWhorter's (45) high vacuum clearing procedure was tried in an attempt to increase visual clarity in the root tissue. Due to the compact nature of the roots, however, no improvement was noted.

Three days after inoculation the same sectioning process was

repeated, though more cross sections were made. Cross sections were easier to prepare and observation of cell contents was equal to longitudinal sections. Longitudinal sections and root segments were mounted on separate slides so the difference in thickness would not present a problem. No clear indication of mycelial penetration of A. fistulosum was found. In over 60cm of root segments examined, only two possible sites of entry were observed and the hyphal tips appeared blunted as if they were walled off. No sites of colonization on the surface of the root tissue were observed. Under ultraviolet illumination strong florescence on those root sections mounted in lacto phenol-cotton blue indicated the possibility of callose (11).

Mycelial strands were prevalent on the root surface of onion breeding line number 223 and occasional hyphal masses were observed with mycelial penetration most frequent at these points. The mycelium did not ramify through the root tissue but was limited to epidermal cells. Florescence observed with line 223 was much less than with A. fistulosum. No pink color was observed in any root sections and all root tissue was firm, indicating no breakdown.

Mycelial masses were common on the root surface of onion breeding line Ia2997B with some hyphal penetration in these areas but the hyphae were restricted usually to a few cells. This variety showed very little florescence under U. V. illumination and was the only onion line to show any indication of pink color during this

experiment.

Mycelial agglomeration occurred frequently on the roots of onion breeding line number 36, but very few hyphae were observed in the epidermal cells. In the few cases where entry was made, a near immediate cessation of hyphal elongation appeared to occur. More florescence was observed than with any other lines examined except A. fistulosum.

In commercial Yellow Sweet Spanish line, P. terrestris mycelium appeared able to penetrate the root tissue at any point. Single hyphae were capable of entry and were not restricted in any apparent way, being able to grow into all the cells except the xylem vessels. Despite the massive invasion of the host, the plants continued to thrive, as judged by visual observation of the top growth. No pink color was observed nor any florescence under U. V. illumination.

Microscopic examination of root sections made from onion breeding lines 248, 249 and B5546B showed essentially an equal response to P. terrestris. Hyphal penetration was made with ease either directly or under mycelial agglomerations which in many cases were nearly continuous over the entire root surface. These lines all retained an intact, or nearly intact, epidermis and vascular ring, but there was a nearly complete breakdown of cortical tissue. Thus the root presented the appearance of a pipe within a pipe. At the time

of sectioning, the tops of these lines were already flacid. No florescence, nor any pink color in the roots was observed.

Subsequent root segments were made only of onion breeding lines 36, 223, A. fistulosum, YSS, and Ia2997B. Five days after inoculation A. fistulosum still was free of fungal growth on the root surface and no root penetration was observed. There was no progression of mycelium within the tissue of breeding lines 36 and 223 though more frequent penetrations were observed. The YSS variety had essentially the same extensive root cell invasion shown in the previous sampling with no indication of limiting action, but new roots were already present. Some invasion of the new roots was observed but was not as extensive as in the more fully developed roots. Onion line Ia2997B had become thoroughly ramified with hyphae and most plants were beginning to desiccate.

A second study using the same methods was initiated to substantiate the above results. Only four onion varieties were used: A. fistulosum, YSS, 223 and 248. Sectioning was done on the third and fifth day after inoculation. Results supported in all details those obtained in the first test. There was no visible hyphal penetration of A. fistulosum roots, and there was no pink color associated with any roots.

From these observations several conclusions may be reached. There appears to be at least two different types of resistance both of

which are biochemical rather than mechanical. The mode of resistance associated with A. fistulosum appears to be partially linked to root exudates, either the lack of required nutrients or the possibility of a fungitoxic exudate. Lack of colonization of the roots of this species suggests that toxic exudates are a factor. The few cases of apparent root colonization have resulted from mechanical entangling in the agitated medium. In the field, A. fistulosum has been reported highly resistant, if not immune to P. terrestris. Previous reports have been based on absence of pink root color (44) but the present results would indicate a complete lack of invasion by the fungus.

The resistance to P. terrestris invasion exhibited by lines 223 and 36, and to a lesser extent by line Ia2997B, indicates an internal biochemical resistance mechanism. It was clear that entry could be made without much apparent difficulty, but once the epidermal barrier was breached little internal mycelial growth occurred. In line Ia2997B, there was an overcoming of the internal resistance but this could be a matter of degree, with the mechanism incapable of fully stopping fungal growth.

A third mechanism was demonstrated, that of tolerance. The Yellow Sweet Spanish line, though permeated by the fungus, showed no immediate ill effects. When the pathogen gained the upper hand, the host produced new roots to support its needs. P. terrestris is a

warm weather pathogen, becoming an economic problem only when soil temperatures approach or exceed 80° F. This condition does not normally occur in eastern Oregon until near mid-season, after the onion has made much of its root growth. At that time the host has made much top growth and is in good condition for maximum root regeneration. As the pathogen invades the roots the host regenerates other roots at a maximum rate, allowing it to secure necessary water and nutrients. Only those conditions reducing host vigor would destroy this balance.

These studies support the implication of pectic enzymes in the disease syndrome, at least with susceptible varieties. The complete destruction of the cortical root tissues of lines 248, 249 and B5546B and their diseased appearance indicate the presence of these enzymes. Keen and Horton (28) have shown that P. terrestris produces pectic enzymes in culture.

The lack of pink color in most infected roots in these experiments shows that a definite time lag occurs between infection and appearance of pigment. Examination of mounted root sections from field grown Yellow Sweet Spanish onions showed that this time lag also occurs in the field. Numerous roots without pink color were found to be invaded by the fungus. In all but a few roots when pink color was observed it was found in the mycelium or in the cell in which mycelium was present. This is in agreement with work done

by Kreutzer (32) who found pigment primarily associated with hyphae. Numerous "pycnidial primordia" (32) were observed in desiccated pink roots. A few were observed in Ia2997B roots grown in culture, indicating only a short time is required for their formation.

Polyphenol Oxidase Enzyme Assay

Phenolic constituents have been implicated in plant disease resistance by many workers but as yet no clear relationship has been established. Farkas and Kiraly (12), O'Hare (21) and Cruickshank and Perrin (6) have presented reviews on the subject. Walker (73), Link, Angell and Walker (37) and Link and Walker (38) have shown that protocatechuic acid and catechol are responsible for prevention of onion smudge disease (Colletotrichum circinans) in pigmented onions. In this instance the phenolic compounds protect the dead scales from the pathogen. No information is available on phenolic constituents of onion roots. In searching for possible mechanisms of pink root resistance in onion roots it was of interest to determine the existence of any potential phenolic reactions.

A broad spectrum of genetic material was selected for these studies. At the pink root breeding trial evaluations in 1966, numerous bulbs were selected for polyphenol oxidase analysis. All bulbs in the trial ground were dug from the soil with minimum root injury and graded. Bulbs selected for analysis were collected

immediately after pink root evaluation, topped and placed in plastic bags which were then placed in a pre-cooled ice box. The bulbs were kept chilled until the roots were excised for analysis, about 48 hours. Commercially grown Yellow Sweet Spanish onion bulbs were also collected from untreated soils. Prior to processing for enzyme assay the root systems of the YSS onions were graded into the 2, 3, and 4 disease categories. These were processed separately and are designated as YSS2, YSS3, and YSS4.

Onion roots were cut from the base plate, being sure to take none of the latter tissue. Root plates were cut from the leaf tissue of the bulb, being sure to cut away all root and leaf tissue. The plates were diced with a razor blade to aid in mascerating the tissue. Five grams fresh weight were homogenized in a pre-chilled Omni-mixer with 10ml (2X tissue weight) of freshly prepared 0.1M phosphate buffer (pH7) containing 0.001M EDTA for 1 minute. The Omni-mixer container was immersed in an ice bath during the entire blending time. The homogenate was pressed through a clean flour sack cloth into a pre-chilled beaker, cooled in an ice bath and centrifuged in a Servall centrifuge at 1700g for five minutes and then at 3800g for 20 minutes at a temperature range of 0-5°C. The supernatant volume was measured by pipetting all of the liquid from the centrifuge tube to a chilled test tube. Care was taken to prevent transfer of the sediment.

A freshly prepared and chilled 0.5% catechol solution was used as the enzyme substrate (43). In initial runs several enzyme preparation:catechol ratios were tested to find a ratio that would give a reading in a reasonably short time. A 2:1 ratio was found best for these preparations. All readings were made on a Beckman DB Spectrophotometer at 400 mμ over a period of at least five minutes. Each sample was run at least twice. No significant differences were found between any of these onion lines.

In 1967 a follow up study was undertaken to verify results obtained in the previous study. Onion seeds were planted in quartz sand and cultured in the same way as onions grown for pink root assay, except they were grown in the greenhouse for three months. After this period the roots were washed free of sand and prepared as previously. When these extracts were combined with catechol substrate prepared in distilled water, no enzyme activity was obtained. However, with catechol dissolved in the pH 7.3 0.1M phosphate buffer with 0.001M EDTA added there was evidence of activity.

This apparent discrepancy was resolved on the basis of pH. The pH of the distilled water was found to vary considerably but a pH of about 6 was encountered most frequently. A study showed a definite influence of pH on the oxidation of catechol. When the solutions were prepared, according to tables in Methods in Enzymology Vol. 1 (15), there was an obvious color break between pH 6.80 and

7.28. A pale yellow color was present in the three lowest pH solutions, while a pale brown color was present in the three higher pH solutions. Enzymic oxidation of catechol was rapid at the three higher pHs and slow or nonexistant at the three lower pHs (Table 6). Mallette (42) points out that a pecularity exists with a number of phenols. Using catechol as the example, oxidation by PPO and oxygen proceeds only after a few molecules of catechol are oxidized. A number of factors influence this "sparking" phase of the reaction, but specifically the lag period can be lengthened and the reaction rate reduced by pH which controls the water reaction of the o-quinone formed in the reaction.

Table 6. Effect of pH on phosphate buffered (0.1M) catechol substrate (0.5% solution) using PPO enzyme from onion line number 223.

Change in O.D.	Dilution Subst.:Enz.	pH		Change in O.D./gr./30 sec.
		Enzyme	Substrate	
0.000	4:1	6.01	6.01	0.000
0.015	4:1	6.01	6.40	0.043
0.065	4:1	6.01	6.80	0.187
0.083	4:1	6.01	7.28	0.777
0.042	8:1	6.01	7.65	0.787
0.040	8:1	6.01	8.01	0.749

A second experiment was undertaken to establish the pH requirement of the catechol substrate and to determine if the pH of enzyme

extraction influenced activity. Onion line number 223 was used in these studies also, with enzyme extraction done as before except the pH of the phosphate buffer was varied. The extraction pH over the range tested does not greatly influence enzyme activity (Table 7). It is recognized that these were crude extracts and that pH would probably exert a greater influence on more purified enzyme preparations. However, for the purposes of this work these preparations were felt to be sufficiently pure.

Table 7. Effect of phosphate buffered extraction pH on PPO enzyme activity using PPO enzyme from onion line number 223. The catechol substrate (0.5%) was buffered with 0.1M PO_4 at pH 7.28. All substrate:enzyme dilutions were 4:1.

Change in O.D.	pH		Change in O.D./gr./30 sec.
	Enzyme	Substrate	
0.090	6.01	7.28	0.421
0.085	6.40	7.28	0.371
0.090	6.80	7.28	0.410
0.095	7.28	7.28	0.398
0.035	7.65	7.28	0.161
0.080	8.01	7.28	0.389

With the means available to test a number of pink root susceptible and resistant onion lines for relative levels of PPO the remaining plants grown in the greenhouse were committed to enzyme extraction. For comparative purposes and to relate the PPO activity

in onion roots to PPO activity already in the literature, common mushroom (psalliota campestris) and potato peelings were also analyzed. Extractions were made as before with a pH of 7.28 in 0.1M phosphate buffer. Polyvinyl pyrolidone (PVP) (40) was used in some extractions and not with others because precipitates were encountered in previous extracts where PVP was used.

Results of these analyses showed that roots of all the onion lines tested possess some PPO activity (Table 8). Though the differences are not large there is an interesting trend of the resistant lines showing a reduced level of PPO, relative to the more susceptible onion lines. Comparison of all onion lines with the mushroom activity shows onions generally have only half as much activity. Comparison with potato peelings shows onions have about 1/15 as much activity and mushroom has about 1/10 as much activity. These data do not indicate any basis of disease resistance directly associated with the PPO system.

Phenolic Constituents

As a second means of determining if phenolic compounds could play a role in resistance to pink root disease, chromatographic analysis of selected onion lines was undertaken. Three onion lines were selected on the basis of disease susceptibility: A. fistulosum--resistant; B5546B--very susceptible; and Yellow Sweet Spanish (YSS)

Utah strain K x L selection--moderately susceptible. The YSS and B5546B onion roots were rated for disease and tissue in the different disease categories was analyzed separately.

Table 8. Comparison of polyphenol oxidase levels in pink root susceptible and resistant onion roots, common mushroom and potato peels. All extractions and substrates (2% catechol) were made in 0.1M PO_4 buffer, pH 7.28.

Source of PPO enzyme	Change in O. D.	Dilution		Change in O. D. /gr. /30 sec.
		Enzyme	Subst:Enz.	
A. fistulosum	0.140	-	4:1	0.504
Line #223	0.107	-	4:1	0.516
Line #248	0.202	-	4:1	1.688
Line #249	0.138	-	4:1	0.935
YSS	0.201	-	4:1	1.528
B5546B	0.162	-	4:1	1.170
Ia 2997B	0.182	-	4:1	1.314
Line #36	0.202	-	4:1	0.727
SWG	0.255	-	4:1	1.122
Common Mushroom	0.031	4:1	8:1	3.286
Potato peels	0.354	4:1	8:1	29.736

The selected onion lines were greenhouse grown in soil infested with P. terrestris (Hess's D-4-5 isolate) cultured on autoclaved barley seeds during the spring and summer of 1966. After the onions began to bulb and B5546B and YSS plants were showing disease symptoms they were removed from the cans. The soil was shaken

and washed off the roots until clean uninjured root systems were obtained. The roots were cut from the bulbs, and 20gr of tissue or more were chopped with a razor blade to lengths of 1/2 - 1cm and plunged into boiling 95% ethanol and boiled five minutes. Four mls of ethanol were used per gram fresh weight of plant material. The extract was vacuum filtered through a Buchner funnel using Whatman #1 filter paper. The tissue was extracted three times by placing it in a Waring blender for two minutes with the same ratio of ethanol-plant tissue (4ml/gr). The extract was vacuum filtered as before. The resulting filtrates were combined and concentrated in-vacuo at 37°C to less than 100ml. The concentrate was poured into a graduated cylinder and brought to 100ml with 95% ethanol. To this concentrate cysteine (0.001M) was added to stabilize the phenolic constituents (3). The samples were stored at -5°C until used.

Thin layer chromatography was used to separate the phenolic constituents. Cellulose (MN-300) plates were prepared by mixing in a Waring blender for two minutes 1 part cellulose powder into 6 parts water. Use of less than 6 parts water yielded lumpy suspensions. The slurries were spread 250 mμ thick with a Desaga applicator on 20 X 20 cm uniformly thick glass plates and dried at 100°C. The plates were held in a desiccator until used.

Plates were spotted using a Hamilton micro-syringe to maintain a small spot. Root extract volumes used to spot plates were

adjusted to maintain equal amounts of tissue applied per plate. Two dimensional ascending development at room temperature (ca. 23°C) was employed in glass developing tanks lined with solvent saturated Whatman #1 paper. Butanol-Acetic acid-Water (4:1:5) (BAW) was used as the first solvent system. Acetic acid-Water (15:85) (HAC) was used as the second solvent system. The solvents were allowed to ascend at least 10 cm above the spot before the plates were removed and air dried. Detection of phenolic compounds was accomplished by examining the plates under long wave ultra violet light over concentrated ammonium hydroxide. Duplicate runs were made of each extract on two different days.

Table 9 shows Rf values obtained from A. fistulosum and YSS extracts. Five spots were obtained from the A. fistulosum extract with spots 1, 2, 3, 4, and 5 being distinct (Figure 1). Three and 4 tended to not always separate completely and on one occasion spots 2, 3, and 4 were imperfectly separated. These results show that three rather distinct chemical entities are present as characterized by their solubilities in these solvent systems.

Chromatography of the YSS extracts showed a changing pattern as disease symptoms progressed from none to severe. Eight distinct spots were present, seven moving as one spot in BAW. Differential separation was obtained with the more highly polar HAC. The extract of slightly diseased tissue (Figure 3) showed nine distinct spots.

Table 9. Average Rf values of phenolic constituents occurring in selected onion root tissue, as determined by TLC.

	Spot location number and developing solvent[1/]																			
	1		2		3		4		5		6		7		8		9		10	
Onion Variety	B	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H
A. fistulosum	.18	.00	.18	.86	.31	.98	.22	.81	.56	.77										
YSS 1	.73	.00	.78	.14	.75	.42	.73	.58	.62	.69	.79	.77	.83	.95	.41	.94				
YSS 2	.24	.00	.71	.00	.79	.16	.75	.48	.75	.61	.79	.79	.26	.45	.32	.75	.37	.92		
YSS 3	.68	.00	.71	.04	.68	.21	.66	.51	.67	.62	.58	.72	.57	.80	.89	.85	.66	.92	.28	.94
YSS 4	.75	.00			.89	.22														

1/ B = Butanol-Acetic acid-Water (4:1:5)

H = Acetic acid-Water (15:85)

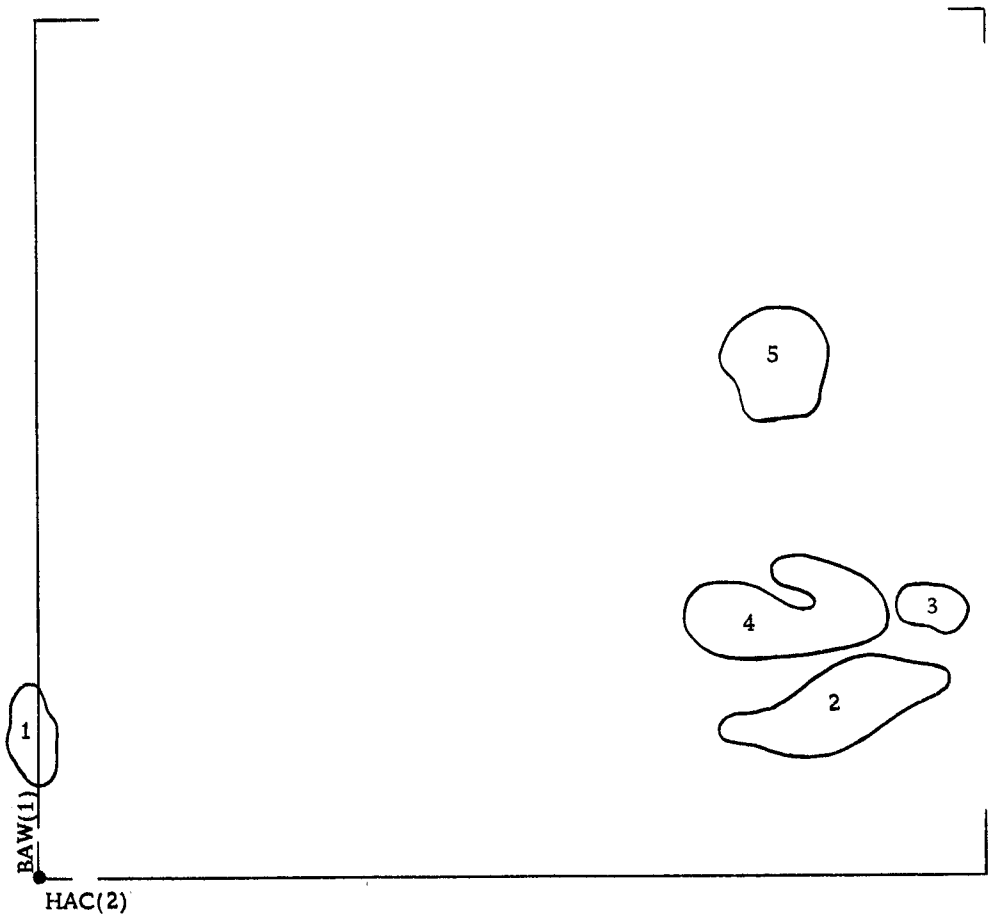

Figure 1. Phenolic distribution pattern obtained from A. fistulosum roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

There was a sharp pattern change from that shown by the non-diseased tissue (Figure 2), but the pattern obtained from extracts of more severely diseased tissue (Figure 4) resembled those obtained from non-diseased tissue. Severely diseased tissue extract showed a near absence of phenolic materials (Figure 5). More work is needed to attach meaning to these results.

Chromatography of B5546B extracts showed no consistant pattern. In nearly all plates an irregular pattern was obtained with BAW but no movement in HAC occurred (Figure 6). Very slightly florescent spots were observed on one plate but were not observed at other times and were disregarded.

Examination of Rf values obtained from A. fistulosum and the various YSS extracts show very few similarities. Similarities do occur among the YSS extracts but significant differences also were present.

These preliminary results indicate a different complement of phenolic constituents exist in these onion roots and the phenolic complement in YSS roots changes during the disease cycle. It is interesting that the most resistant variety, A. fistulosum, shows fewer constituents than the commercial variety and that the most susceptible onion, B5546B, showed no phenolic constituents with this method.

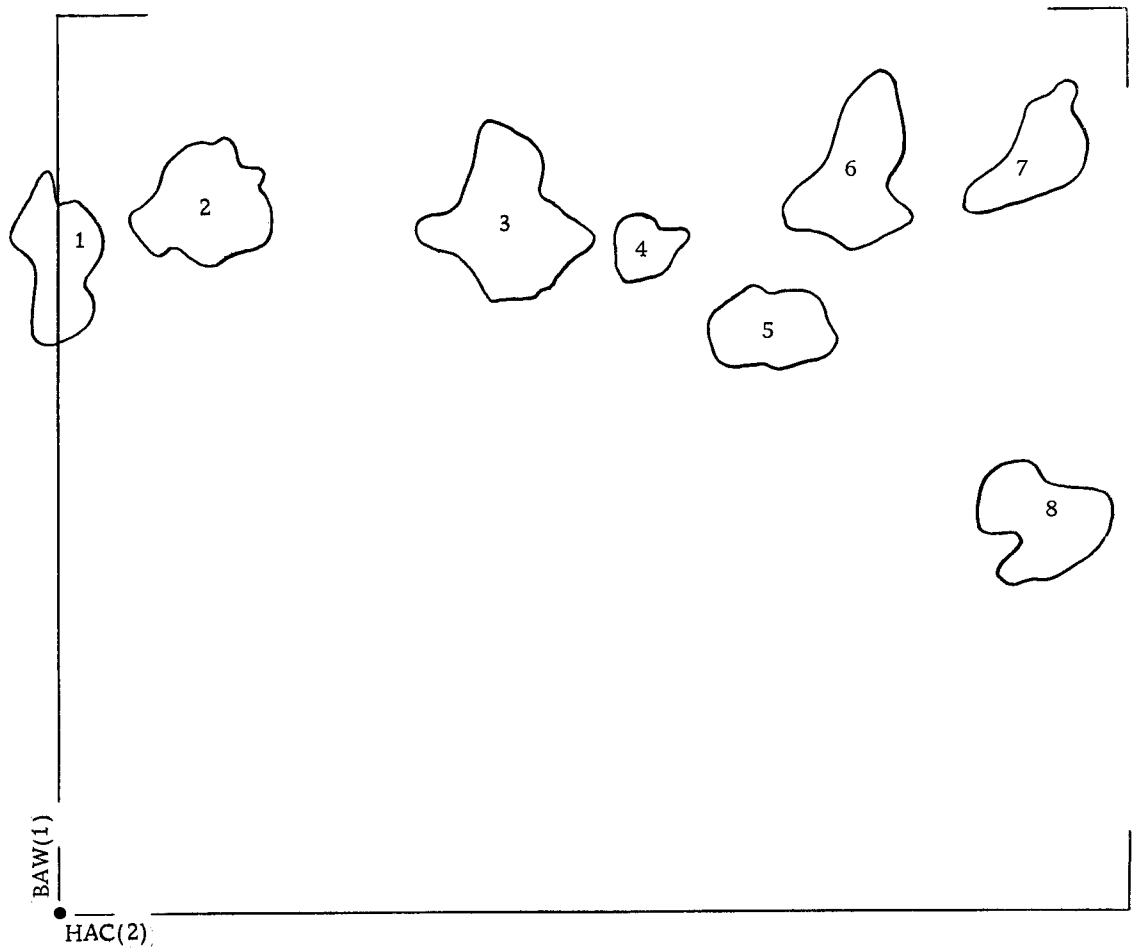

Figure 2. Phenolic distribution pattern obtained from YSS1 roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

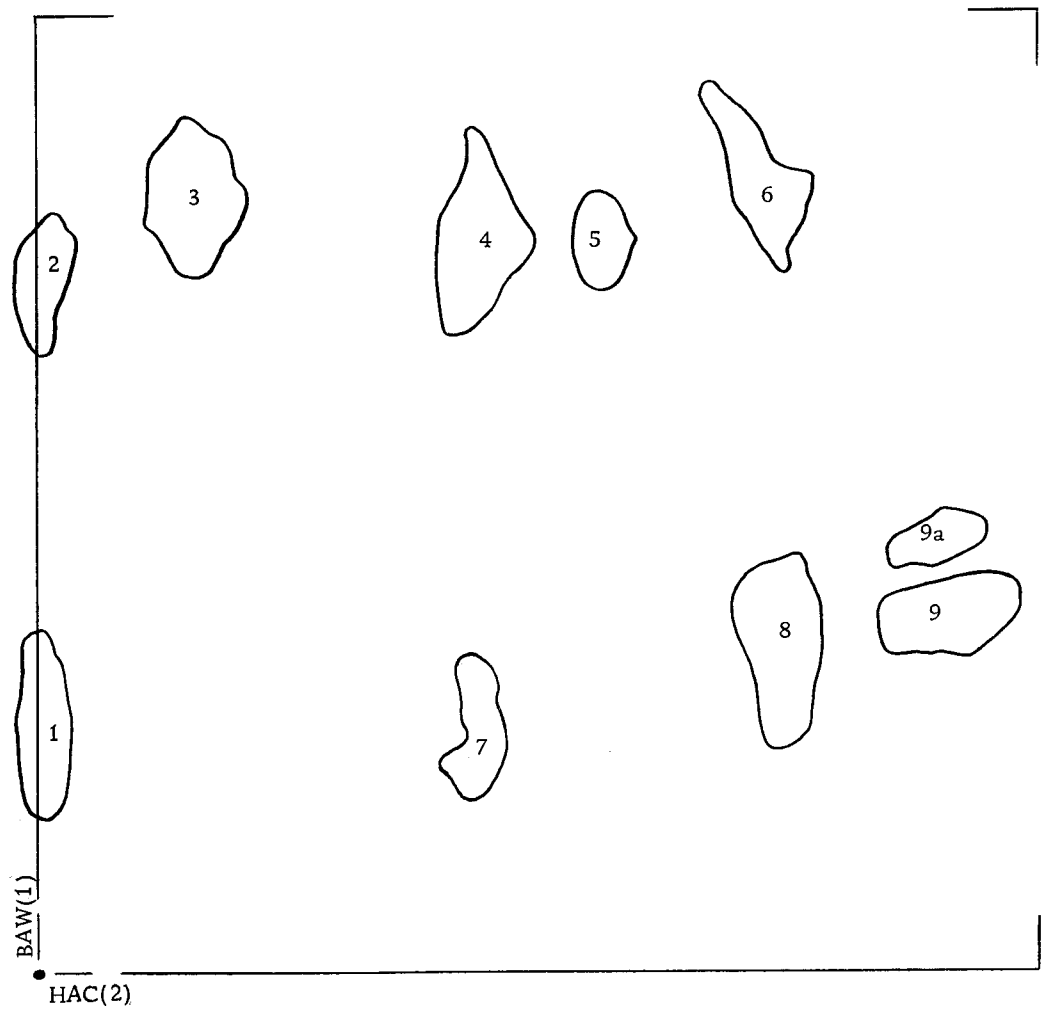

Figure 3. Phenolic distribution pattern obtained from YSS2 roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

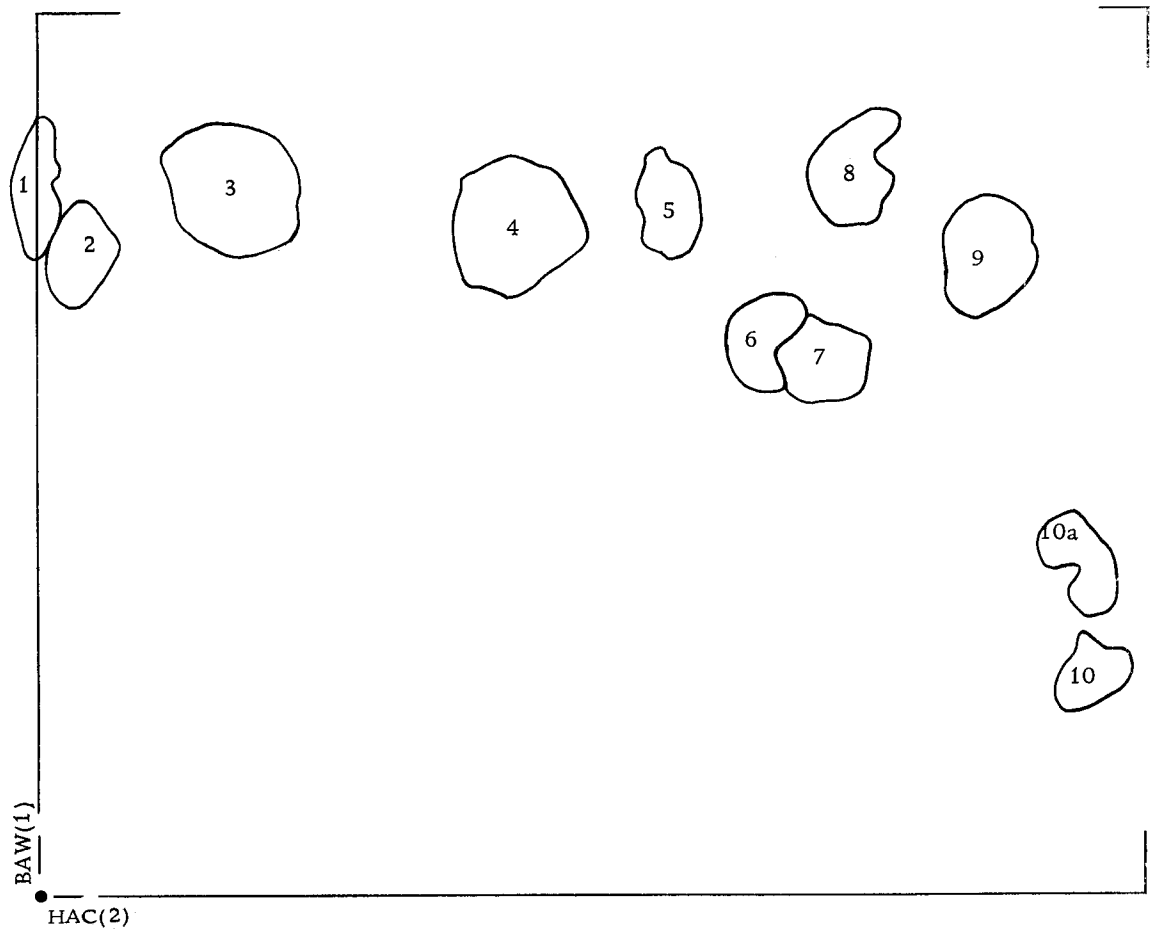

Figure 4. Phenolic distribution pattern obtained from YSS3 roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

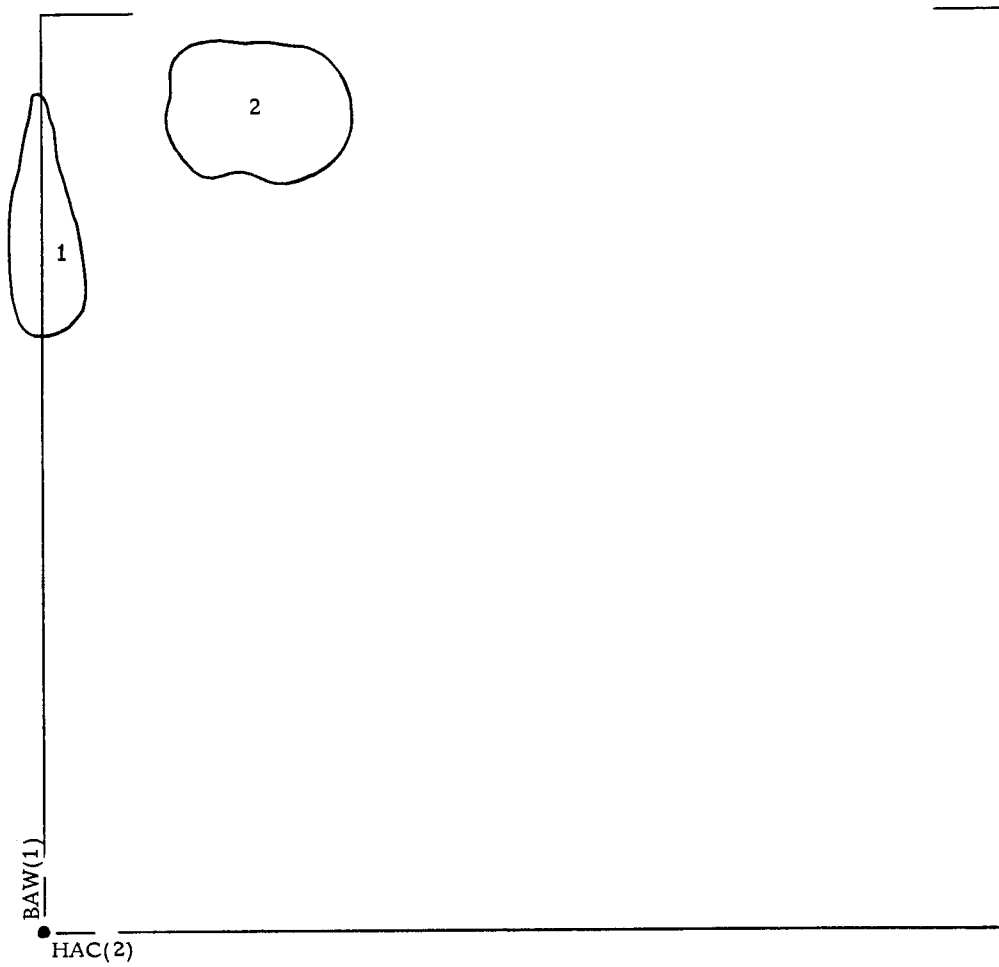

Figure 5. Phenolic distribution pattern obtained from YSS4 roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

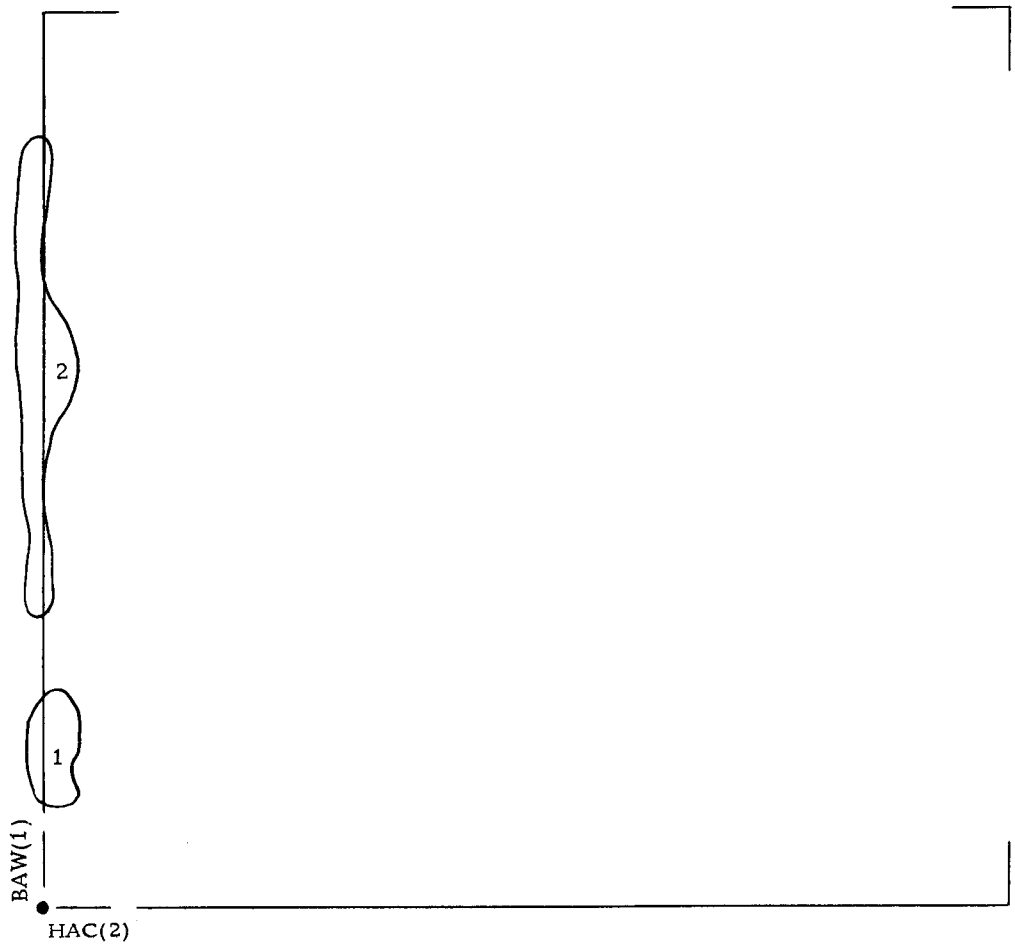

Figure 6. Phenolic distribution pattern obtained from B5546B roots using TLC. (Cellulose; (1) BAW 4:1:5; (2) HAC 15:85.)

Direct Assay of P. terrestris Infective Propagules in Soil

Watson (75) has published a method for quick identification of P. terrestris isolates using wheat straw. Also Hess (23) and Hess, Vaughan and Leach (24) have used near ultraviolet light to induce sporulation for positive identification of P. terrestris. While these methods aid in identification of individual cultures, there is at present no means available to determine the level of P. terrestris inoculum in soil. Obviously a method of determining the number of propagules per unit of soil would be of value, allowing more knowledgeable interpretation of onion breeding experiments, more intelligent use of crop rotation, and perhaps facilitating basic studies not now practical. The purpose of the following study was to determine whether P. terrestris levels could be assessed in a fashion similar to that used originally by Schmitthener and Williams (57).

Preliminary Study

Three composite soil samples were collected for preliminary study, one each from three fields with a known pink root history. The soils were air dried, and passed through a 2mm screen. Weighed portions of soil were diluted with clean Del Monte El-20 mesh quartz sand to give 500gr of soil:sand at dilutions of 1 part soil to 1, 2, 5, 10, 50, or 100 parts sand. The weighed portions of soil

and sand were placed in a jar, tumbled until thoroughly mixed and placed in cardboard ice cream containers. Twenty B5546B onion seed, selected because of high disease susceptibility, were planted in the diluted soil to a depth of about one-quarter inch. All containers thus prepared were watered with Hoagland's solution and covered with kraft paper for about four days to allow germination of the shallowly planted seed.

Subsequent waterings were made as required, usually every other day, with additional Hoagland's solution added at seven day intervals. Four 40-watt Gro-lux florescent tubes were used to supplement available light on a photoperiod of 14 hours light, 10 hours dark. No temperature control was employed, but ambient room temperature was between 70 and 80° F. Evaluations were made six weeks after planting by reading the root system of each plant as infected or not infected. The percentage of infection was calculated from these readings and was used to compare the infective level of the three soils.

The preliminary results, Table 10, were encouraging. Definite trends were indicated, though numerical reversals were present. It was determined that greater dilution was needed and that with the lower dilutions there were factors other than pink root disease to consider. Stands were reduced and were less vigorous at low levels of soil dilution. The number of seed planted yielded a stand too small to be

Table 10. Preliminary soil dilution study for determination of *P. terrestris* inoculum level as assayed by root readings of B5546B onion seedlings six weeks after planting.

Dilution	Source of Soil Samples (Grower fields)											
	Nakada				Teramura 1				Teramura 2			
Sand:Soil	Non-Infected	Infected	Total	% Disease	Non-Infected	Infected	Total	% Disease	Non-Infected	Infected	Total	% Disease
1:1	1	5	6	83	3	7	10	70	0	8	8	100
1:2	0	8	8	100	1	7	8	83	0	5	5	100
1:5	1	10	11	91	6	5	11	45	1	7	8	88
1:10	3	12	15	80	9	2	11	18	16	1	17	6
1:50	3	3	6	50	5	3	8	37	9	5	14	36
1:100	14	4	18	22	5	10	15	50	5	3	8	37

of value. The cardboard containers promoted fungal and probably bacterial growth that could confuse any results. After six weeks only a few roots were showing pink color, but the color was bright pink. Many roots were showing indistinct disease symptoms, as indicated by yellow to brown color. In contrast, onions grown in pure sand had bright white roots. These observations in conjunction with earlier observations of root sections devoid of pink color, but clearly parasitized by P. terrestris mycelium, make it clear that reading pink color for disease yields a conservative estimate of true disease. Even so, the over-all result of this test seemed encouraging, recognizing the possible inherent variation in a biological method of this type, and further studies were conducted.

Soil samples were collected from fumigation trials by randomly taking five surface soil samples (0-3 inches deep) from each plot and combining these samples into one composite sample. Each field treatment was replicated three or four times and, since each treatment replicate was sampled separately, the greenhouse dilution trials were also replicated three or four times. The soil from the pink root testing grounds at the Malheur branch station was also sampled. This field was subdivided into quadrants and one composite soil sample (five samplings) was collected from each quarter. The soil was collected from the surface three inches when fields were dry, placed in paper bags and air dried.

As with previous sample preparations, all soil was passed through a 2mm screen and diluted 1 part soil to 1, 10, 100, 1,000, or 10,000 parts clean quartz sand. The soil-sand mixtures were adjusted to 1,000 gms, allowing a four inch depth in each eight inch diameter plastic pot. Drain holes in the pots were sealed with tape to prevent loss of soil-sand and water.

Onion Variety Response

Two commercial onion varieties were used to test their value in this procedure. They were Yellow Sweet Spanish (YSS) (Utah strain, K x L selection) and Southport White Globe (SWG). Both lines are pink root susceptible, but the latter is much more susceptible as shown in field plantings. These varieties were selected because of their present and future availability. Seed of the B5546B variety, though this variety is perhaps more susceptible, are available only in small lots from plant breeders.

Fifty seed were counted for the first ten lots of each variety. These were weighed on a Mettler balance to the nearest mg. The average weight determined for each variety was used to measure out the remaining seed lots. To test the accuracy of the procedure, ten packets of each variety chosen at random were subsequently counted and the average number of seed was 52 for SWG, 53 for YSS. Seed from one packet of each variety were planted in each pot by using a

template to cover one half of the surface and scattering the seed over the surface of the exposed half. The other half was planted by placing the template over the seeded half and scattering seed over the unplanted surface. An identifying marker was placed in each pot to maintain the identity of the varieties. The seed were covered with 1/8-1/4 inch of the soil-sand mix reserved for this purpose.

All pots were filled and seeded before being watered with Hoagland's solution. In this way a common starting point was achieved for all dilutions. The pots were covered with kraft paper for five days, during which time nearly all seed germinated.

No supplemental light was needed. The photoperiod was about 16 hours light and 8 hours dark. The air temperature was held about 70°F and rarely went above 95°F. Tap water was used to maintain necessary moisture. Hoagland's solution was added once a week. Care was taken not to waterlog the soil-sand mixture.

Evaluation was made six weeks after the initial watering. As before, plants were judged infected or not infected according to pink color, and the percentage of infection was determined (Tables 11 and 12). In these tests onion stands were generally reduced in the 1:1 planting and the onions had a reduced growth rate compared to the greater dilutions. This was partly due to soil compaction but other non-determined factors appeared to be acting also. The 1:1 dilution is presented from one test only since these data appeared to be in line

Table 11. Average percentage of infection obtained from field treated soil samples with the indicated soil:sand dilutions using onions as biological indicators (Nakada trial)

Treatment	Rate Gal./A.	Onion Variety and Soil:Sand Dilution Level									
		Southport White Globe					Yellow Sweet Spanish				
		1:1	1:10	1:100	1:1,000	1:10,000	1:1	1:10	1:100	1:1,000	1:10,000
Untreated-1	-	80	77	25	19	9	39	39	24	14	1
Telone	40	95	71	31	20	2	77	43	24	1	5
Vorlex	10	76	72	36	17	4	63	47	20	24	6
Vorlex	15	70	68	36	9	4	84	41	14	8	2
Vorlex	20	80	72	28	8	5	60	30	21	12	10
Untreated-2	-	48	83	46	38	9	71	48	47	25	2
Vorlex	30	80	75	19	11	0	49	48	51	9	5
Untreated-3	-	87	53	36	11	2	58	6	35	9	2
Telone PBC	40	63	73	22	28	2	75	49	28	15	0
LSD 5%			26	42	34			36	38	25	

Table 12. Average[1/] percentage of infection obtained from field treated soil samples with the indicated soil:sand dilutions using onion varieties as biological indicators. (Teramura trial)

Treatment	Rate Gal./A.	Onion variety and Soil:Sand Dilution Level							
		Southport White Globe				Yellow Sweet Spanish			
		1:10	1:100	1:1,000	1:10,000	1:10	1:100	1:1,000	1:10,000
Untreated	--	36	30	8	2	16	17	4	0
Telone	40	35	6	1	2	16	7	1	4
Vorlex	10	38	1	8	4	17	4	2	5
Vorlex	15	27	10	7	1	8	15	0	1
Vorlex	20	12	4	0	0	18	2	0	0
Vorlex	30	34	6	1	0	17	10	1	1
LSD 5%		50	23	16		31	19	4	

[1/] Average values were obtained from three field replicates, each one assayed separately.

with the results of the higher dilutions. Little significance can be attached to these data however because of the erratic nature of this dilution level. At the 1:10 dilution the disease level with SWG was often nearly twice that of the yellows. At greater dilution levels the difference was not as evident, which might be expected as the inoculum dilution increases. Yellow onions showed no difference between most treatments at the 1:10 dilution level, but one check was far below all other treatments. This check was completely at variance with results obtained with the white onions. The width of the numerical range of the total replicated treatments at each dilution level was not significantly different at the 5% level, therefore the differences in disease level were due to increased susceptibility of the white onions.

At the 1:100 dilution level one of two trials showed significant differences existed between treatments. With soil from the fumigation trial conducted on the Teramura ranch (Table 12), assays using white onions showed all treatments significantly reduced the inoculum level below the level present in the untreated soil. Assay of the same soil using the yellow onion showed no significant differences. At a dilution of 1:1000, assay with white onions showed no significant differences were present, while assay with yellow onions showed statistically significant inoculum level reduction occurred with Vorlex treatments of 15 and 20 gal/A.

A second test was initiated to further test the method and to attempt to ascertain the inoculum level in the field during the growing season. Soils were collected as before but only from the Nakada fumigation plots and the pink root trial ground at the Malheur Experiment Station. The sample preparation was as described for the first dilution assay. As before, the 1:1 dilution was affected by factors other than P. terrestris, confirming results previously obtained. Comparison of root disease levels of white and yellow onions showed closer agreement than in the previous test, though the disease level occurring on onions was lower (Table 13).

The 1:10 dilution showed Vorlex at 15 gal/A reduced the fungal inoculum below that present in the Telone 40 gal/A treatment and the untreated-2 sampling, but it was not different than the rest of the treatments. At the 1:100 dilution, inoculum in the Vorlex 30 gal/A treatment was significantly lower than other treatments. The yellow onions at the 1:100 dilution level only showed a significant difference between the Vorlex 30 gal/A treatment and the rest of the treatments.

The infection level of soils from untreated plots of two fumigation test fields and two soil samples from the Malheur Experiment Station pink root test field are compared in Table 14. Data obtained with SWG at the 1:10 soil:sand dilution level showed the Nakada soil had significantly more inoculum than the Teramura soil. The Experiment Station east sample had significantly less inoculum than the west

Table 13. Average percentage of infection obtained from the second assay of field treated soils, using the indicated soil:sand dilutions and onions as biological indicators.

Material	Rate Gal./A.	Onion Variety and Soil:Sand Dilution Level							
		Southport White Globe				Yellow Sweet Spanish			
		1:10	1:100	1:1,000	1:10,000	1:10	1:100	1:1,000	1:10,000
Untreated-1	-	43	19	12	0	23	22	18	0
Telone	40	55	27	10	15	48	27	23	0
Vorlex	10	50	27	10	3	51	38	10	26
Vorlex	15	12	22	8	3	28	17	15	0
Vorlex	20	47	12	10	14	29	16	15	9
Untreated-2	-	53	32	16	2	38	19	24	4
Vorlex	30	20	4	19	0	26	6	10	0
Untreated-3	-	37	29	15	1	46	19	13	0
PBC	40	45	27	10	4	38	29	14	3
LSD 5%		40	28	25		55	29	23	

Table 14. Average percentage of infection, comparing four untreated field soils for P. terrestris infestation using soil:sand dilutions with onions used as the biological indicator.

	Onion Variety and Soil:Sand Dilution Level							
	Southport White Globe				Yellow Sweet Spanish			
Field	1:10	1:100	1:1,000	1:10,000	1:10	1:100	1:1,000	1:10,000
Nakada	77	25	19	9	39	24	14	1
Teramura	32	26	10	2	15	14	3	0
Malheur Expt. Sta. East	22	5	4	3	0	3	5	4
West	85	26	20	0	63	22	0	0
LSD 5%	33	42	22		24	34	26	

sample. The inoculum level of the Nakada field was similar to the west soil sample from the station, while the Teramura field and the east soil sample from the station had comparable inoculum levels. Results obtained at the same dilution level with the YSS onion variety were the same, though the level of infection was consistantly lower. The higher dilution levels showed that the dilution end points of the four soil samples were about the same, however the dilutions were not great enough to prove this.

These data further support that differences exist in the disease level of different fields and point to the importance of having a basis for comparison of different fields for purposes of chemical testing or hybrid onion variety evaluation. Obtaining data of this type before selecting fields for test could allow pairing fields on the basis of their inoculum levels. Additional research into the subject of inoculum level required for disease would allow fumigation of only those fields most likely to benefit from treatment.

Depth of Inoculum in the Soil

It was of interest to determine at what soil levels P. terrestris inoculum is most prevelent. Soil samples were collected from two sites of the Malheur Experiment Station pink root test field, both, coincidentally, on the east side of the field. Two holes were dug more than 18 inches deep, one at the upper (south) end of the field,

the other at the lower (north) end. Loose soil was moved away from the edge of the hole. The face of one side of the hole was then freshly cut with a knife, and soil samples were taken from the 3, 6, 9, 12, and 18 inch levels. Soil was removed in a two inch horizontal band, one inch above and one inch below the designated level. A one inch sample was taken from the surface. The soil was placed in paper bags, kneaded to break up soil structure and air dried. Three replicates of each sample were assayed.

Inoculum appeared to be concentrated in the top six inches with a rapid decline from this level downward (Table 15). At nine and 12 inches there was a low level of inoculum and at 18 inches little or none. In this respect, results with both white and yellow onions agreed.

These results are compatible with what might be expected, since the soil is plowed to a depth of 6-9 inches every year, and there should be a near homogeneous mixing of P. terrestris propagules through the upper layer. Existence of inoculum at depths of 18 inches would not necessarily be expected but might explain, at least in part, the lack of disease control with fumigants applied 8-10 inches deep in the soil. These data can have a profound effect on the approach to fumigation of the soil, since it is a near certainty that fumigation will at best give only one year's benefit. Sufficient inoculum may be present in the lower soil horizons to reinfest the treated area. Nearly a full

Table 15. Distribution of pathogenic inoculum of P. terrestris in a soil profile, using the indicated soil:sand dilutions and onions as biological indicators.

Soil Depth-inches	Onion Variety and Soil:Sand Dilution Level[1/]									
	Southport White Globe					Yellow Sweet Spanish				
	1:1	1:10	1:100	1:1,000	1:10,000	1:1	1:10	1:100	1:1,000	1:10,000
1	16	15	10	8	0	10	3	15	11	1
3	21	21	5	12	1	16	20	20	8	8
6	34	7	9	0	3	11	18	19	0	0
9	5	3	0	2	1	13	2	6	2	6
12	10	3	1	6	0	9	4	7	1	0
18	6	3	0	0	0	1	0	0	0	0
LSD 5%	31	20	11	12	3	28	22	28	14	10

[1/] Figures show average percent infection.

year passes between treatment and harvest and during this time the deeper soil is well mixed with the more shallow soil layers by plowing and discing. Also, some onion roots will penetrate deep into the soil (64) and allow the fungus to move through them to the upper soil levels.

Optimum Time for Reading Assays

The six week reading time was determined arbitrarily, though preliminary studies showed it was successful. The shortest possible exposure of onion roots to infective P. terrestris propagules would be most desirable since it would minimize occurrence of multiple infections and underreading of disease severity. However, a compromise must be reached, since sufficient time must pass to allow symptom expression. A study was initiated to determine the optimum time for readings to be made.

Two untreated soils, which in previous tests showed a high inoculum level, were prepared at 1:10 and 1:100 dilutions and planted with SWG onions. Disease readings were made after 3, 4, 5, and 6 weeks. The results (Table 16) indicate that the shortest time to obtain meaningful results would be five weeks, and that six weeks gives a higher disease rating. The higher rating would show greater differences and at this time appears the more desirable to use. There was no more precision achieved at a period of less than five weeks,

in fact there was less since a small numerical change between small numbers has a greater effect on percentage than a similar numerical change between large numbers.

Table 16. Effect of time of exposure on infection levels.[1/] Two soil:sand dilution levels were used with the Southport White Globe onion as the biological indicator.

1:10				1:100			
weeks after inoculation				weeks after inoculation			
3	4	5	6	3	4	5	6
2.3	7.7	26.3	50.0	1.3	2.3	11.7	24.0
0.7	6.7	30.1	43.1	2.0	5.0	15.3	28.0

[1/] Figures show the average of three replications. Two different soils were used. (Average percent infection)

Relation of Soil Particle Size to Inoculum Density Ratings

Although the nature of the infectious propagules has not been determined it was of interest to attempt to determine whether propagules of P. terrestris are associated with large particles or exist in the soil as spores, pycnidia or minute mycelial fragments.

Soil from the Malheur Experiment Station onion breeding plot was air dried and passed through a 2mm screen. The sieved soil was then passed through Tyler Standard Screens, sizes 14, 28, 48,

100, 200, and 400 and each soil fraction was diluted 1:100 with clean quartz sand. About 50 SWG seed were planted per pot of soil.

Examination of the average infection percentage of all soil samples (Table 17) showed a slight increase in infection with decreasing soil particle size to the 100/200 (soil passed the 100 mesh screen, held on the 200 mesh screen) fraction, then the infection percentage decreased. This result might be expected since the largest soil particles can contain numerous propagules, some of which will not be in contact with the root of the host. As the soil particle size decreases, more infective propagules will come in contact with the host as more soil surface will be exposed. Another way to state this is, more infective inoculum will be in a position to contact the host as the volume of each soil particle decreases, reducing its ability to mask propagules internally located in the soil particle. With the smaller soil fraction the masking effect may be expected to be reduced to a minimum.

The data show that infection falls rather sharply in soil fractions 0.074mm and smaller. This result indicates that the infective propagules are generally greater in size than 0.074mm, or that numerous smaller propagules associated with larger soil particles are necessary for successful infection. The pycnidia of P. terrestris are generally 120-450 mμ (17) or 0.120-0.450mm. There is the possibility that pycnidia are being separated from the smaller soil

particle fractions, but these propagules have been observed very rarely, except in culture. Certainly lack of observation does not rule out pycnidial implication as the infective propagule, but it does support the theory that another propagule is more strongly involved in infection. Though these data do not give direct support for determining the form of the infective propagule, they do show that using a soil fraction size of 0.5-1.0 mm will give a better measure of the infective potential associated with a soil sample.

Table 17. Average[1/] percent infection determined from seven soil particle size fractions. The 1:10 soil:sand dilution level was used with the Southport White Globe onion as the biological indicator.

Soil fraction size in mm.	Fraction	Malheur Exp. Sta. Soil Sample Location			Avg. % Infection
		East	West	West	
>1.168	/14	7	11	8	8.6
1.168	14/28	15	13	3	10.3
0.589	28/48	4	27	8	13.0
0.295	48/100	1	16	6	7.6
0.147	100/200	8	17	1	8.6
0.074	200/400	0	1	4	1.6
0.038	400/	2	3	1	2.0

[1/] Average determined from three replicates per soil particle size fraction.

Possibility of Contamination

To determine the significance of contamination with this method, an untreated soil sample was sieved and split into two samples. Each sample was diluted with quartz sand as before. After dilution, one dilution series was placed in plastic pots, the other was placed in pint canning jars, watered and autoclaved for one hour at 121°C and 15 psi. Each series was replicated five times as was a sand check potted in plastic pots. Onion seed was planted (SWG variety) several days later and all pots were watered with Hoagland's solution and covered with kraft paper.

Results showed that the sand represents a minor source of contamination (Table 18). The seed lot used in this work was not a source of contamination. The autoclaved soil:sand dilutions showed a low level of contamination and this possibly resulted from one or more propagules not being killed by the autoclave treatment. Since contamination occurred only once in sand and once in soil that had been autoclaved, both cases probably resulted from unclean pots, or from chance contamination.

Table 18. A comparison of autoclaved, non-autoclaved soil:sand and non-autoclaved quartz sand to evaluate the biological assay procedure. The Southport White Globe onion was used as the biological indicator.

	Test medium and Soil:Sand dilution level [1]									
		Non-autoclaved					Autoclaved			
Replicate	Sand only	1:10	1:100	1:1,000	1:10,000	1:100,000	1:10	1:100	1:1,000	1:10,000	1:100,000
1	0	38	20	8	3	0	0	0	0	0	0
2	0	40	18	4	0	0	0	0	0	0	0
3	0	18	27	4	0	0	0	10	0	0	0
4	0	11	16	8	13	0	0	0	0	0	0
5	3	23	10	0	0	0	0	0	0	0	0

[1] Figures show average percent infection.

Field Research

Methods of Soil Fumigant Application

Broadcast Fumigation - 1966 Studies

Non-residual Studies. Crop increases have been obtained with spring fumigation (22) but the practice is incompatible with current farming practices in the Snake River Valley. Soil temperatures are too low in the early spring to permit clearing of the toxic fumes from the soil in time for an acceptable early planting. Fall fumigation offered promise of fitting into cultural practice but the time interval between treatment, planting and especially harvest, seemed excessive. Initial trials conducted by Pack (51) and Vaughan and Fisher (72) gave erratic results.

Murata Test. To evaluate soil fumigation more thoroughly, a replicated trial was initiated in a field having a past history of pink root disease. Treatments were applied in a non-randomized design with four replicates (12' X 75') on September 31, 1965. Eight fumigants or combinations of them were shank injected at 40 gallons per acre with fumigation shanks set eight inches deep on eight inch centers. A John Blue, ground driven, positive displacement pump applicator was used.

Soil preparation prior to treatment consisted of pre-irrigating,

deep plowing, discing and harrowing to achieve a texture equivalent to a fine seed bed with uniform moisture. When the soil was treated, the soil temperature at a depth of six inches was 56°F at 10 A. M. Immediately after application, the soil in each plot was compacted with two passes of a cultipacker. The soil was not disturbed until the following spring when it was lightly disced before planting. Discing and furrow irrigation was done parallel to the long axis of the plots to minimize cross contamination. On February 2, 1966 the field was planted with approximately two pounds per acre of Yellow Sweet Spanish onion seed. Normal cultural practices were pursued with no regard to the fumigation treatments.

Evaluation on August 8 showed three compounds reduced disease significantly (Table 19). These were combinations of methyl isothiocyanate (MIT) and dichloropropanes-dichloropropenes (DD) (EP201, EP248, EP297, and Vorlex) and Telone plus chloropicrin. Telone, a material very similar to DD, and Vapam, a material with essentially all of its practical fungicidal activity associated with MIT, caused little or no decrease in disease level. Lanstan (1-chloro, 2-nitropropane) also caused little or no disease reduction. The extreme bulb size variation encountered made interpretation difficult if not meaningless. There appeared to be a trend of increased bulb size associated with compounds having a higher level of 1, 3-dichloropropene (1, 3-D).

Table 19. Effect of broadcast fumigation on Yellow Sweet Spanish onions.

	Bulb Ratings		Onion Yield (pounds)/10' of Row			
	Disease	Size				
Material	8/8	8/8	#1 Jumbo	#1 Medium	#2 Jumbo	#2 Medium
Check	3.87 g	5.62 d	11.47 cde	1.19 abcd	9.35 d	2.12 a
EP 201	2.81 ab	6.12 abc	14.87 ab	0.56 d	13.44 abc	1.56 abc
EP 248	3.25 cde	6.19 abc	14.65 ab	1.12 abcd	11.28 cd	1.44 abc
EP 297	2.50 a	6.44 a	11.19 de	0.62 cd	16.06 a	1.44 abc
Lanstan[1/]	3.62 efg	5.81 cd	11.90 bcde	1.40 a	9.53 d	1.65 abc
Lanstan	3.75 fg	5.81 cd	14.15 abcd	1.19 abcd	9.03 d	1.84 abc
Telone	3.69 fg	6.12 abc	14.37 abc	1.59 a	11.72 bcd	1.62 abc
Telone plus Chloropicrin (4:1)	3.19 bcd	6.25 ab	15.00 a	1.25 abc	12.19 bcd	1.15 bc
Vapam	3.69 fg	5.87 bcd	14.31 abc	1.12 abcd	12.62 abcd	1.75 abc
Vapam	3.44 cdef	6.06 abc	11.94 bcde	1.34 ab	11.19 cd	2.19 a
Vorlex	3.06 bc	6.19 abc	14.00 abcd	1.06 abcd	15.25 ab	1.06 c
LSD 10%	0.40	0.40	3.02	0.65	3.63	0.79

[1/] This Lanstan treatment was applied at 18 gallons per acre, all other treatments were made at 40 gallons per acre.

Harvest data collected September 9, 10, and 11, 1966 are presented in Table 19 as pounds of onions in the respective grades per ten feet of row, the actual area sampled. A detailed discussion of these data is not warranted since the results are so heavily influenced by the price obtained for the various grades. Three points are pertinent: (1) Most chemical treatments resulted in an increase in the #1 Jumbo category; (2) these increases were obtained in spite of the high level of variation; and (3) statistical analysis of yield data and disease and bulb size data showed no correlation between methods of evaluation with any combination of comparisons.

Only four materials are commercially available, and two, Lanstan and Vapam, were not acceptable at this time. Lanstan was considered uneconomical and the formulation of Vapam presented application problems. The yield data for Telone and Vorlex, the two most readily acceptable materials, were converted to tons per acre and the percentage of onions in each grade and the dollars that would have been returned on two market days for each of four years was determined (Table 20). Plots treated with Telone had a greater percentage of onions in the more desirable #1 Jumbo category. Treatment with both materials gave a high percentage of onions greater than three inches in diameter; Vorlex 91%, Telone 86%. The check plot yielded 84% Jumbo onions but total yield per acre was five tons less than from the Telone plots and seven tons less than the Vorlex

Table 20. Effect of broadcast soil fumigation on dollar return from Yellow Sweet Spanish onions. Both materials were applied at 40 gallons per acre.

Material	Onion Grade	% Onions in Grade	Tons/A in Grade	Per Acre Dollar Value of Crop on 1963 9-1	1963 12-31	1964 9-1	1964 12-31	1965 9-1	1965 12-31	1966 9-1	1966 10-4	4 yr. Avg Value
Untreated	1 jumbo	38.78	13.51	1080.80	1323.98	756.56	1269.94	675.50	540.40	945.70	972.72	
Check	1 medium	3.53	1.22	48.80	122.00	41.48	63.44	51.24	34.16	75.64	78.08	
	2 jumbo	45.78	15.94	605.72	0	414.44	733.24	382.56	350.68	541.96	573.84	
	2 medium	7.94	2.76	49.68	0	27.60	66.24	55.20	27.60	82.80	82.80	
	Culls	3.95	1.37									
Total		99.98	34.80	1785.00	1445.98	1240.08	2132.86	1164.50	952.84	1646.10	1707.44	1509.35
Vorlex	1 jumbo	43.60	18.34	1467.20	1797.32	1027.04	1723.96	917.00	733.60	1283.80	1320.48	
	1 medium	3.30	1.38	55.20	138.00	46.92	71.76	57.96	38.64	85.56	88.32	
	2 jumbo	47.50	19.98	759.24	0	519.48	919.08	479.52	439.56	679.32	719.28	
	2 medium	3.30	1.38	24.84	0	13.80	33.12	27.60	13.80	41.40	41.40	
	Culls	2.21	0.92									
Total		99.91	42.08	2306.48	1935.32	1607.24	2747.92	1482.08	1225.60	2090.08	2169.48	1945.77
Telone	1 jumbo	47.33	18.85	1508.00	1847.30	1055.60	1771.90	942.50	754.00	1319.50	1357.20	
	1 medium	5.23	2.08	83.20	208.00	70.72	108.16	87.36	58.24	128.96	133.12	
	2 jumbo	38.57	15.36	583.68	0	399.36	706.56	368.64	337.92	522.24	552.96	
	2 medium	5.33	2.12	38.16	0	21.20	50.88	42.40	21.20	63.60	63.60	
	Culls	3.49	1.39									
Total		99.95	39.83	2213.04	2055.30	1547.15	2637.50	1440.90	1171.36	2034.30	2106.88	1900.80

plots. The dollar values for the selected market periods reflect the interaction between total yield and percent in the various grades, with more dollars returned for large onions. These figures also point out the importance of large onions, since little or nothing is paid for small onions.

The comparison between Vorlex and Telone is continued in Table 21 which shows a more true dollar return by deducting the cost of the material. The four year average return and the lowest return during this period were selected because they reflect the average expectation and the extreme. Here it can be seen that for fumigation with Telone there is a sustained high return on investment while fumigation with Vorlex is less desirable. The high cost of Vorlex counter balances the increased yield obtained with its use. Telone, through its lower cost, returns more interest on investment even though it is less effective biologically.

Table 21. Summary of the effect of broadcast soil fumigation on dollar return from Yellow Sweet Spanish onions.

Treatment	Rate Gal./A.	Total Dollars/A. Based on the Avg. Yield		Change from Check After Material Cost Deducted	
		Four year Market		Four year Market	
		Low	Avg.	Low	Avg.
Check	--	953	1509	--	--
Vorlex	30	1225	1946	+72	+237
Telone	40	1171	1901	+150	+324

Residual Studies

Saito Test. Powelson (55) has shown that fumigation for Verticilium wilt control with Vorlex or Telone enhances potato yield for a period up to three years after application. It was of interest to determine if this same effect could be anticipated with onion pink root disease.

Telone and Vorlex were reapplied in September 1965 to a plot established in March 1965 by Dr. Powelson. Broadcast treatments (Table 22) were made perpendicular to Powelson's randomized strip plots. Results generally showed disease reduction was most strongly associated with Vorlex and increased bulb size associated with Telone (Table 23). Disease ratings made in July and August showed the spring and fall application of Vorlex at 30 and 30 gal/A significantly reduced disease and was better than all other treatments. Oddly, the fall treatment of Vorlex 30 gal/A was no better than the check. Telone treatments gave less disease control than some Vorlex treatments but were nearly always significantly better than the untreated check. On August 8 the disease level in the plots treated with Telone in the spring was not different from that in the check. Plots treated with Telone in the fall produced bulbs significantly larger than any other, as rated in July and August. Other Telone treatments as well as some Vorlex treatments increased bulb size early in the season, but

August bulb size ratings showed only the fall treatment with Telone was better than the check. Telone at 40 gal/A and Vorlex at 15 gal/A as spring treatments resulted in significantly reduced bulb size.

Table 22. Treatments used to evaluate carry-over effect of Telone and Vorlex for control of onion pink root disease.

Material	Rate Gal./A.	Time of Application	
		Spring	Fall
Telone	40	X	
Telone	40		X
Telone	40-40	X	X
Vorlex	15	X	
Vorlex	30	X	
Vorlex	30		X
Vorlex	15-30	X	X
Vorlex	30-30	X	X

Yield evaluations showed Vorlex applied at 30 gal/A in the spring significantly increased the yield of #1 Jumbos above all other treatments. Yield of #2 Jumbos was significantly increased by Telone treatments in the fall. All other treatments gave comparable results and all yielded better than the check. Combining #1 and #2 Jumbo yields the Telone fall treatment was slightly better than the best Vorlex treatment, though it was not significantly different. Yield analysis of medium onion grades showed the check had a greater weight in these catagories than other treatments.

Table 23. The long and short term effect of broadcast fumigation on Yellow Sweet Spanish onions.

Material	Rate Gal./A. and time Applied[1/]	Bulb Ratings: Disease 7/6	Disease 8/8	Size 7/6	Size 8/8	Onion Yield (pounds)/10' of Row: #1 Jumbo	#1 Medium	#2 Jumbo	#2 Medium
Check	-	2.4 e	4.1 de	2.2 c	5.1 bc	6.7 ab	2.4 a	6.5 c	6.4 ab
Telone	40S	2.0 c	3.7 d	2.6 b	4.2 e	7.7 ab	1.7 ab	8.6 bc	3.6 ab
Telone	40F	2.0 c	3.4 c	3.0 a	5.4 a	6.5 ab	1.8 ab	15.2 a	6.1 ab
Telone	40SF	1.9 c	2.4 b	2.6 b	5.3 ab	7.7 ab	1.0 ab	12.3 abc	3.5 ab
Vorlex	15S	2.0 c	3.0 c	2.0 c	4.7 d	6.3 b	2.0 ab	10.0 abc	3.9 ab
Vorlex	30S	1.5 ab	2.3 b	2.4 bc	4.9 cd	11.2 a	0.3 b	10.1 abc	3.3 b
Vorlex	30F	2.5 e	4.1 e	2.5 b	5.0 bcd	7.5 ab	2.0 ab	7.9 abc	4.5 ab
Vorlex	15S 30F	1.8 bc	2.2 ab	2.6 b	5.0 bc	6.2 b	0.7 ab	14.4 ab	6.6 a
Vorlex	30F 30S	1.3 a	2.0 a	2.6 b	4.9 cd	8.2 ab	2.2 a	9.8 abc	5.5 ab
LSD 10%		0.32	0.30	0.30	0.30	3.8	1.5	5.5	2.5

1/ S = Spring treatment
F = Fall treatment
SF = Spring and Fall treatments

Table 24 shows that the influence of the various treatments on dollars returned followed closely the biological data. Telone as a fall treatment yielded the largest return on investment, while Vorlex at 30 gal/A in the spring was second best. All other treatments resulted in losses.

From these results it may be concluded that (1) Telone at 40 gallons per acre does not have appreciable residual effect on pink root disease, while Vorlex at 30 gallons per acre may have; (2) Telone applied in the fall was approximately equal to any Vorlex treatment tested; and (3) Vorlex appeared to reduce pink root disease with some residual effect, while Telone seemed to have little or no direct effect on the disease. A reverse effect appeared to occur on the plants, i.e. Telone increased bulb size over the check, Vorlex did not. The result of the Vorlex 30 gal/A fall treatment is unexplainable at this time.

Broadcast Fumigation - 1967 Studies

Conclusions based on 1966 field results indicated that Telone applied in the fall at 40 gal/A was most promising and should be tested further. Vorlex at 30 gal/A applied in the spring or fall was not commercially practical and therefore lower than recommended fungicidal rates would have to be examined or the material eliminated from further study. It was decided to examine Vorlex further at a

Table 24. Summary of long and short term effect of broadcast soil fumigation on dollar return from Yellow Sweet Spanish onion.

Material	Time and Date of Application	Rate/A Gallons	4 Year Avg. Return/A.	Change[1] From Check After Material Cost Deducted	Lowest Return in 4 Years 12-31-65	Change[1] From Check After Material Cost Deducted
Telone	Fall 9-65	40	1433.17	+157.65	929.70	+ 72.79
Telone	Spring 3-65	40	1305.48	- 50.04	846.44	- 90.47
	Fall 9-65	40				
Telone	Spring 3-65	40	1258.07	- 17.45	766.84	- 18.07
Vorlex	Spring 3-65	15	1184.64	-125.88	746.80	-145.11
Vorlex	Spring 3-65	15	1343.54	-176.98	856.94	-244.97
	Fall 9-65	30				
Vorlex	Spring 3-65	30	1513.63	+ 78.11	1018.54	+ 21.53
Vorlex	Fall 9-65	30	1266.61	-148.91	785.78	-211.13
Vorlex	Spring 3-65	30	1409.06	-226.46	892.18	-324.73
	Fall 9-65	30				
Untreated Check			1195.52		776.91	

[1] Cost of materials and application were figured as follows: Telone 40 gpA = $ 70
Vorlex 15 gpA = $105
Vorlex 30 gpA = $210

rate that would place it on a dollar par with Telone. This decision was not entirely dollar oriented since MIT bearing materials had shown a capability to reduce the disease and Telone had not. Also, there were indications that Vorlex at 30 gal/A in the fall, the most practical time to fumigate, may be too high a dosage, resulting in marginal phytotoxicity.

With these considerations, three tests were initiated with application conditions very similar, if not identical, to the 1966 field trials. Telone at 40 gal/A and Vorlex at 10, 15, 20 and 30 gal/A applied in the fall were compared, using Southport White Globe (SWG) and Yellow Sweet Spanish onions. A comparison was also made between DD (approximately 65%, 1, 3-D) and Telone (guaranteed to be 85% or more of 1, 3-D) to determine the effect of relative levels of 1, 3-D on bulb size.

Nakada Test. Root disease ratings made July 19 on SWG onions showed no treatment reduced pink root disease but that significantly more disease was associated with the Telone 40 gal/A and Vorlex 15 gal/A treatments (Table 25). Similar readings made August 8 showed that disease was more severe as a result of treatment since all treatments resulted in significantly higher disease indices than the check. Bulb size index readings made July 19 showed all treatments except the highest Vorlex rate promoted some bulb size increase. August 8 readings however, showed that no differences in bulb size

Table 25. The effect of broadcast fumigation on Southport White Globe onions, treated in 1967.

Material	Rate Gal./A.	Bulb Rating: Disease 7/19	Disease 8/8	Size 7/19	Size 8/8	Yield (pounds)/10' of Row: #1 Jumbo	#1 Medium	#2 Jumbo	#2 Medium	Cull
Check	--	3.1 a	4.5 a	3.3 b	4.8 a	1.8 b	3.3 a	1.8 bc	1.8 ab	0.5 ab
Telone	40	3.7 b	4.8 b	3.4 ab	4.7 a	2.0 b	3.6 a	2.5 ab	1.4 b	0.4 b
Vorlex	10	3.0 a	4.8 b	3.4 ab	4.8 a	2.3 ab	3.3 a	1.8 c	2.7 a	0.7 a
Vorlex	15	3.6 b	4.9 b	3.6 a	4.8 a	2.9 a	3.6 a	2.2 abc	1.6 b	0.4 b
Vorlex	20	3.1 a	4.8 b	3.3 b	4.8 a	2.8 a	3.5 a	2.7 a	2.0 ab	0.3 b
LSD 10%		0.42	0.32	0.18	0.47	0.66	0.84	0.72	1.00	0.21

were present.

Higher yields of #1 Jumbo onions were associated with Vorlex treatments. This trend was also evident with the #2 Jumbo size; the lower rates had lower yields in this grade category. Telone fumigation promoted yield of more #2 Jumbo onions than the check but not significantly more.

All treatments, except Vorlex at 15 gal/A on the lowest market, resulted in a return on investment (Table 26). Vorlex at 20 gal/A made the best return followed by Telone at 40 gal/A and Vorlex at 10 gal/A which were equal. Vorlex at 15 gal/A gave the poorest return. Considering the higher investment required by the higher rate and the increased capital risk associated with it, the lower Vorlex rate and Telone are probably best.

Teramura Test. Root disease index ratings made on YSS onions on July 19 showed disease reduction associated with lower Vorlex rates but none with Telone or the 30 gal/A Vorlex treatment (Table 27). On August 8, disease reduction was most strongly associated with the highest rates of Vorlex with a slight reduction from Telone treatment. Bulb size indices on July 19 were only slightly different, with Vorlex at 15 gal/A better than all other treatments. The later readings showed little difference, with Vorlex at 10 gal/A showing the smallest onions.

Yield analysis showed that the check had more #1 Jumbo onions

Table 26. The influence of broadcast fumigation treatments on dollar return to the grower. The prices used were obtained from the past four years for Southport White Globe onions.

Treatment	Rate Gal./A.	Total Dollars/A. Based on Avg. Yield			Change from Check After Material Cost Deducted		
		Low	High	Avg.	Low	High	Avg.
Check	-	516	921	758	--	--	--
Telone	40	592	1048	861	+ 8	+59	+35
Vorlex	10	586	1042	857	+ 1	+52	+30
Vorlex	15	597	1048	862	-22	+24	+ 1
Vorlex	20	683	1204	990	+29	+145	+94

Table 27. The effect of broadcast fumigation on Yellow Sweet Spanish onions, treated in 1967.

Treatment	Rate Gal./A.	Bulb Rating: Disease 7/19	Bulb Rating: Disease 8/8	Bulb Rating: Size 7/19	Bulb Rating: Size 8/8	Yield (pounds)/10' of Row: #1 Jumbo	Yield (pounds)/10' of Row: #1 Medium	Yield (pounds)/10' of Row: #2 Jumbo	Yield (pounds)/10' of Row: # 2 Medium
Check	--	2.6 b	4.5 c	4.4 b	5.5 ab	13.1 a	0.3 ab	9.8 c	1.8 ab
Telone	40	2.8 c	4.4 bc	4.5 b	5.6 ab	10.5 bc	0.5 a	15.8 ab	1.4 b
Vorlex	10	2.2 a	4.5 c	4.2 cd	5.4 b	12.0 ab	0.4 ab	12.6 bc	2.7 a
Vorlex	15	2.1 a	4.6 c	4.6 a	5.6 ab	12.1 ab	0.2 ab	15.7 b	1.6 b
Vorlex	20	2.1 a	4.3 b	4.3 bc	5.6 ab	9.6 c	0.3 ab	18.6 a	2.0 ab
Vorlex	30	2.6 b	4.0 a	4.2 cd	5.7 a	12.4 ab	0.1 b	12.9 bc	1.9 ab
LSD 10%		0.24	0.17	0.13	0.22	1.93	0.32	3.54	0.94

and Vorlex at 20 gal/A had the least (Table 27). Vorlex at 20 gal/A and Telone yielded more #2 Jumbo onions while the check had the least. Little difference existed between treatments in the medium grade categories. Several treatments yielded more #1 mediums but others yielded more in the #2 grade so that the combined Jumbo grade totals showed little change.

Analysis of return on investment showed that Vorlex at 15 gal/A returned more than other treatments in most cases, but was not enough better than Telone or Vorlex at 10 gal/A to warrant the additional material cost (Table 28). Telone had a greater return than Vorlex at 10 gal/A on all markets making it the preferred treatment.

Wada Trial. Readings early in the season indicated that DD reduced pink root disease but by August 8 there were no differences in disease ratings between plots fumigated with DD and Telone (Table 29). On the other hand harvest evaluations showed greater yields of #1 and #2 Jumbo onions in the Telone treated than in the DD treated area.

Results obtained from the above trials indicate that Telone exerts little or no effect on P. terrestris or on pink root disease, while Vorlex at higher rates may. Reduced rates of Vorlex appear to exert little effect on the fungus or on the disease. Bulb size increases seem to be associated more strongly with Telone but

Table 28. The influence of broadcast fumigation treatments on the dollar return to the grower. The prices used were obtained from the past four years for Yellow Sweet Spanish onions.

Treatment	Rate Gal/A	Total Dollars/A Based on Avg. Yield			Change from Check After Material Cost Deducted		
		Four year market			Four year market		
		Low	High	Avg.	Low	High	Avg.
Check	--	764	1913	1339	--	--	--
Telone	40	862	2168	1520	+30	+187	+113
Vorlex	10	836	2120	1473	+ 3	+138	+ 65
Vorlex	15	909	2271	1595	+42	+255	+153
Vorlex	20	930	2342	1640	+28	+291	+163
Vorlex	30	844	2097	1468	-127	- 23	- 78

Table 29. A comparison of Telone with DD as a means of comparing different levels of 1, 3-dichloropropene for its effect on Yellow Sweet Spanish onion yields when both materials are applied at 40 gal/A as broadcast fumigation treatments.

	Bulb Rating				Yield (pounds)/10' of Row			
	Disease		Size					
Treatment	7/19	8/8	7/19	8/8	#1 Jumbo	#1 Medium	#2 Jumbo	#2 Medium
Telone	1.4	2.4	3.0	4.7	18.1	0.5	9.4	0.7
DD	1.2	2.5	2.0	4.2	13.9	1.9	4.0	0.9
LSD 10%	0.1	0.5	0.1	0.1	0.4	4.1	0.5	1.9

considerable variation was apparent. Telone gave a significantly greater increase in bulb size than DD, indicating the amount of 1,3-D plays a direct role in plant vigor if not in disease control.

Some, if not all, of the discrepancy between 1966 and 1967 results may be explained on the basis of climate. The 1966 season progressed in a normal way with spring temperatures increasing gradually to the normal high summer temperatures. The 1967 spring-summer temperature transition, however, was atypical. The early season was very cool, the temperature remaining low until about June 16 at which time an abnormally sustained hot period developed. This resulted in slow plant growth early in the season and in water stress later. These conditions reduced the early growth surge usually associated with fumigation, and particularly Telone treatment.

Pre-formed Bed Fumigation

Murata Test. Fumigation of pre-formed beds was undertaken as an effort to retain the efficacy of broadcast treatment but to reduce the material cost and therefore increase the attractiveness of the practice to growers. Since with pre-formed bed treatment only the area under the row is treated, the potential saving is substantial in comparison with broadcast treatment which treats all of the soil.

Pre-formed bed treatments were established in the same field

as the broadcast test, using the same application equipment. Seven fumigant materials were shank injected into the soil, at the same rate per shank as for 40 gallons per acre broadcast, directly under the row to be planted the following spring. Material placement was 8-10 inches below the finished bed level. Shank spacing was 18 inches between the two rows on each bed and 22 inches between bed centers (18"-22"-18"), the same spacing used for planting rows on the beds. Beds were formed by mounting listers and injector shanks on the same tool bar. The depth at which the guide fins were set was below the disturbed soil level. Movement of the fins through the undisturbed soil left a slot in the soil into which the same fins mounted in the spring on the seeder tool bar would reposition themselves and the seeder for precise seed placement, relative to fumigant placement.

Immediately after soil fumigation and bed formation, the beds were rolled twice with a smooth roller to pack the soil and seal the fumigant vapors into the soil. Normally, use of a smooth roller to pack soil after fumigation is not desirable because maximum compaction occurs on the high points and little or none in the lower areas. For pre-formed beds, however, packing of the raised areas is particularly desirable since this is the only area treated. Also, use of a cultipacker roller breaks down the beds which defeats the purpose of forming them. The surface of the beds was harrowed lightly February 2, 1966 to break the soil crust and planted. As in the rest

of the field, normal cultural practices were followed without regard to the treatments.

Readings on July 6 showed that fumigants containing the largest amounts of 1, 3-D reduced disease most, while compounds having a combination of 1, 3-D and MIT reduced disease less but significantly more than the 1-chloro, 2-nitropropane which was no better than the check (Table 30). A second root reading made August 8 showed Telone combined with chloropicrin significantly reduced disease.

It is interesting that EP201 did not perform as well as the Telone-chloropicrin even though these compounds are similar in composition. EP201 had 6, 30 and 4 gal/A of chloropicrin, DD and MIT respectively, while Telone-chloropicrin had 8 and 32 gal/A of chloropicrin and Telone respectively. DD and Telone are essentially the same material except that Telone contains approximately 25% more 1, 3-dichloropropene.

Bulb size ratings showed treatments containing chloropicrin in combination with 1, 3-D, with or without MIT, induced significant bulb size increase earlier than any other treatment. Telone exerted an effect nearly equal to the Telone-chloropicrin combination and EP201. EP297 was less effective. The second bulb size reading showed all treatments were comparable; whatever effect was exerted by the better treatments early in the season was not sustained.

Onion yield measurements were made at the same time as

Table 30. The effect of pre-formed bed fumigation on the yield of Yellow Sweet Spanish onions.

	Bulb Rating				Yield (pounds)/10' of Row			
	Disease		Size					
Treatment	7/6	8/8	7/6	8/8	#1 Jumbo	#1 Medium	#2 Jumbo	#2 Medium
Check	2.7 e	3.9 c	3.2 a	5.7 ab	13.1 de	3.0 a	6.7 a	3.2 a
EP 201	2.1 bcd	3.4 bc	4.0 ed	5.6 ab	13.0 e	0.9 d	5.4 a	1.5 b
EP 248	2.4 cde	3.9 c	3.5 abc	5.3 b	13.2 de	1.2 cd	5.6 a	0.5 f
EP 297	2.5 de	3.7 bc	3.6 abcd	5.5 ab	13.9 cde	0.7 d	7.1 a	0.6 de
Lanstan	2.6 e	3.7 c	3.5 abc	5.8 ab	14.3 bcde	1.7 b	6.9 a	0.7 d
Telone	2.0 bc	3.2 abc	3.9 d	5.9 ab	17.2 a	1.1 cd	6.2 a	0.5 ef
Telone + Chloropicrin (4:1)	1.5 a	2.5 a	4.5 e	6.0 a	16.8 ab	0.3 e	7.7 a	0.0 h
Vorlex	2.5 e	3.3 bc	3.4 ab	5.9 ab	15.9 abcd	1.5 bc	5.5 a	0.1 g
Vorlex	2.3 cde	3.1 ab	3.5 abc	6.0 a	16.2 abc	1.8 b	5.9 a	1.0 c
LSD 10%	0.43	0.69	0.46	0.62	2.84	0.45	2.80	0.12

broadcast treatments. Telone, Telone-chloropicrin and both Vorlex plots yielded significantly more #1 Jumbo onions, while plots treated with the other materials were no better than the check. Telone treatment was superior to all others in this grade category. There were no differences between treatments in the #2 Jumbo category. The untreated check had significantly more onions in the smaller medium grades.

Dollar return figures were computed only for Telone and Vorlex (Table 31). Telone treated plots placed a higher percentage of onions in the Jumbo category than did those treated with Vorlex. This fact combined with a greater total yield made Telone the preferred treatment. Other figures show how market demand affects the dollar return. Table 32 shows the relative return achieved with the respective treatments, after material costs were deducted. Telone returned more than 100% on investment from the four year average market value and the lowest market during the sample period. Vorlex however caused a loss of dollars under both market conditions.

Mid-season Below-the-row Fumigation - 1965 Studies

A different approach to onion soil fumigation was tested in 1965. An L-shaped shank, used in conjunction with a logarithmic sprayer, was designed to place fungicides or fumigants under the row during the growing season. The main purpose of the initial trials was to

Table 31. Effect of pre-formed bed soil fumigation on dollar return from Yellow Sweet Spanish onions. Both materials were applied at 40 gallons per acre.

Material	Onion Grade	% Onions in Grade	Tons/A in Grade	Per Acre Dollar Value of Crop on 1963 9-1	1963 12-31	1964 9-1	1964 12-31	1965 9-1	1965 12-31	1966 9-1	1966 10-4	4 yr. Avg Value
Untreated Check	1 jumbo	50.68	17.39	1391.20	1704.22	973.84	1634.66	869.50	695.50	1217.30	1252.08	
	1 medium	8.63	2.96	118.40	296.00	100.64	153.92	124.32	82.88	183.52	189.44	
	2 jumbo	28.68	9.88	375.44	0	256.88	454.48	237.12	217.36	335.92	355.68	
	2 medium	8.14	2.79	50.22	0	27.90	66.96	55.80	27.90	83.70	83.70	
	Culls	3.66	1.26									
Total		99.89	34.28	1935.26	2000.22	1359.26	2310.02	1286.74	1023.64	1820.44	1880.90	1702.06
Vorlex	1 jumbo	64.18	20.51	1640.80	2009.98	1148.56	1927.94	1025.50	820.40	1435.40	1476.72	
	1 medium	5.76	1.84	73.60	184.00	62.56	95.68	77.28	51.52	114.80	117.76	
	2 jumbo	22.28	7.12	270.56	0	185.12	327.52	170.88	156.64	242.08	256.32	
	2 medium	3.94	1.26	22.68	0	12.60	30.24	25.20	12.60	37.80	37.80	
	Culls	3.80	1.21									
Total		99.96	31.94	2007.64	2193.98	1408.84	2381.38	1298.86	1041.16	1826.66	1888.60	1755.89
Telone	1 jumbo	65.63	22.58	1806.40	2212.84	1264.48	2122.52	1129.00	903.20	1580.60	1625.76	
	1 medium	4.38	1.51	60.40	151.00	51.34	78.52	63.42	42.28	93.62	96.64	
	2 jumbo	23.79	8.18	310.84	0	212.68	376.28	196.32	179.96	278.12	294.18	
	2 medium	2.59	0.89	16.02	0	8.90	21.36	17.80	8.90	26.70	26.70	
	Culls	3.47	1.19									
Total		99.86	34.35	2193.66	2363.84	1534.40	2598.68	1406.54	1134.34	1979.04	2043.58	1906.76

determine whether the shank could be used without excessive injury to the growing plants and whether selected compounds would give disease reduction without phytotoxicity.

Table 32. Summary of the effect of pre-formed bed soil fumigation on dollar return from Yellow Sweet Spanish onions.

Treatment	Rate Gal./A.	Total Dollars/A. Based on the Avg. Yield — Four year Market		Change from Check After Material Cost Deducted — Four year Market	
		Low	Avg.	Low	Avg.
Check	--	1023	1702	--	--
Vorlex	30	1041	1756	-67	-31
Telone	40	1134	1907	+70	+164

Two sites were selected, one field having just been irrigated and the other to be irrigated immediately after fumigation, thus representing the extremes of conditions likely to be encountered. Two shanks were mounted on the tractor tool bar to give a six inch treatment depth under each of two beds. In this way, two rows of onions were treated, one row on each of two beds, leaving one row on each bed untreated. A logarithmic sprayer was mounted on the tool bar and connected to the delivery tube of each shank to make the treatments. For the wettable powder or emulsifiable formulations, water was used as the diluent. Diesel fuel was used for the non-emulsifiable fumigant compounds, e. g. Telone and Vorlex.

The initial rate was eight pounds actual per acre for Demosan, Daconyl and Lanstan. Telone and Vorlex had initial rates of 14 gallons per acre. The half-rate length of the logarithmic sprayer was 23.7 feet and the non-replicated plots were 100 feet long allowing four full half-rate dilutions.

Prior to the use of the shanks, onions were removed with a shovel from numerous places in several fields in a way to minimize root disturbance. These evaluations showed that approximately 80% of the root system was concentrated above the six inch level. Strydom (64) has shown that onion root systems will penetrate at least to the two foot level, with 80% of the roots occurring in the top 12 inches of a sandy loam soil. In most fields examined in the present work however, there was a definite compaction layer at about three inches. Because of this layer, most roots developed laterally, with only a few roots (ca. 20%) growing below the six inch level.

Some bulb size reduction was caused by the shank. Diesel oil alone had no apparent effect on the onions. Neither Demosan nor Daconyl influenced bulb size or the disease level. Lanstan at the higher rates caused no phytotoxicity and appeared to reduce disease. Lower rates, below four pounds, had no affect. Vorlex at ten gallons per acre promoted uniform bulb size and root development but did not overcome the reduction in bulb size caused by disturbance of the roots. Rates higher than ten gallons per acre were phytotoxic. Telone caused

no phytotoxicity, indicating higher rates could be used.

It was concluded that there may be some potential merit in this method of application of fungicides, and that of the materials tested, only those having a low vapor pressure offered greater movement potential in the soil, and should be studied further.

Mid-season Below-the-row Fumigation - 1966 Studies

P. terrestris causes disease only after soil temperatures approach 80°F. And, treatments, to be effective, must be made before the fungus enters the roots.

In 1966 a U shaped bar was used to overcome problems associated with torque that occurred with the previous shank. Seven compounds, all possessing fumigant properties were applied with a logarithmic sprayer on May 21. Two, low pressure, high volume Tee-jet nozzles were positioned on this blade, one under each row. A complete bed with two rows per bed was treated with an initial gallonage of 18.4 gallons per acre. However, the sprayer-nozzle combination delivered 72 gallons per acre. In order to obtain the desired initial fumigant gallonage, the centrifigal pump housing the sprayer was filled with diesel fuel, purged of air and a measured amount of fuel withdrawn. The pump housing was then refilled with the required amount of toxicant and again purged of air. Each plot was 50 feet long and replicated three times. The half-rate distance

was 8.6 feet so the following half-rates were applied for each material: 9.2, 4.6, 2.3, 1.2, 0.6, and 0.3 gal/A.

At 5 P.M. the soil temperature at six inches was 70°F. The soil was in a good moist condition having been irrigated four days previously. The onions were about eight inches high and showed no indication of beginning to bulb.

A second application was made in the same field on June 14. These treatments were made under beds adjacent to those previously treated and were identical to the earlier applications. The field had been irrigated 14 days earlier but moisture was still good near the surface and was excellent at the six inch level. The 5 P.M. soil temperature at six inches was 78°F. The onions were just beginning to bulb, with the average diameter about 1/2 inch. The tops were approximately 18 inches high. Random root evaluations made across the field at this time showed 40% of the plants had very faint indications of pink in their root systems. Only a very few roots were severely infected.

Twenty four hours after treatment and again about three weeks later, phytotoxicity of the chemicals was judged by observing wilting of the leaves. The maximum rate tolerated initially or after three weeks, in which time some recovery had occurred, was determined by measuring the distance between the initial point of application and the tolerance point. These distances were then converted to gallonages

from Figure 7. Harvest evaluations of root disease and bulb size were made at 10, 20, and 30 feet from the initial point. To compensate for rate differences associated with the method of application, five plants from each side of the measured rate were examined.

Table 33 shows the distance from the initial point to the point at which there was no apparent effect, and the gallonage is indicated. The dosage of each chemical from which onions recovered from initial phytotoxicity is also shown. Table 34 shows disease and bulb indices obtained. The data indicate that use of the treatment blade resulted in very little damage to the crop.

Lanstan caused no phytotoxicity and had no apparent effect on the disease or bulb size. These data support unpublished results (56) that Lanstan moves very little in the soil. The highest gallonage rate read was equivalent to 73.6 pounds of active ingredient per acre, which on many other crops would be highly phytotoxic if the material contacted the plants (61).

Vorlex applied in May caused initial wilting but no permanent phytotoxicity as measured by bulb size at harvest time. Recovery appeared complete about three weeks after treatment. Disease reduction was effected as was some bulb size increase through the lowest dosage for which readings were made. The June application was phytotoxic initially and recovery was not as complete as that occurring after the earlier treatment. This is supported by harvest

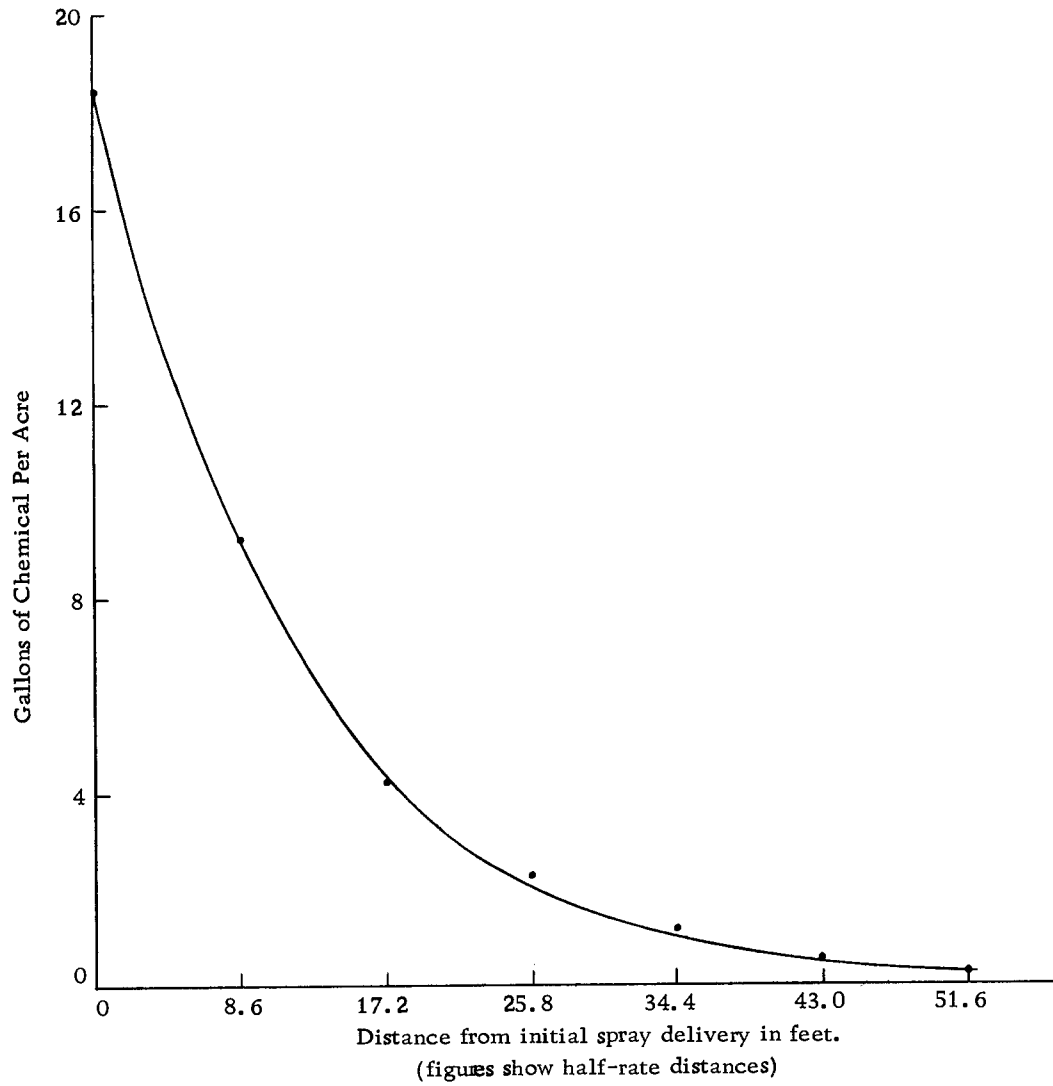

Figure 7. Standard curve used to determine rate of application in conjunction with the logarithmic sprayer applications.

Table 33. Mid-season evaluation of fumigants applied below the onion rows on May 16 and June 14, showing the distance from the initial application point that onions showed no chemical injury from treatment and the maximum rates from which treated plants recovered.

	Observation Date							
	June 14				July 6			
	Tolerant		Recovered		Tolerant		Recovered	
Treatment	Distance[1]	Rate[2]	Distance	Rate	Distance	Rate	Distance	Rate
Lanstan	0	18.4	-	-	0	18.4	-	-
Vorlex	31	1.4	6	11.8	26	2.2	11	7.2
Chloropicrin	43	0.6	32	1.4	32	1.4	50	0.0
Telone	12	6.6	0	18.4	35	1.2	21	3.2
Telone + Chloropicrin (4:1)	56	0.1	11	7.2	28	1.8	13	6.0
Telone PCB	24	2.5	NO READING		32	1.4	9	8.8
Vapam	9	8.8	None		9	8.8	None	

[1] Distance shows point where onions no longer showed chemical injury.

[2] Rates shown are maximum tolerated.

Table 34. Harvest evaluation of fumigants applied below the onion row. Root disease and bulb size indices were obtained as before and are shown in this table, the index of each treatment compared with a corresponding check reading.

Rate Gal./A.	Date Treated	Materials											
		Lanstan		Vorlex		Chloropicrin		Telone + Chloropicrin (4:1)		Telone		Vapam	
		Disease	Size	Disease	Size	Disease	Size	Diseases	Size	Disease	Size	Disease	Size
8	Check	3.7	5.9	4.0	5.5			2.6	6.1	4.2	5.1	4.3	5.6
	5/21	3.0	6.4	2.3	6.7	p*	p	3.5	6.2	4.1	6.2	3.1	6.7
	Check	3.9	5.7	2.9	5.6			3.9	6.8	3.9	4.4	2.5	5.0
	6/14	4.3	5.1	p	p	p	p	4.5	3.5	p	p	4.1	6.8
3.5	Check	3.9	6.1	3.6	4.6			4.7	5.9	4.9	4.8	4.4	5.1
	5/21	3.5	5.2	2.3	5.9	p	p	3.5	5.7	4.8	5.9	2.7	7.0
	Check	3.2	4.9	2.9	6.6			4.6	6.4	4.5	4.7	3.2	6.7
	6/14	3.3	5.3	2.2	6.6	p	p	3.5	4.8	p	p	2.6	5.3
1.6	Check	3.7	6.5	4.2	5.9			3.7	6.2	4.5	5.4	5.0	5.4
	5/21	3.2	6.3	2.6	6.5	p	p	3.6	5.9	4.1	6.0	2.8	6.3
	Check	4.4	5.5	3.1	5.6			4.5	5.9	4.1	5.5	3.7	5.2
	6/14	4.6	5.3	3.1	6.7	p	p	4.5	5.6	4.4	4.1	2.4	6.7

* P = Treatment was phytotoxic, most plants dead or severely stunted.

readings. Onions that received the eight gallons per acre treatment never did recover. Disease control was not evident at lower rates, and little bulb size increase resulted. These results may be explained on the basis of the entry of P. terrestris before treatment and the shortened time for plant recovery between treatment and harvest.

Chloropicrin was the most phytotoxic of the materials tested, causing severe and, for the most part, permanent injury. Following the earliest treatment some recovery was observed, but with the later treatment no recovery was noted. No harvest data were recorded.

Telone caused an initial shock but the onions recovered from the effects of the first treatment. There was no apparent disease control but there was an increase in bulb size. The second treatment resulted in much more severe phytotoxicity although some recovery occurred. No disease control was observed and no increased bulb size was indicated.

The first application of Telone-chloropicrin (4:1) combination resulted in severe initial phytotoxicity but some recovery resulted. There was an increased disease reading on treated onions, indicating plant injury or creation of a favorable environment for the pathogen, but bulb size was not affected. The second treatment caused less initial phytotoxicity but recovery was about the same. At the highest rate the disease index was higher, as in the early treatment. A

slight disease reduction was encountered at the median rate, with no effect at the low rate. Bulb size was depressed at all rates evaluated, indicating phytotoxicity rather than enhancement of the pathogen.

These results show that a stunting effect occurred which could be due to a sublethal effect on the roots, or an effect on root regeneration. Wilting symptoms were not as severe with this treatment as with others.

Reaction to Telone-PBC treatment was similar to that for Vorlex, Telone and chloropicrin but the area of the field where these plots were located was disturbed, making accurate readings impossible.

Vapam caused little phytotoxicity, but what did occur was non-reversible. The first treatment resulted in encouraging disease reduction and a consistant increase in bulb size at all rates. The second application resulted in disease reduction at all but the highest rate which is unexplainable, unless one would do so on the basis of marginal phytotoxicity. Marginal toxicity could mask fungicidal effects of fumigation. Bulb size was increased by two of the three rates.

With few exceptions late treatments caused greater injury and fewer of the injured plants recovered. This may be accounted for by any of the following factors. Following the second treatment the

plants had about one month less time to recover from the setback associated with application. The soil was drier when the late application was made, allowing more rapid root exposure and reducing the vapor holding power of the soil below the onion roots. The soil temperature was eight degrees higher when the late treatments were made, allowing more complete volatilization of the material in a shorter period, increasing the short term toxicant concentration. Increased root disease in plots that received the later treatments can be explained on the basis of pathogen entry prior to treatment. Difference in root and plant development would also exert an undetermined effect and increased foliage of the larger onions would greatly increase water loss.

Based on results of early applications, Vapam would merit further study. Telone and perhaps Lanstan would be worth further evaluation. This approach appears to have merit if no other alternative exists. The initial phytotoxicity associated with certain treatments would present a definite disadvantage, but onions will survive mid-season treatment. More importantly, with additional effort this method could possibly be made to work effectively.

DISCUSSION

One of the objects of this study was to determine if there were differences in the mode and level of fungal penetration into roots of onion lines suited to Oregon growing conditions. The experimental results show that at least three levels, if not types, of resistance are present. The onion variety A. fistulosum resists colonization or penetration. The extent of resistance demonstrated suggests true immunity. The inability of the fungus to colonize the tissue indicates lack of required exudates or perhaps even release of an inhibitor. Since all nutrients essential for growth and reproduction of this isolate were supplied in the media, it is unlikely that exudate deficiency accounts for the failure of the fungus to colonize roots of this species. Synthesis of an inhibitor by A. fistulosum could explain the complete lack of fungal colonization observed, which was in strong contrast with all other onion lines.

There is little evidence of an endogenous inhibitive substance in the epidermal walls. Hess (22), by treating resistant onion roots with lethal levels of propylene oxide, showed that tissue resistant before treatment is susceptible to colonization after it is killed. The author produced a similar effect with A. fistulosum roots treated with lethal levels of sodium hypochlorite, when the treated tissue was placed on agar inoculated with a pathogenic isolate of P. terrestris.

These results are indicative of a living resistance mechanism rather than the impregnation of epidermal cells with a fungitoxic compound. The possibility of a toxic exudate is the most likely explanation at this time for resistance of A. fistulosum to P. terrestris.

A second form of resistance to P. terrestris was shown to occur in several onion lines tested and was similar in nature to that demonstrated by Struckmeyer et al. (63) with Texas Hybrid 28 and Excel. This was a biochemical form of resistance that was active after mycelial entry into the epidermal cells. External colonization occurred on all onions with this type resistance suggesting no differences in this respect. Colonization characteristics were similar to those reported by Kreutzer (32). Once epidermal walls were penetrated the individual hyphae were stopped without any external morphological changes. Onion line #223 exerted the strongest level of resistance followed by #36 and Ia2997B. The genetic backgrounds of these three breeding lines are apparently quite diverse, with one from private industry, one based on new plant introductions of the U. S. D. A. and the other based on the Idaho state breeding program. A study of correlation between the level of root penetration in various resistant vs. susceptible genetic crosses could offer guidance to the onion breeder.

A third type of response to invasion was found in this series of experiments, that of tolerance. The response appeared to be total

tolerance to mycelial invasion by P. terrestris into the roots of the Yellow Sweet Spanish onion line. During the time of these tests, hyphae were apparently able to move in any direction and into any tissue except the xylem vessels. Even with massive invasion the young plants showed no evidence of wilting, in fact new roots were generated during the relatively short duration of the tests. Certainly energy was being directed to root development that otherwise would have gone to more balanced plant growth in the absence of disease. However, the high level of vigor was not visibly reduced by the massive infection present. Evidently, lack of penetration of xylem vessels allowed sufficient root function to meet plant needs. This plant reaction to the entry of the pathogen would form a reasonable basis for the Sweet Spanish variety being considered only moderately susceptible as far back as 1933 (54) instead of susceptible.

In susceptible lines there was rapid and nearly complete collapse of infected roots, but as before the xylem vessels were never seen to be penetrated. This reaction was similar in appearance to what occurred in the YSS tissue but the plant's reaction was entirely different since the susceptible plants collapsed within a short time after inoculation.

It was interesting that Ia2997B was the only onion line that showed evidence of pink color. As Kreutzer (32) observed previously, pink color was associated only with the mycelium. Perhaps color

would have diffused into the cell if the experiment had been continued longer. Appearance of color in such a short time is indicative that symptom expression can occur shortly after infection but it appears that symptom expression is related to the onion line itself and perhaps also to the extent of infection. Lack of color in roots of onion lines strongly infused with mycelium proves a definite time lag occurs between infection and appearance of color. In agar culture, color is most strongly associated with areas habited by older mycelium.

According to Eschrich and Currier (11), florescence in U. V. irradiated tissue treated with cotton blue is indicative of the presence of callose. Under U. V. light, florescence appeared to be correlated with resistance, since the strongest florescence was associated with A. fistulosum and decreased florescence was observed as resistance decreased. This suggests resistance based on a greater callose synthesis ability in resistant onion lines. An increased callose synthesis ability may explain results obtained by Struckmeyer et al. (63) who mention observing a stretching and thickening of the epidermal cell walls under infection pegs, thus preventing entry. They also mentioned the presence of what appeared to be enlargements over the ends of infection pegs penetrating into epidermal cells.

During observations of many infected root tissues from eastern Oregon, no pycnidia were ever observed, yet it was a rare infected root that was void of what Kreutzer (32) called "pycnidial primordia"

(after Hansen). In only two cases in the literature are pycnidial primordia reported to have given rise to pycnidia. In one instance pycnidial formation was associated with a specific isolate (32); Struckmeyer et al. (63), mention "a knot-like structure which later developed into a pycnidium. The cells within the pycnidium were short and rounded." Since no mention was made of the presence of characteristic setae on the pycnidium, the question arises as to whether these were not pycnidia-like bodies encountered by Kulik and Tims (35). In the current work many pycnidial primordia were observed in diseased roots from the field and a few were observed in the young root tissue of the Ia2997B onion line grown in culture. Their occurrence in the young root tissue was as a result of invasion by an isolate of P. terrestris selected because of the ease with which it formed characteristic pycnidia. It would seem that use of the term microsclerotia would better describe these mycelial agglomerations than the many terms now used. The term pycnidia could then be reserved to describe the structure bearing characteristic setae and spores. According to Ainsworth and Bisby (1) the term microsclerotium would correctly describe the structures found by many workers, and would be correct even for a structure bearing spores or giving rise to a pycnidium. The term pycnidial primordia would not be correct unless these bodies always gave rise to characteristic pycnidia, and Kulik and Tims (35) have shown that these bodies do not

meet this requirement.

A second objective of these studies was to determine if a polyphenol oxidase system was functional in onion roots, and if so was the activity of the system and the level of the phenolic constituents sufficiently different in pink root resistant and susceptible onions to offer a basis for explaining resistance. The data collected showed a functional PPO system in onion roots but the activity level was low and insufficient differences were found to explain resistance based on this factor alone. Onion PPO activity was low compared to that in mushroom or potato peelings, which are both strongly suspected of having a PPO system operating as a defense mechanism.

The different phenolic patterns obtained from the onions studied were interesting. The presence of a phenolic chromatographic pattern in A. fistulosum so different from others studied may offer a basis of resistance and would be worth further pursuit. It is conceivable that a toxic level of an endogenous phenolic constituent could be responsible for resistance, alone or in conjunction with other systems. The changing phenolic patterns associated with disease progression in YSS are also interesting, but the metabolism of nondiseased tissue may be expected to change with the advent of disease and it may be as a result of disease, not a response to disease. Much more effort is needed here to clarify the situation.

A major difficulty associated with research studies of P.

terrestris has been the extreme difficulty of isolating the organism in any quantitative way. Watson (75), Hess (23) and Hess, Vaughan and Leach (24) have devised procedures to aid isolate identification but these do not allow assessment of the inoculum potential. Anderson and Huber (2) using a modification of the soil sampling tube, approached some quantitation. Direct isolation procedures using various chemical modifications of the soil dilution technique were frustrated by lack of precision and ready ability to identify P. terrestris isolates on the plates. Identification, using the above procedures for isolate identification, required far more time than was merited. The alternative seemed to be a biological assay.

A soil-sand dilution method using onion seedlings as biological indicators seems to have sound applicability. The method, as presently conceived, suffers from the lack of a high level of precision, as do many biological assays, but offers a useable research tool. A minimum of three replicates should be used and the test should be repeated at least twice. The sensitivity is increased by using a soil fraction having an average particle size between 0.5 and 1.0mm. The method was used to evaluate chemical treatments of field soil that were rated for efficacy in the field with a different procedure. Although both methods were variable, the agreement between the two methods was excellent. Both showed which treatments gave disease control, or no control, and showed gradations due to differences in

rates of fumigant applications.

Evaluation of the method showed that six weeks is probably the optimum growing period for maximum symptom expression. This was a necessary compromise between sufficient symptom expression, for accurate and easy reading, and secondary root development and multiple infections, both of which lead to inaccuracies. Since the method rates a plant as infected or not infected (+ or -), it may be anticipated that underestimation of the pathogenic inoculum potential will occur. The method indicates only those propagules pathogenic to onion, limiting the methods usefulness, but substitution of another sensitive biological indicator, perhaps a sensitive tomato variety, may overcome this limitation. The SWG onion was selected in these studies for its availability and because of its known susceptibility to P. terrestris. Duplicate evaluation of SWG and YSS consistantly showed SWG produced a higher percentage of disease, making it the more desirable biological indicator.

Though underestimation of disease may be a recognized limitation, use of the dilution end point as a relative indicator of inoculum levels can help to overcome this objection. It was possible to establish that P. terrestris survives at a considerable depth in the soil. This is a result similar to the findings of Wilhelm (77) in work on Verticillium wilt of strawberry. Similar usage of the method showed that considerable disparity exists between the inoculum levels of

different fields, in fact between different portions of a single field. Observed inequality of infection in the field, and the behavior of other soil borne organisms, would indicate this type of disparity must be expected.

Examination of soil particle size fractions showed P. terrestris inoculum was most strongly associated with the larger soil particles. This was expected since the larger particles are agglomerates of smaller soil particles and fungal propagules, but as the soil particle size decreases, each particle may be expected to function independently as will the fungal inoculum. A relatively sharp reduction in percentage of infection was found associated with soil fraction sizes smaller than 0.147mm. This suggests that single spores or small mycelial fragments are seldom responsible for infections and that the infective units are pycnidia, microsclerotia, or mycelial and/or spore agglomerates. Use of the soil-sand dilution method could contribute meaningfully to elucidating the biology of P. terrestris.

Comparison of washed, non-autoclaved quartz sand, non-autoclaved and autoclaved soils showed the need for replication and multiple tests to attain a meaningful result. And, as would be expected, this comparison showed this method is open to chance contamination from outside sources, requiring clean test areas as well as sufficient checks.

Perhaps the most significant observation to come from the use

of this method was in showing the extreme difference that existed between the east and west sides of the pink root test ground at the Malheur Experiment Station in 1967. Since the field was split in half for the first time in 1967, these results may be only a reflection of the difference in irrigation practices. However, the eastern half of the field was maintained in a relatively dry condition with the idea of making pink root disease more severe, yet this side of the field showed a lower level of inoculum in the assay tests. The west side, which was maintained with higher moisture to represent commercial conditions, showed a higher level of inoculum in the assay. Since the method has proved reasonably accurate under circumstances where it was possible to cross-check it, more study should be exerted to determining the inoculum distribution within the test field. This is especially critical since selection of onion lines for further crossing is made on the basis of results from the trial ground.

This method would seem to have application in studies not approached in the current work. A number of possible areas of continued study that would offer valuable results are presented below. A study of the fluctuation of inoculum levels of pathogenic lines of P. terrestris from year to year, combined with observation of an onion variety's disease susceptibility may show why a resistant onion variety is not always resistant when grown in the same field during consecutive years. Perhaps the inoculum level becomes so high as

to overcome the variety's ability to resist pathogen entry, as has been pointed out by Kreutzer (32).

An interesting and potentially profitable line of research would be an attempt to determine if all isolates of P. terrestris are equally pathogenic on all of the varied hosts that have been shown to be colonized by this fungus. Use of different biological indicator plants could be of value in this type of work. Kulik and Tims (35) did extensive work which indicated that isolates vary in their ability to attack onion, but all of their isolates came from a field cropped to shallots, a very close relative of onion. A very natural extension of these studies would be to determine what crops, if any, increase or decrease P. terrestris inoculum pathogenic to onion. Taubenhaus (65) working in Texas showed short term crop rotations were of little value, but it is possible that differences could exist in different geographical areas. Preliminary studies, initiated after the current work was completed, indicate that differences do exist.

It would seem possible and logical that use of the soil:sand dilution method would allow meaningful screening of chemicals for control of this disease with more precision than is possible in the field. The method would allow more realistic assessment of chemical efficacy under actual conditions, since treatment can be made in the field but biological assay would be carried out in the greenhouse. Not only more realistic results should be achieved, but

the time required per test would be drastically reduced. The test procedure could be shortened by only preparing and reading low dilutions or, if the effect on inoculum potential was desired, an extended dilution series could be used.

A system needs to be worked out whereby the level of pathogenic propagules can be pre-determined to direct fumigant application to those fields most apt to benefit from treatment. In order to do this an economic threshold must be determined for this fungus.

The determination of a means to control pink root disease was a major objective of this research. Field studies were undertaken to evaluate the efficacy of a number of materials and application practices.

Several methods of application were evaluated for their ability to fit into established cultural practices. Three application methods were shown to be potentially adaptable to use in eastern Oregon. Mid-season below-the-row fumigation would be the cheapest, being a band application and using lower amounts of materials, but the phytotoxic potential was great. This method would require much more research to move it into the practical realm. Pre-formed bed treatment was shown to be as effective as broadcast applications and it is approximately one-third cheaper, depending on recommended treatment procedures for the respective materials. However, a rather unique problem arose. It would fit well into cultural practices,

in fact it allows more ground preparation in the fall when time is more plentiful, but the practice is dependent on the occurrence of freezing and thawing in the winter to prevent serious crusting of the beds. If the crust is not mellowed, as it was not in the winter of 1966-67, the field would have to be worked to allow planting the fine onion seed. Since any soil preparation, other than a light harrowing, would destroy the beds and prevent planting into treated bands, the beneficial effects of fumigation would be lost.

Broadcast fumigation received most attention and probably offers the most benefits in the long run, though its problem is expense. Two years of testing with well replicated and well sampled tests have shown that fumigation is practical.

Many chemicals were tested, several were ineffective and many offered nothing over the two registered and readily available compounds, Telone and Vorlex. Telone is cheaper though it requires more material per acre (40 gal) and appears to have little or no effect on P. terrestris, but it does increase the early vigor of the YSS onion. The dollar returns with Telone are variable but the investment level is more in line with the hazards involved.

Telone applied in the fall has not failed to make a positive dollar return in six trials in two consecutive years. Even in a year when the early season was unfavorable for plant growth, and thus minimized the effect Telone exerts on early growth, treatment was still

profitable.

Vorlex is about four times more expensive than Telone and, at rates presently recommended by the manufacturer, requires only one-fourth less material. It offered some disease control but induced little increased early vigor. Vorlex has actually produced more onions in the desirable grades than Telone but the increase was not sufficient, nor sufficiently dependable, to offset the high (ca. $200/A) cost of treatment. Research was initiated to evaluate lower rates of Vorlex, and promising results were obtained, but insufficient data are available to make conclusions on the lower rates.

Several methods of treatment evaluation were used to measure chemical efficacy and to determine the value of the different methods of field evaluation. In future studies it should not be necessary to use all of them. The disease index system worked well and gave a rapidly obtained and useful index. This should be the preferred method of field evaluation of P. terrestris infection levels, if for no other reason than to give comparable data from one year to the next. This is essential in the onion breeding program where continuous comparisons are made. Use of this method by all onion breeders would allow valid cross comparisons so long as several reference varieties were used at the different locations. So far as disease indexing in chemical control tests is concerned, the same results can be obtained by taking soil samples from field treated plots and

assaying them in the greenhouse. The expenditure of time would be greatly reduced and greenhouse testing would be more realistic in the early phase of evaluation. Field observations indicated that root systems become more susceptible to disease as the bulbs become more mature. Assaying soil with non-mature onions should yield more accurate comparative results by removing plant maturity as a variable.

Bulb size indexing was useful for certain purposes, but offered very little in making gross chemical efficacy determinations for which harvest measurements were the only valid measurement. Statistical correlation studies showed no relationship between bulb size indexing and yield, measured at harvest, so a determination of the type and extent of the desired data is necessary.

Total yield alone is of no practical value for measuring the economic return from onions in eastern Oregon. Harvest data must be broken down into grades or there is no basis for making meaningful economic determinations. This is essential because the market places a premium on size.

In the final analysis, dollar return is the ultimate basis of a material's usefulness. The extreme variation of the market from day to day, combined with the grower's decision to sell or not sell on any given day, nullifies the application of normal statistical procedures to evaluate a material. Statistical analysis of the biological

data for reproducibility of a result is useful, but the high level of biological variation prevents analysis at high levels of significance. Even so, a sufficient dollar return may be associated with the variable biological effect to make it attractive to the grower.

SUMMARY

1. A study of P. terrestris penetration into onion roots of selected onion lines showed four host-pathogen relationships: immunity, varying degrees of resistance, tolerance, and complete susceptibility.

2. Microscopic examination of infected onion roots from the field showed numerous microsclerotia (or "pycnidial primordia") associated with the roots, forming a possible basis for carryover inoculum.

3. A functional polyphenol oxidase enzyme system was present in onion roots but not at a level sufficient to be effective in disease resistance. Only small differences were present between disease resistant and susceptible onion lines. Comparison showed onions had about one-half as much PPO activity as common mushrocm and about one-fifteenth as much as potato peelings.

4. Thin layer chromatography (TLC) studies showed that phenolic constituent patterns of A. fistulosum were different from those of YSS which had a changing phenolic pattern with the progression of disease. The onion line B5546B did not show any distribution of phenolic constituents with the TLC procedures employed.

5. A soil dilution bioassay procedure was developed to allow quantitative study of the inoculum potential of P. terrestris. The

results obtained by this method supported field observation regarding lack of disease control with Telone, while high rates of Vorlex were shown to reduce disease. P. terrestris inoculum was shown to survive at depths of 18 inches and infective inoculum was found to be most strongly associated with soil particles greater than 0.147mm in diameter. Extreme variations in inoculum level were shown to exist in the pink root test grounds at the Malheur Experiment Station.

6. Three methods of soil fumigation for pink root control were evaluated. These were mid-season below-the-row, pre-formed bed and broadcast treatment. Only broadcast fumigation is currently feasible.

7. Many soil fumigants were evaluated for pink root disease control. Telone and Vorlex were most effective, resulting in increased onion yields and enhanced dollar returns.

8. Use of a combination of disease and bulb size index resulted in convenient data collection and meaningful indices.

9. Evaluation of fumigation in terms of dollar returns is discussed bringing out various considerations associated with this practice.

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