BLACK ROOT OF RADISH CAUSED BY *APHANOMYCES RAPHANI* KENDRICK IN OREGON

by

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INTRODUCTION

Red radishes are grown in the truck farming area around Portland, Oregon and distributed to many parts of the Willamette Valley. The gross value of the crop that can be grown in a single season may be as high as $4000.00 per acre, but the acreage in radishes is small. Using natural rainfall and over-head irrigation three to four crops are grown each year.

In the past few years a condition characterized by discoloration, distortion and rotting has appeared in many radishes. The condition has become so bad in some fields that the radish crop has been plowed under. The investigations reported in this paper were undertaken in an effort to find the cause of and possible controls for the disease.

The first disease symptom is a light brown vertical lesion on the main root where a lateral root forces its way out. Under conditions of heavy watering a dark lesion around the base of the stem may develop, stunt the plant and inhibit the enlargement of the hypocotyl and upper root into a globe. Although the globe develops from both the hypocotyl and upper root it will be referred to as an enlarged hypocotyl. On the enlarged hypocotyl faint to dark purple blotches or streaks usually appear near the surface and cracks and/or a constricted region may develop in one

or more places. As the disease progresses, bluish-black areas appear beneath the purple surfaces and extend inward toward the center of the radish. At any time other fungi or bacteria may enter the openings created by the primary parasite and cause rotting.

The results of this research show that the cause of the disease is Aphanomyces raphani Kendrick, the cause of black root of radish. This phycomycete is prevalent in many areas of the United States and is found in Germany and Canada. Attempts to control the disease have not been successful.

Figure 1. Left to right, three radishes with the purple blotch phase of black root and one healthy radish, the third radish is developing cracks.

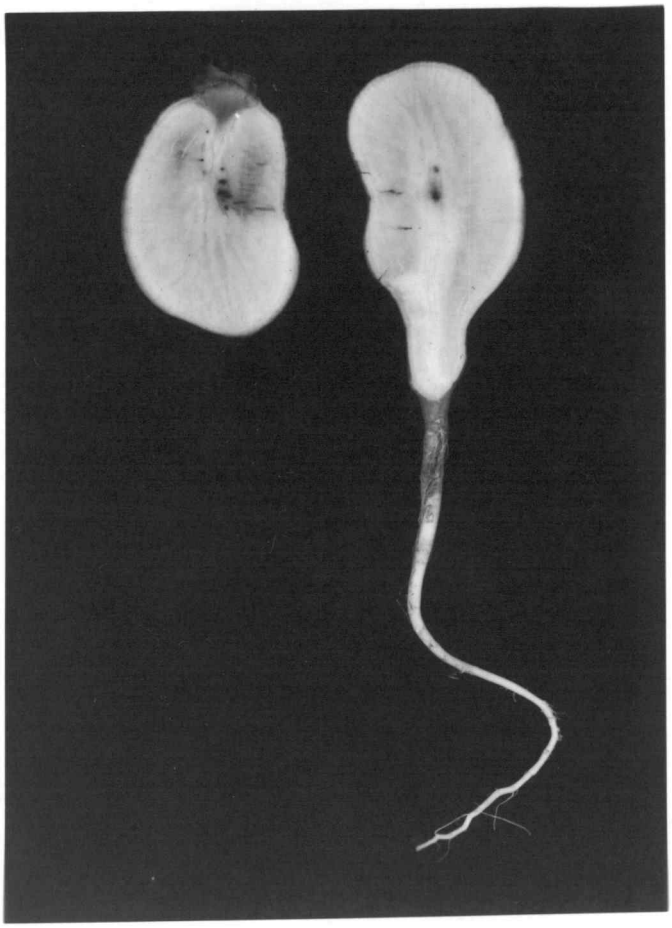

Figure 2. Bluish-black areas extending inward from the purple blotch beneath the surface of a radish infected with black root.

LITERATURE REVIEW

Black root of radish was first reported by Barrett (1) in 1912, who found that the disease was caused by Aphanomyces laevis de Bary. In 1915 Edson (7) reported that black root of radish was caused by a fungus now known as Pythium aphanidermatum (Edson) Fitzpatrick (2). In 1927 Kendrick (9) produced black root of radish using a fungus he had isolated from diseased radishes but failed to produce black root using isolates of Pythium aphanidermatum. He decided that his fungus was a new species and named it Aphanomyces raphani Kendrick. In 1932 Boning (3) reported black root caused by Aphanomyces raphani in Bavaria, Germany. Weber (11) in 1932 stated that the disease was present in Florida. Conners (6) found the disease in Quebec, Canada in 1938. In 1950 the United States Plant Disease Survey reported black root of radish, Raphanus sativus, caused by Aphanomyces raphani to be present on the eastern seaboard from Maine to Florida and in Oklahoma, Iowa and California. The disease reported to be caused by Pythium aphanidermatum was present in Indiana, Kansas, Massachusetts, Michigan, Mississippi, New York, Ohio, Oklahoma, Pennsylvania, South Carolina and Wisconsin.

The disease relationships, the causal fungus and the effect of soil additives on severity of disease have been studied to obtain a better understanding of the factors involved in this parasitism and to obtain possible controls for the disease.

Barrett (1) reported that under certain conditions the seedling stem became shrunken at the surface of the ground, and the plant finally fell over. Kendrick (9) isolated the fungus from dark streaks in or extending throughout the seedling hypocotyl, cotyledons, petioles and true leaves. Infection of the petiole or leaf resulted in a yellowing and death of the leaf. In the greenhouse an infected condition of the hypocotyl resulted in a long, constricted dark hypocotyl and a stunting of the entire seedling. Both Kendrick (9) and Boning (3) found that the radish was attacked during all periods of its growth.

The fungus penetrates the radish through wounds and may attack sound tissue. Kendrick (9) found that most incipient lesions occur at the base of secondary roots and concluded that the natural wound at the base was the most common avenue of penetration. Boning (3) reported that infection occured through the wound at the base of the lateral root, through the ruptured parts of the primary hypocotyl, through insect and implement wounds and that the fungus seemed to attack directly through the uninjured tissues.

The soil is infested with oospores of the fungus. Kendrick (9) observed a few oospores in the disintegrating outer layer of diseased radishes. Boning (3) found that the decay of the radish released oospores to the soil where they became sources of inoculum for primary infection. The soil will remain infested for several and perhaps many years (9). Continuous cultivation of radishes promoted the disease, and this promotion was probably related to an

increase in inoculum in the soil (9). Herold (8) reported crop losses from black root of fifty to eighty percent in southern Germany. Disease incidence was higher in sand than in compost soil or loam.

High temperature and moisture increase the severity of disease. Boning (3) stated that the severity of disease was greater during the midsummer heat than during the cool of autumn. Herold (8) found that high soil moisture promoted the disease.

On artificial media, fungus growth and reproduction are best near or above room temperature. Kendrick (9) found that the highest rate of increase in colony diameter was at temperatures between 23 and 27 degrees centigrade. Zoospore production was best at 20 and 25 degrees. After three days oogonia and antheridia developed in cultures held at 20 and 25 degrees centigrade.

The medium greatly influences the growth and reproduction of the fungus. Kendrick (9) found that mycelial growth was abundant on dextrose agar and potato dextrose agars (PDA) and poor on slants of corn meal agar. While many oospores developed in corn meal agar only a few were produced in PDA. When the agars were very moist a few zoosporangia developed. In radish root decoction zoospores and oospores were produced abundantly. Herold (8) reported that aerial mycelium grew to a height of six millimeters but produced no oospores when the fungus was grown on a medium of equal parts of cherry, oatmeal and peptone-glucose-saccharose agars. Profuse, zoned colonies arose in 0.1% peptone solution. In radish root decoctions the fungus produced

good mycelial growth, but in hypocotyl and leaf extracts it made scanty and almost zoneless growth. Decoctions of radish leaves and hypocotyls and 0.001% peptone solution were the best media for zoospore emission and oospore production. After a dormancy of at least six weeks, oospores in radish debris and in oatmeal agar germinated to produce germ tubes.

The fungus is not seed borne and has no known host besides radish. Kendrick (9) planted seeds from many infected and uninfected radishes from several different sources in sterile soil. When the radishes grew they were healthy. Turnips, carrots, parsnips, peas, garden beets and yellow mangels grown in infested soil developed no symptoms of disease.

Some varieties of radish are resistant to penetration by and development of the fungus in their tissues. Kendrick (9) stated that long white radish varieties were severely infected in their edible part while infection seldom reached the enlarged globe of the red radish. A satisfactory crop of red radishes could often be grown on infested soil. Of many radish varieties tested only White Chinese was consistently resistant to infection and disease development. Boning (3) reported that late summer red radishes were fairly resistant to the disease.

Various additives to the soil affect the severity of the disease. In 1932 Boning (3) found that soil treatment with 2% formalin or calcium cyanamide (50 gm. per sq. meter) gave moderately successful control of the disease. In 1936 Boning (4) stated that

calcium cyanamide, ammonium sulphate, and formalin completely controlled the disease, but calcium cyanamide and formalin checked the growth of the plants. Urea treated plots produced only 3% infection as compared to the 39% in untreated soil. Other chemicals gave less promising results. In 1949 the same writer (5) reported that his previous attempts to control black root had proven inadequate or impracticable. He found that the fungus was favored by a high humus content of the soil and by the use of only stable manure for fertilizer. Acid fertilizers reduced the amount of black root while lime had no effect. High potash lowered the percentage of infection and increased the radish yield. High nitrogen increased yield, and calcium nitrate decreased the percentage of black root. In 1952 Herold (8) reported that neither mineral fertilizer or chemical seed treatment controlled the disease.

METHODS AND MATERIALS

The methods and materials used in more than one part of this research will be presented here and those used only in a particular experiment will be presented with the discussion of that experiment.

ISOLATION

After only occasional success with procedures employing mild surface sterilization a high percentage of individual cultures was obtained by thoroughly surface sterilizing washed pieces of infected seedling stem or washed pieces of infected enlarged hypocotyl and

placing the pieces on potato dextrose agar (PDA) or white bean agar (WBA) in petri plates. Sections of seedling stem, above the basal lesion indicating infection, were surface sterilized for 30 seconds in 1 or 2 percent sodium hypochlorite and placed on PDA or WBA. Enlarged hypocotyls were surface sterilized for one minute in 1 or 2 percent hypochlorite, after which sections of diseased tissue were removed, dropped into 1 or 2 percent hypochlorite for about five seconds and then placed on PDA or WBA. The fungus grew from the stem sections in four to five days, but it required two to thirty eight days (average 16) to grow from the pieces of enlarged hypocotyl. It is not obvious to the author why it should take so long a time for the fungus to grow out from some pieces of tissue. Using the thorough surface sterilization, cultures of the fungus were obtained from a total of nine infected radish seedlings and from many diseased radishes grown on infested soil in the greenhouse. While determining pathogenicity the fungus was obtained routinely from the enlarged hypocotyls of many diseased radishes.

CULTURE

The following media were used to isolate, to culture and to induce the formation of reproductive structures in Aphanomyces raphani. Potato dextrose agar (PDA) and white bean agar (WBA) were employed in isolating and culturing the fungus. Radish agar was resorted to for consistant, rapid and plentiful production of oogonia, antheridia and oospores. Asexual reproduction was obtained

by placing a piece of any of the above media, covered with growing fungus mycelium, in a petri plate of sterile distilled water. Oogonia, antheridia and oospores developed on some plates of PDA and WBA. On radish agar sexual organs and oospores occurred on all plates in great numbers within five days after the transfer of mycelium from established cultures. The time required for asexual reproduction was quite consistent and will be reported later in this paper.

The PDA was freshly prepared standard laboratory potato dextrose agar containing zero to one hundred PPM streptomycin nitrate. The WBA was prepared in exactly the same way as the PDA excepting that 50 gm. of small white Navy Pea bean and 2.5 gm. of dextrose were used instead of 200 gm. of potato and 20 gm. of dextrose per liter of water. Radish agar was prepared by adding 15 gm. of agar to the liquid from 200 gm. of enlarged radish hypocotyl. The hypocotyls had been blended with 700 ml. of distilled water in a Waring blender. The resultant mixture was brought to one liter with distilled water, autoclaved for 15 minutes at 115 degrees C., cooled and poured into petri plates or stored.

IDENTIFICATION OF THE FUNGUS

The fungus mycelium is hyaline, non-septate and prostrate. The hyphae branch at right or lesser angles. Slight aerial mycelium may develop in a young culture on PDA, but as the culture grows older the mycelium becomes dense and prostrate. The side hyphae are sometimes

short and conspicuous and sometimes long and indistinguishable from other vegetative hyphae. The diameters of the hyphae on PDA vary from 3.9 to 15.9 microns, and with a sample of 30 observations the sample mean and variance are respectively 9.4 microns and 0.78.

When medium containing growing mycelium is placed in sterile distilled water the mycelium produces complex reproductive hyphae or zoosporangia which have more than one lateral evacuation hypha. These zoosporangia are very similar in appearance to the vegetative hyphae. The evacuation hyphae are thinner than the vegetative and vary in appearance from a long tightly twisted corkscrew to a gently curving or straight tube. Within the first twelve hours after transfer to water the protoplasm in a reproductive hypha condenses to form a single chain of long, thick, flexible, cylindrical units. These units flow out through one or more evacuation hyphae. Emerging from the apical tip of the evacuation hypha each unit curls up or rounds up to form a sphere. Soon a cluster of spheres gathers at the tip. The diameters of these spheres or encysted zoospores vary from 8.1 to 13.0 microns, and with a sample of 50 observations the sample mean and variance are respectively 9.75 microns and 0.097. A few hours after cluster formation motile zoospores begin to emerge from the spheres. One zoospore comes from each sphere and leaves behind a round transparent covering. When immobilized with 1% silver nitrate and observed under an ordinary or a phase microscope, the motile zoospore appears to be flattened and pear shaped and has two long flagella. The motile spore swims for a few minutes or longer, then

throws off its flagella and becomes a sphere or encysted spore. This sphere is smaller than the encysted spore in the cluster; the measured diameter varys from 4.2 to 8.1 microns, and with a sample of 50 observations the sample mean and variance are respectively 5.9 microns and 0.056. This smaller encysted spore germinates to form a germ tube. It is assumed that this germ tube becomes a mycelium. The condensed protoplasm in the hypha or the individual spore in an encysted cluster sometimes produce a germ tube.

On a short lateral hypha attached to a mycelial hypha there develops an oogonium, whose walls are smooth on the outside and irregular on the inside. On white bean agar the oogonial diameters vary from 26.0 to 33.8 microns, and with a sample of 30 observations the sample mean and variance are respectively 30.3 microns and 4.1. On radish agar the oogonial diameters vary from 22.8 to 32.5 microns, and with a sample of 30 observations the sample mean and variance are respectively 27.4 microns and 0.56. One to three club shaped and often dichotomously branched antheridia come from the oogonial hypha and/or a near by hypha and attach to the oogonium. Within each oogonium develops a single dense but colorless oospore with a thick hyaline wall. During formation of the oospore the contents of the oogonium can sometimes be seen as a sperical mass covered with and perhaps completely composed of motile particles surrounded by a colorless and transparent material that extends to the oogonial wall. Neither the immature nor mature oospore fills the oogonium. On WBA the diameters of the oospores vary from 20.2 to 26.7 microns,

and with a sample of 30 observations the sample mean and variance are respectively 24.4 microns and 0.28. On radish agar the oospore diameters vary from 19.5 to 26.0 microns, and with a sample of 30 observations the sample mean and variance are respectively 21.8 microns and 0.30. On WBA the hyaline wall of the oospore has a thickness of 0.65 to 3.25 microns, and with a sample of 30 observations the sample mean and variance are respectively 1.46 microns and 0.074. On radish agar the thickness of the hyaline wall varies from 0.65 to 3.25 microns, and with a sample of 30 observations the sample mean and variance are respectively 1.9 microns and 0.065. These traits place the fungus in the genus Aphanomyces of the Saprolegniaceae (2, 12).

The smooth, round oogonial wall separates the fungus from the several Aphanomyces species which have wavy or spiny walls (9). The complex branched sporangium distinguishes it from Aphanomyces laevis de Bary which has a simple sporangium (12). The lack of one to four numerous lateral protuberances on the distal portion of the evacuation hyphae and the lack of numerous single apical prolongations (3.5 x 13 microns) on the antheridia separates the fungus from A. camptostylus and A. cladogamus Drechsler (12). The fungus resembles A. euteiches except that A. euteiches sometimes has a columella-like structure protruding into the oogonial cavity at the stalk and does not cause a black root disease of radish (9). Aphanomyces raphani Kendrick and the fungus isolated in this study are similar in all characteristics except for the dimensions of

certain organs.

Following is Kendrick's description of A. raphani N. sp., the cause of radish black root.

> "The vegetative mycelium is hyaline, non-septate, and profusely branched at right angles, and bears short, conspicuous side branches. The hyphae are 8.2 to 11.3 microns (average 9.2) in diameter. The mycelium is slightly aerial in early stages of growth on potato dextrose agar, but soon becomes a prostrate, dense, flat, tough mat.
>
> The zoosporangia are terminal or intercalary, long and profusely branched, and differ from the vegetative hyphae only in the spiral twisting of the branches from which the zoospores are discharged. The zoospores, which are produced in a single row in the zoosporangium by segmentation of its contents, are cylindrical, 6.9 to 26 microns in length and escape from one to several, tapering more or less spirally twisted, lateral discharge hyphae. The zoospores become globose and encysted on emergence, are 8.8 to 12.7 microns (average 10.2) in diameter, and remain in a clump for a short time at the outlet of the discharge tube. The zoospores soon emerge from their cysts in motile form with two laterally attached cilia, swim about for a short period, come to rest, round up, and germinate by sending out a slender germ tube.
>
> The oogonia are globose 32 to 44.9 microns in diameter (average 37.4) and are terminal on short side branches and thick-walled when mature. The oogonial wall has a smooth outer surface and an irregularly contoured inner surface. The antheridia are produced on simple or bifurcated slender stalks which arise from the hyphae bearing the oogonial stalk or from a near-by hypha. The antheridial stalk is often closely associated with the oogonial stalk but never twines about it. The antheridia are club-shaped, typically one to three, usually two to each oogonium, and lie flat on the surface of the oogonium. The oospores are single, globose, 21.4 to 29.8 microns (average 25.7) in diameter, with a hyaline wall 2.5 to 4.5 microns thick. Germination of the oospore has not been observed.
>
> The fungus is parasitic on Raphanus sativus L. Type locality: Lafayette, Ind."

Since the isolated fungus and Aphanomyces raphani are morphologically similar and both cause black root of radish it is concluded that the fungus from the farming area around Portland,

Oregon is Aphanomyces raphani Kendrick.

PROOF OF PATHOGENICITY

Pieces of PDA about 0.5 cm. sq. were cut from actively growing cultures of the fungus. Each piece was placed in a 500 ml. erlenmeyer flask containing 250 ml. of potato dextrose broth. The broth was standard PDA less the agar. Several flasks were placed on a shaker and shaken until the mycelium formed a white lacy ball about four centimeters in diameter. This took about seven days. Each ball was transferred to another flask containing 250 ml. of sterile distilled water. After three days on the shaker the water was filled with encysted zoospores. Since the zoospores were not in clusters it was assumed that they were in the second encysted state. Spore suspensions of two concentrations were prepared from these flasks. Suspension A consisted of the contents of three flasks blended in a Waring blender to make 730 ml. of water, mycelium and encysted zoospores. Suspension B consisted of the contents of two flasks blended with a liter of distilled water.

Radishes were infected by pouring suspension A on seedlings growing in cans. Ten cans were filled with greenhouse potting soil, five with infested farm soil and five with infested farm soil treated with chloropricrin (35 gal. per acre) in the field during the fall previous to this spring experiment. The chloropicrin was applied by Jack Fisher of Dow Chemical Company. Ten radish seeds of the variety Red Comet were planted in each can. When the

seedlings developed one true leaf, 100 ml. of suspension A was poured into each of five cans of the greenhouse potting soil.

Radishes were infected by pouring suspension B on soil containing radish seeds and/or by placing strips of PDA covered with growing mycelium in the wet soil near seedlings. Ten cans of naturally infested farm soil were autoclaved for two hours at 115 degrees centigrade, and five more cans of infested farm soil were left unsterilized. More than ten seeds were planted in each can, and on the day of planting 200 ml. of one day old suspension B was poured into each of five cans of autoclaved soil. When seedlings in the cans receiving suspension B were starting to form globes, the soil around the seedlings was inoculated with strips of PDA covered with growing mycelium.

To prevent cross contamination between cans of different soil treatment each treatment group of five cans was placed at least eighteen centimeters away from every other group. All cans were number ten cans filled with soil to a depth of six inches.

When the radishes were of market size the plants were removed from the cans, washed and examined for disease. Radishes with the characteristic purple blotching or streaking on the enlarged hypocotyl or light brown vertical streaks surrounding lateral roots on the tap root were counted as having the black root disease. Five pieces of tissue were cut from each of two radishes from each can, surface sterilized, and plated onto PDA. All fungi growing out of pieces of radish, having identical growth characteristics with that

of the inoculum were considered to be Aphanomyces raphani. Many of the isolates were checked for asexual reproduction and all corresponded to that of A. raphani.

Table 1: Percent of black root in and percent recovery of Aphanomyces raphani from radishes in infested and non-infested soils.

Soil	Amount of disease*	Percent diseased	Amount of recovery**	Percent recovery of fungus
Greenhouse soil + suspension A	20/29	69	6/10	60
Greenhouse soil	0/39	0	0/10	0
Infested farm soil	15/33	45	6/10	60
Infested farm soil treated with chloropricrin	32/36	89	1/10	10***
Autoclaved infested farm soil	0/75	0	0/10	0
Autoclaved infested farm soil + suspension B and strips	50/66	76	4/10	40
Infested farm soil	55/59	93	6/10	60

* Disease is reported as the number of diseased radishes over the total number of radishes in five cans.

** Recovery is reported as the number of radishes from which the fungus grew, over the total number of radishes used in isolation.

*** The radishes in this soil were so badly infected and filled with secondary invaders that the fungus was not regularly isolated.

For the first three treatments listed in Table 1 the isolation of the fungus was repeated using only one radish from each can.

The percent recovery of the fungus was 60, 0 and 40 percent, respectively.

The fungus grew from a total of 54 pieces of tissue. Inoculated greenhouse soil, inoculated autoclaved naturally infested soil and naturally infested soil produced high percentages of diseased radishes from which the fungus was recovered in high percentages. Greenhouse soil and autoclaved naturally infested soil produced only healthy radishes from which no fungus was isolated. The presence of high percentages of disease and of the fungus in radishes grown in infested soils together with the lack of disease and of the fungus in non-infested or sterilized soils show that the disease, black root of radish, is caused by Aphanomyces raphani Kendr. The severity of the disease in soil treated with chloropicrin might be related to a killing of the natural enemies of A. raphani combined with a failure to kill its thick walled oospore. The above results indicate that the oospore, which Boning (3) reports as the source of primary infection, may be killed by steam sterilization.

FUNGUS HYPHAE IN THE RADISH

In January of 1958 radishes with the purple blotch of black root disease were purchased at a store in Long Beach, California. Pieces of infected enlarged hypocotyl were killed and fixed in 50% FAA and embedded in paraffin. Sections were cut 8-15 microns in thickness and stained with aniline blue in clove oil. In and

near the purple blotch a sparse non-septate mycelium was found between and within the parenchyma cells of the pericycle, phloem and xylem but not in the cambial zone. Barrett (1) in 1912 and Kendrick (9) in 1927 found an intercellular mycelium in the diseased radish. Perhaps there is some relationship between the short conspicuous side hyphae sometimes seen in culture and the hyphae or haustoria in the parenchyma cells. Boning (3) reported that when the blackened areas extended in towards the center of the radish they followed the medullary rays and often left the cambial zone intact.

ATTEMPTED CONTROLS

CHECKING FOR A MINOR ELEMENT DEFICIENCY

Plots, 5' x 15', of radish seedlings were sprayed with Solubor or with Solubor in combination with a sequestrene chelated minor element. The treatments in pounds per acre were Solubor 12.5; Solubor 25; Solubor 75; Solubor 50, 138 Fe 1 pound; Solubor 50, Na_2Zn 4; Solubor 50, Na_2Mn 4; Solubor 50, Na_2Ca 1; Solubor 50, Na_2Mg 1; Solubor 50, Na_2Cu 0.25. The percentage of active metal in the Solubor was B 20%, in 138 Fe 6%, in Na_2Zn 14%, in Na_2Mn 12%, in Na_2Ca 8.5% in Na_2Mg 5.5% and in Na_2Cu 13%. Between each treated plot there was an untreated check plot. The chelates were found to have no effect on disease incidence but did stunt the growth of radish foliage and roots.

SOIL ANALYSIS FOR MAJOR ELEMENTS

The Soils Department at Oregon State College tested the farm soil which produced the affected radishes. Following their test, plots in this soil were used in checking for a minor element deficiency and plots were treated with organism killing chemicals in an attempt to reduce the severity of the disease. The deficiency and soil treatment experiments are discussed in other sections of this report. The Soils Department analysis of the soil is given in Table 2.

Table 2. Soil analysis of infested soil.

Lab no.	Field no.	Soil pH	Lime Requ't T/A	Phosphorus lbs/A	Phosphorus level	Potassium lbs/A	Potassium level	Calcium lbs/A	Calcium level	Mg lbs/A	Boron ppm
4647	Pitton	5.3	3	109	VH	710	VH	3460	M	730	0.86

While Boning (5) obtained a disease reduction with acid fertilizers and high potash, here we have an acid soil high in potassium that produces high percentages of severely infected radishes.

SOIL TREATMENT

Infested soil was treated in an attempt to eliminate or reduce the severity of the disease. In the summer of 1957 autoclaved number ten cans were filled to a depth of six inches with well mixed infested farm soil. Three rates of dichlone and three of

2-pyridenethione 1 oxide zinc salt were mixed as a powder with the soil, and each mixture was placed in five cans. Each of three rates of vapam were pipetted to the middle of five cans of soil. Ten cans of soil were autoclaved for four hours at nine pounds pressure. Ten cans of untreated soil were left as checks. After treatment all the cans of soil containing chemical were covered with a layer of water. The dichlone, zinc compound and vapam contained 50%, 50% and 31% active ingredients respectively, but all treatments were conducted on a basis of 100% active ingredients. Six days after treatment the cans were moved into the greenhouse and planted with radishes of the variety Red Comet. A flat containing greenhouse potting soil was also seeded. By thinning and by transplanting from the flat the number of seedlings in each can was brought to five. When the radishes were about two thirds market size they were sprayed with benzene hexachloride to kill cabbage moth larvae. When the radishes in the check and autoclaved soils were of market size, all the radishes were removed from the cans and washed, and the number of diseased radishes was determined (see Table 3).

Table 3. Treatment of infested soil in an attempt to reduce the severity of black root of radish

Soil treatment	Rate lbs*/A	Replication (can)** 1	2	3	4	5	Total	Percent diseased	Stunting
Dichlone	50	2/5	3/5	4/5	3/5	5/5	17/25	68	
	100	1/5	5/5	2/5	2/5	1/5	11/25	44	
	200	0/5	3/5	0/5	0/5	0/5	3/5	12	severe
Zinc compound	50	3/5	2/5	1/5	3/5	1/5	10/25	40	moderate
	100	1/5	1/5	3/5	0/5	3/5	8/25	32	moderate
	200	1/5	2/5	2/5	2/5	3/5	10/25	40	severe
Vapam	50	1/5	0/5	4/5	4/5	1/5	10/25	40	
	100	1/5	1/5	2/5	1/5	1/5	6/25	24	
	200	0/5	2/5	1/5	3/5	3/5	9/25	36	
Steam		0/5	1/5	3/5	1/5	4/5	12/50	24	
		3/5	0/5	0/5	0/5	0/5			
Check		4/5	5/5	3/5	4/5	5/5	42/50	84	
		5/5	3/5	4/5	4/5	5/5			

* 100% active ingredients.

** The amount of disease is reported as the number of diseased radishes over the total number of radishes in each can.

Increasing rates of dichlone seem to reduce the percentage of disease. However twenty-four percent of the radishes in the steam sterilized soil were diseased. In later experiments radishes in steam sterilized soil were not diseased. The cans in this experiment were placed close together and were moved once by other people while the radishes were growing. It is believed that there was cross contamination between cans caused by splashing water. Therefore, the validity of the results is questionable, and no attempt was made to determine their statistical significance.

DISCUSSION AND CONCLUSIONS

High percentages of individual A. raphani cultures were obtained when infected radish tissues were surface sterilized with 1 or 2% sodium hypochlorite. Thorough surface sterilization of some parasitized roots may kill secondary invaders in the outer layers of the infected roots and make it possible to obtain many pure cultures of the primary parasite from deep within the living roots.

Chemicals may kill the vegetative and asexual stages of the fungus and may kill the enemies of the fungus while leaving the fungus oospore untouched. The soil treatment results such as twelve percent diseased in soil treated with dichlone at a rate of 200 lbs per acre as compared to eighty-four percent diseased in untreated soil indicate that there may be an initial reduction in disease in treated soils. Radishes grown in the field fumigated by Jack Fisher and discussed under proof of pathogenicity were in the first month and a half after treatment almost completely free from disease. In the following spring radishes grown on the same soil in the greenhouse were severely infected (see Table 1). Also, that spring, when Mr. Ned W. Frandeen, County Extension Agent at Gresham, again had chloropicrin applied to the field the radishes grown on the treated ground were severely diseased. The fact that the radish is attacked in all stages of its development and that the fungus may remain in the soil for

many years indicates that the thick walled oospores do not all germinate at the same time or in the same year. Thus, any chemical applied to the soil must be capable of killing the oospore when it is in a dormant condition. Steam sterilization of the soil should kill the fungus (see Table 1), but at present this would be very expensive.

The radish variety White Chinese is resistant to fungus invasion and development. Breeding resistant qualities into commercially desirable radishes may be possible. However, Kendrick (9) found that the fungus often did not reach the enlarged hypocotyl of red radishes, while the writer has found the enlarged hypocotyl highly infected. Either the radish variety Red Comet is less resistant than other red radish varieties or a more virulent strain of the fungus has appeared.

SUMMARY

Red radishes growing in the truck farming area of Portland, Oregon are severely affected by a condition characterized by a discoloration, distortion and rotting of the mature root. Any part of the seedling may be attacked and damaged. A fungus was isolated from affected roots and seedlings and identified as Aphanomyces raphani Kendrick, the cause of black root of radish. Immobilization and staining with 1% silver nitrate revealed a flattened, pear shaped zoospore with two long flagella. Using Koch's postulates it was proven that the fungus causes the abnormal

condition, the black root, by penetrating and growing within the radish. Permanent slides of infected radishes show in the enlarged hypocotyl a non-septate fungus mycelium between and within the parenchyma cells of the pericycle, phloem and xylem but not in the cambial zones. A thorough surface sterilization with 1 or 2% sodium hypochlorite makes possible the isolation of the fungus from high percentages of diseased radishes. In culture the fungus is very quickly killed by some bacteria.

Treatment of cans of infested soil with dichlone, with 2-pyridenethione 1 oxide zinc salt and with vapam gave indications of an initial disease reduction. Subsequent treatments and radish plantings in greenhouse and field did not result in a significant reduction in the number of diseased radishes. Steam sterilization completely eradicated the fungus. Chelated minor elements applied to plots of seedlings in an infested field did not affect the development of the disease but did stunt the radishes. The Soils Department of Oregon State College tested the infested soil and found it to be high in phosphorus and potassium. The soil Ph was 5.3.

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