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STUDIES CONCERNING PHYTOPHTHORA ROOT-ROT OF SAFFLOWER

(*CARTHAMUS TINCTORIUS* L.)

by

Bill B. Berkenkamp

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT PATHOLOGY

In Partial Fulfillment of the Requirements For the
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GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Bill B. Berkenkamp entitled STUDIES CONCERNING PHYTOPHTHORA ROOT-ROT OF SAFFLOWER (CARTHAMUS TINCTORIUS L.) be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

STUDIES CONCERNING PHYTOPHTHORA ROOT ROT OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.). Bill B. Berkenkamp - Ph D Dissertation - Department of Plant Pathology, The University of Arizona.

Phytophthora drechsleri Tucker was isolated repeatedly from infested soil. Several isolates and one from the American Type Culture Collection were tested for pathogenicity on various plants and tissues and differences were found indicating physiological strains. Production of sporangia was stimulated by growing the fungus on dilute tomato juice and transferring the mycelium to distilled water. All attempts to stimulate oospore production failed. Synthetic media would not support growth, unless they contained calcium and thiamine in addition to other commonly used ingredients. The ability of one strain to utilize various carbon sources was tested. All of the sugars, starch, and some pectic products tested were utilized. Highly acidic pectic products did not support growth. All nitrogen sources tested were utilized except potassium nitrite. No great differences in the efficiency of utilization of proteinaceous materials, amino acids, and inorganic nitrogen were found.

Safflower plants growing in hydroponic culture were inoculated and roots were collected and sectioned after various intervals. Upon

examination, it was found that infection hyphae penetrate directly and grow rapidly through the root cortex. Culture filtrates were found to stimulate germination of safflower and to cause no injury to seedlings. Discs of stem tissue were inoculated and placed on other discs, separated by filters and membranes. The inoculated pieces were darkened and decomposed. Those separated were not discolored but were macerated indicating probably pectolytic activity.

Various extracts from diseased and healthy safflower of both resistant and susceptible varieties were tested for fungus inhibition. The fungus was not inhibited by any of the extracts; however, extracts from diseased plants caused extensive discoloration when injected into safflower plants in the field.

The effect of root pruning was tested on plants in a root box supplied with a spray of water. It was found that pruning had little effect, but resistant and susceptible varieties of safflower had significantly different root:shoot ratios. A low root:shoot ratio was found to be related to resistance.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) has been cultivated since antiquity. It is native to the mountains of Southwest Asia and Ethiopia and in ancient times became established around the Mediterranean Sea, and throughout India and Southern China. Carthamin, a red dye extracted from the flowers, was used as a substitute for saffron. Egyptian mummies have been found wrapped in cloth dyed with carthamin. More recently this natural dye has been replaced with synthetic derivatives but the flowers are still used as food coloring, a pigment for rouge, and a mild laxative in some countries. Safflower seed was used as a source of oil for cooking and lamps early in its history. The first introduction into North America was probably from Spain into Mexico where it has been grown for its flowers. The first mention as safflower as a crop, in the United States, was in 1895. Rabak (30) pioneered safflower development in the United States but failed to establish it commercially. Claassen (5, 6, 7) continued the work of Rabak and developed safflower lines with up to thirty-six per cent oil content, as well as higher seed yields. The crop became commercially established in California in 1949 (21) and many processing plants now exist. In a compilation of world acreage estimates, Knowles (21) reported 1,023,298 acres in India in 1948; 121,000 acres in Russia in 1932; and 65,000 acres in the United States in 1953. Estimates were very difficult in India because of the use of safflower as a border barrier around other crops to protect them from animals. Introductions into Australia and Canada were not commercial until 1956. Presently safflower oil is used as a drying component in paints and varnish, and in the manufacture of alkyd resins where it has steadily replaced hard resins in enamels. The use of safflower oil in food products has recently begun in the United States. Previously exports of oil from this country have been used as food oils in Europe. Decorticated meal is an equivalent of other high protein meals in fattening animals, but has been found to be deficient in lysine and methionine.

Phytophthora root rot is the most important disease of safflower in the United States. The disease was first observed in Nebraska in 1947 (13) as a root rot, and also as the cause of damping off of seedlings. Severe losses of up to eighty per cent of the plants in a field were reported (14). Recently developed resistant varieties have reduced losses from Phytophthora, but no immunity has been found. This root rot is a major factor limiting safflower production under irrigation, and a practical control is lacking.

Four species of Phytophthora are known to cause root rots and blights of safflower: Phytophthora drechsleri Tucker, P. parasitica Dast., P. cactorum (Leb and Cohn) Schroet, and P. palmivora Butl. Of these, P. cactorum is least pathogenic (33). Phytophthora parasitica is known only from artificial inoculations (33), while P. palmivora has been reported only from South America (26) and India (1). Phytophthora drechsleri is the most commonly found causal agent in the United States. Recently it has been reported on tomatoes, potatoes, and honeydew (10, 16, 37, 38). The first isolation of P.

drechsleri was made in Idaho by Charles Drechsler in 1922 (39) from potato tubers. Drechsler tentatively identified the fungus as Phythohthora erythroseptica Pethyb, due to its similarity to a culture obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (11). This culture was originally described and supplied by G. H. Pethybridge, who found it causing a pink rot of potato tubers in Ireland (28). In 1927 C. M. Tompkins et al (36) isolated a Phytophthora from sugar beet roots in Utah, which resembled Drechsler's isolate in cultural and morphological characteristics. He gave an excellent description of Phytophthora root rot of sugar beets and of the causal organism, Phytophthora drechsleri, and described the unique lens-shaped apical thickening in the sporangium and substituted this for the usual term "nonpapillate." A Phytophthora was isolated from diseased tomato fruit in 1928 by G. B. Ramsey in California who regarded it as conspecific with the isolates from Idaho potatoes and Utah sugar beets (11). The original culture from Idaho potatoes was sent to Dr. C. M. Tucker, who is presently a leading authority on the genus Phytophthora. He described it as Phytophthora drechsleri (39). Tucker classified his new species in the group made up of P. cinnamoni, P. erythroseptica, P. cambivora, P. cryptogea and P. richardiae. Phytophthora drechsleri was the first of this group to be isolated in the United States and is distinguished from the other members of this group by a high optimum temperature (30-32.5 deg C) for growth, as well as the ability to grow at 35 deg C, and some differences in pathogenicity. **This isolate was virulent** on potato tubers, fruits of eggplant and apple, as well as seedlings of papaw and tomato. These features along with the rare production of sporangia, and their nonpapillate form were sufficient in Tucker's opinion to distinguish it from all other Phytophthoras. The first isolation of P. drechsleri from safflower in the United States was probably in 1948 by Claassen et al. (8), who isolated a "phycomycete" from safflower. D. C. Erwin, who had observed a root rot of safflower in the field, as early as 1947, isolated P. drechsleri from safflower in 1950 (13) and his identification of the fungus was verified by Tucker. Inoculations made under greenhouse conditions showed that P. drechsleri was pathogenic to both seedlings and older plants of safflower. The optimum temperature for infection and pathogenesis was 25-30 deg. C. A small number of plants were killed at 20, but none at 17 deg. C. This study was continued and it was found that high soil moisture favored the disease (14). The pathogen was also shown to be very virulent on plants grown in nutrient solutions.

In 1952, Erwin (14) found that temperatures favoring radial growth of P. drechsleri in culture was closely correlated with soil temperature favoring pathogenicity. Braun (4) found that P. drechsleri attacking guayule was also limited by low temperatures. A minimum temperature of 16 deg. C. was necessary for disease development and pathogenicity was severe at 19 deg. C. The optimum pH for growth of the fungus was found to be 7.0. Growth was slightly less at pH 6, and 8, and still less at 4 (14). Final pH readings after the termination of the experiment showed a slight decrease in acidity had occurred for all values below 5, and a more pronounced increase in acidity has occurred in cultures initially at 6 and above. These results did not agree with the findings of Tompkins _et al._ (36) who found that good growth occurred from 3.6 to 8.4 with no indication that the limiting pH values were being approached. Similar effects of temperature on growth were reported by Erwin (14) and by Tompkins _et al._ (36) who found the minimum temperature for growth of _P. drechsleri_ was near 8 deg. C.; the maximum about 36 deg. C., and the optimum temperature for growth was between 28 and 34 deg. C. Tucker (39) found the cardinal temperatures to be 10 deg., between 30 and 32.5 deg. C. and 37.5 deg. C. on corn meal agar.

Claassen _et al._ (8) in 1948 observed resistance in some safflower introductions. Those from India and Africa seemed to have no resistance to root rot. In 1950 Thomas (32) found safflower root rot widely distributed over the United States. The disease was less serious in Nebraska due to the planting of susceptible varieties on dry land and the use of resistant varieties where the crop was irrigated. In California, Thomas noted that poor cultural practices, over irrigation, and "long runs", increased the severity of the disease. In 1952, Thomas (34) observed differences in resistance in the field between varieties, selections and foreign introductions of safflower. Later (35) he found a method of evaluating resistance of varieties of safflower in the greenhouse and showed that it was closely correlated with the reaction of these varieties in the field.

METHODS AND RESULTS

Varieties of Safflower

Safflower seed used in the studies reported in this paper was furnished by D. S. Black[1]. The varieties were N 8, N 10, WO 14, Gila, and US 10. The Chemurgy Project of the University of Nebraska developed many varieties, all of which are designated by the prefix "N". N 8 is a selection for resistance from Special Russian. N 10 is a plant selection for yield from N 852 which was originally a mass selection from an introduction from the Anglo-Egyptian Sudan. WO 14 is a selection from a cross between N 8 and N 977-16-1 which was backcrossed to N 8 four times and selected for resistance to rust. This variety was developed by Pacific Oilseeds, Inc. (formerly Western Oilseeds Co.). N 977-16-1 is a selection from N 977 which was originally introduced from Romania. Gila, was developed by D. D. Rubis[2] in Arizona. It is a selection from a cross between N 10 and WO 14. The progeny was backcrossed to N 10 and selfed for four generations with selection for root rot, and rust resistance, and good agronomic characteristics (31). US 10 was developed by the U. S. Department of Agriculture. It was derived in the same

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manner as Gila with the exception that it was not selected for yield or other desirable agronomic characteristics. All of the varieties described except N 10 are resistant to Phytophtera root rot.

Selection of Isolates

Isolates of *P. drechsleri* used in the studies reported upon here, were obtained from soil from a severely infested field[3]. The infested soil was placed in pots and potato tubers and safflower were planted. When safflower plants began showing disease symptoms, they were removed and parts of the roots cultured. Isolations were also made from the potato tubers after the same length of exposure. Isolates from diseased potato tubers and safflower roots were similar in cultural characteristics. The isolates were then inoculated into wounded and unwounded tomato fruit, cotton bolls, Bryophyllum leaves, potato tubers, onion bulbs, and saguaro seedlings. Pathogenicity to these plants and plant parts varied somewhat. Results of these tests are shown in Table 1. The culture with the broadest spectrum of high virulence was selected for study. This culture is subsequently referred to as No. 3, and was deposited with The American Type Culture Collections as Culture No. 14509. Culture No. 10923, *P. drechsleri* was obtained from The American Type Culture Collection and used in these studies for comparison with No. 3. This culture was originally supplied by W. F. Jeffers, of the University of Maryland, in 1951.[4]

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[3]Field H Border 12 W, University of Arizona Agric. Exp. Sta., Mesa, Arizona.

[4]American Type Culture Collection, Catalogue of Cultures 6th Ed., 1958.

TABLE 1

Pathogenicity[a] of *Phytophthora drechsleri* to Various Hosts

Host	Type of Inoculation	Culture No. 3	4	5	6	10923
Tomato fruit	Wounded	**	**	**	**	*
(*Lycopersicon esculentum*)	Unwounded	*	*	*	*	-
Cotton bolls	Wounded	**	*	-	*	*
(*Gossypium hirsutum*)	Unwounded	**	-	-	*	*
Bryophyllum leaves (detached)	Wounded	**	**	**	**	-
(*Bryophyllum* sp.)	Unwounded	**	**	**	**	-
Potato tubers	Wounded	*	**	**	**	-
(*Solanum tuberosum*)	Unwounded	-	-	-	-	-
Onion bulbs	Wounded	*	-	-	-	-
(*Allium cepa*)	Unwounded	-	-	-	-	-
Saguaro seedlings	Wounded	**				*
(*Carnegiea gigantea*)	Unwounded	-				-

[a]Ratings

- Non-pathogenic

* Weakly pathogenic

** Strongly pathogenic

The pathogenicity tests on saguaro plants were conducted on plants growing in soil in the greenhouse. The plant parts were surface sterilized with mercuric chloride, 1:1000, rinsed with sterile water and placed in plastic dishes with added moisture. Inoculations were made on both wounded and unwounded plant parts. The wound inoculations were made with a sterile scapel by cutting a small slit in the tissue and inserting a piece of a mycelial mat. The unwounded series was inoculated by placing a piece of mycelium on the surface of the plant parts.

Growth

It was necessary to culture the fungus aseptically to identify it, to produce inoculum for greenhouse trials, and for use in vitro. Phytophthora drechsleri grew well on both potato dextrose agar and corn meal agar[5] but on the latter, growth was thin and spreading. On lima bean agar and wort agar the growth was slow. Growth was very limited in nutrient broth, Richard's solution and Hoagland's mineral solution to which 20 gm dextrose per liter was added. The isolate grew well on filtered canned tomato juice diluted with distilled water. The dilutions were 1:1, 2:3, and 3:7. The 3:7 dilution of tomato juice was utilized to provide inoculum for use throughout the study.

Sporulation

The isolates of P. drechsleri did not sporulate on any of the media used. Since sporulation was necessary for identification, and

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[5]All standard media were "Difco" prepared media, or prepared according to the "Difco" manual.

for use in certain inoculations the mycelium was transferred from tomato juice to distilled water. With this treatment sporulation was stimulated and an abundance of sporangia were produced (Fig. 1). This technique to induce production of sporangia had already been reported (15) for various species of Phytophthora.

Zoospores were produced by transferring well-developed mats of mycelium grown on a liquid medium to a petri dish containing sterile distilled water. After two or three days when sporangia had developed, zoospore release was stimulated by transferring a mat to fresh distilled water, or by changing the water in the dish. After about one hour the water contained numerous active zoospores. This liquid was poured off, and the process was repeated for a further supply of zoospores.

Oospores were rarely produced in culture. They were observed in only one case in an almost completely dehydrated plate culture of the fungus on potato dextrose agar. Pairing all isolates in all possible combinations did not stimulate oospore production, as has been reported for other species of this genus (2). The use of avacado root extracts, which Zentmeyer reported as stimulatory (40), also failed.

Numerous sporangia were measured; the measurements being in general agreement with those of other workers for P. drechsleri. Leonian and Greer (23) have agreed however, that sporangial dimensions can not be of primary importance in taxonomy. Germinating sporangia are shown in Figure 1. Abberant sporangia were found germinating from more than one opening. One of these is shown in Figure 1. As the

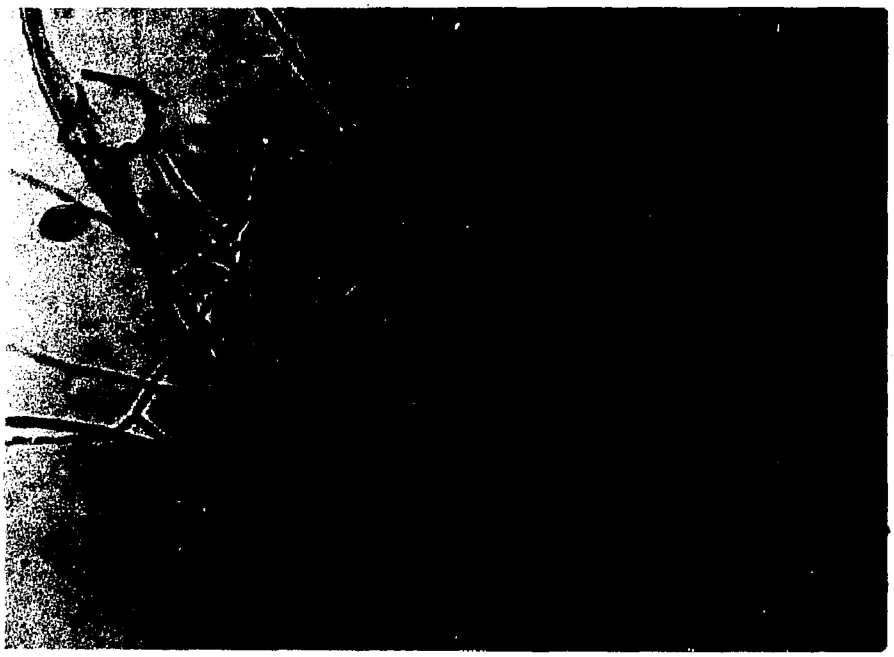

Figure 1. Sporangia in various stages of maturity. One is releasing zoospores (A), and another has germinated by more than one opening (B). Approx. 210 X

sporangia approached maturity the contents showed some movement. The apex then ruptured and several zoospores were ejected as if by internal pressure. The remainder of the zoospores could be seen swimming inside the sporangium for a time and then swimming out through the small apical opening. After they were free of the sporangium the zoospores swam for about 20 to 90 minutes and came to rest against a surface. The flagella were then lost, the cell wall became thicker, and the spores proceeded to germinate by a germ tube. These spores were strongly adherent. Slides, placed under colonies sporulating in water, could be removed, rinsed and the zoospores stained.

Nutritional Requirements

To learn something about the nutritional requirements of *Phytophthora drechsleri*, a basal medium was devised following suggestions given by several authors (9, 18, 22, 25, 27). The basal medium contained the following in grams per liter: KH_2PO_4 1.36, Mg $SO_4 \cdot 7H_2O$ 2.47, Ca Cl_2 2.78, Thiamine 0.1, Sucrose 40.0, Asparagine 4.0 and a trace of $FeCl_3$, Cu SO_4, $MnCl_2$, and $ZnSO_4$. It was found that in addition to commonly used mineral salts, calcium was necessary for growth. Also, no growth could be obtained on any synthetic medium without the addition of thiamine. This explains the poor growth on modified Hoagland's and Richard's solution. Sucrose was used as a standard carbon source at 40 grams per liter, and asparagine as a standard nitrogen source at 4.0 grams per liter. The activity of the fungus on various carbon sources substituted for sucrose at the same concentration is shown in Table 2. Four replications of 10 ml of

TABLE 2

Effect of Carbon Sources on Growth and Sporulation of *Phytophthora drechsleri*

Carbon Source	Linear Growth[a]	Dry Weight	Sporulation[b]
	cm	gm	
Sucrose	4.3	.0104	3
Lactose	3.7	.0027	3
Maltose	3.3	.0116	3
Mannitol	4.6	.0029	3
Xylose	3.3	.0037	3
Cellobiose	2.8	.0046	3
Soluble Starch	4.4	.0058	3
Pectin (Citrus)	1.7	.0019	2
Sodium Pectate	1.2	.0023	2
Pectic Acid	.0	-	-
Polygalacturonic Acid	.0	-	-
Galacturonic Acid	.0	-	-

[a]Growth after four days measured in cm up the tube.

[b]Sporulation was estimated:

1. Sparse
2. Moderate
3. Profuse

liquid medium in standard 18 by 150 mm culture tubes were capped with a stainless steel cover and sterilized. The inoculum was mycelium from a liquid culture which had been rinsed in sterile distilled water. Small pieces which were not floating were selected and transferred aseptically to the tubes. After four days, linear growth was measured from the bottom of the tube to the tips of the advancing hyphae. The growth in weight was measured after four days by removing the mycelium from the tubes, rinsing in distilled water and placing the mycelial mats on tared two-inch squares of aluminum foil. After drying the mycelium at 80 deg C for 1 hour, the weight of growth in each tube was calculated. The mycelium from one replication was transferred to petri dishes of sterile distilled water incubated for two days and sporulation estimated as follows: 1 sparse, 2 moderate, 3 profuse.

In the course of the experiment, the density of the mycelium as observed in the cultures was noticeably different in some of the treatments. The mycelial density was calculated in milligrams dry weight per centimeter linear growth, but no relationship to the various treatments could be found.

Phytophthora drechsleri has the ability to utilize a variety of carbon sources. However, no growth occurred on pectic acid, polygalacturonic acid, or galacturonic acid. These acidic pectic products probably lowered the pH of the media to a level unfavorable for growth. The pH values of the media containing acidic pectic products after termination of the experiment were found to be about 3.0. Media containing pectic acid and polygalacturonic acid had a relatively large amount of precipitate, which may have been calcium pectate and calcium polygalacturonate. This would reduce the soluble calcium which has been suggested as a protectant against other cations as hydrogen, sodium and potassium (9). This could explain the selective growth on certain pectic materials which did not precipitate with calcium.

The ability to utilize various nitrogen sources was tested by substituting them in the basal medium in place of asparagine at the same concentration of 4 gm per liter. The experiment was run using the same methods as were used in the carbon utilization tests. The nitrogen sources utilized are shown in Table 3. Glycine was found to be the best for growth in dry weight, and gelatin was the best source for linear growth. The viscosity of the gelatin might account for high linear growth, since it may help support the mycelium as it grew up the tube. Inorganic nitrogen sources supported growth as well as organic sources, with the exception of potassium nitrite. No relationship between the amount of nitrogen in the nitrogen source and growth could be found. This experiment was modified, with some deletions and additions of nitrogen sources. In the latter experiment the nitrogen sources were added so that each treatment received the same weight of nitrogen as was added in 4 gm of asparagine per liter. This experiment was carried out and read in the same manner as described for the carbon source experiment except that all treatments were adjusted to pH 6.0. The results are shown in Table 4. In both cases when tyrosine was used the amount added was greater than was soluble and crystals were present. When

TABLE 3

Effect of Nitrogen Sources on Growth and Sporulation of *Phytophthora drechsleri*

Nitrogen Source	Linear Growth[a]	Dry Weight	Sporulation[b]
4 gm /liter	cm	gm	
Potassium Nitrate	2.0	.0057	3
Potassium Nitrite	.0	-	-
Ammonium Chloride	1.5	.0035	1
Ammonium Nitrate	1.2	.0031	1
Gelatin	2.4	.0049	3
Casein	1.7	.0036	3
Peptone	0.9	.0039	3
Glycine	2.0	.0100	3
Asparagine	1.9	.0038	3
Proline	1.8	.0037	3
Arginine	0.2	.0003	3
Tyrosine	1.9	.0017	2
Glutamic Acid	0.6	.0014	3
Aspartic Acid	0.6	.0027	2
Tryptophane	0.8	.0027	3
Leucine	1.8	.0027	3
Methionine	1.8	.0026	1
Yeast Extract	1.7	.0048	3
Cystine	0.4	.0016	2
Glutamine	1.2	.0036	2

[a]Growth after four days measured in cm up the tube.

[b]Sporulation was estimated:

1. Sparse
2. Moderate
3. Profuse

TABLE 4

Effect of Nitrogen Sources on Growth and Sporulation of *Phytophthora drechsleri*

Using .061 M Nitrogen Concentration

Nitrogen Source	Average Linear Growth	Average Dry Weight	Sporulation[a]	Final pH
	cm	gm		
Potassium Nitrate	2.3	.0088	3	4.7
Ammonium Chloride	1.7	.0049	1	3.9
Ammonium Nitrate	2.7	.0049	3	4.6
Peptone	1.3	.0062	3	4.9
Yeast Extract	2.5	.0037	3	4.9
Asparagine	2.0	.0061	3	5.0
Alanine	2.0	.0052	3	4.8
Arginine	2.0	.0041	3	4.7
Aspartic Acid	1.4	.0026	3	5.2
Cystine	1.2	.0027	3	4.9
Glutamic Acid	1.3	.0030	3	5.2
Glycine	1.9	.0018	3	4.7
Isoleucine	1.4	.0031	3	4.7
Leucine	1.5	.0022	3	4.9
Methionine	1.6	.0047	3	4.8
Phenylalauine	1.4	.0056	3	4.8
Proline	1.9	.0047	3	4.5
Serine	1.8	.0085	3	4.8
Tryptophane	1.0	.0040	3	4.7
Tyrosine	1.2	.0024	1	4.2
Valine	1.9	.0084	3	4.7
Combination of all Acids	2.1	.0083	3	4.9

[a]Sporulation was estimated:

1. Sparse
2. Moderate
3. Profuse

the pH was adjusted to 6.0 a small amount of precipitate resulted. No growth occurred in potassium nitrite. Its use may have resulted in a medium with an oxidation potential too high for growth. Potassium nitrate was found to support growth as well as any other source of organic or inorganic nitrogen tested. Ammonium chloride supported growth but was found to be inhibitory to sporulation.

Infection Studies

In order to follow the progress of infection, plants were grown in aerated nutrient solution in five liter aluminum cans, 11.6 inches deep. Two holes were drilled in the lids of the cans, one to support the plants and a second to allow aeration. Cotton or glass wool was wrapped around the hypocotyl of seedlings grown in quartz sand, and the plants were placed so that the roots were suspended in the solution. To aerate the solution, glass tubing 1/8 of an inch in diameter was drawn out and cut off so that the small end was constricted. The glass tubing was put through the second hole in the lid so that the tip was within one inch of the bottom of the can. Cotton was wrapped around the tubing where it entered the lid so that dust was excluded and contamination was reduced. The cans were filled with Hoagland's solution (17) and the apparatus was sterilized. The plants grew well and did not show any deficiency symptoms. However, a powdery mildew not previously reported in the United States (3), appeared on the older leaves. Distilled water was added to the tanks during the experiments to keep the solution level to within 2 to 3 inches from the top of the cans.

Inoculations and Examination

The temperature was not controlled in the cans and the temperature of the nutrient solution varied between 19 and 24 deg C. Air temperature in the greenhouse varied between 16 and 26 deg C. Inoculations were made by adding a zoospore suspension to the nutrient solutions in the tanks. Samples of roots were harvested at intervals during the course of the experiment. The roots were killed and fixed in formal acetic alcohol[6]. The roots were then dehydrated using Johansen's (19) tertiary butyl alcohol method, sectioned and stained with safranin and fast green. The sections were examined microscopically and infections were found in roots harvested after four and one-half hours exposure to zoospores. The zoospores lost their flagella, rounded up and germinated. The germ tube was formed either in the portion adhering to the root or on a non-adhering portion of the spore toward the root epidermis. Penetration was direct without the formation of appressoria or other auxiliary structures. After entry the infection hyphae appeared to be reduced in their diameter throughout. After gaining entrance to the root the hyphae were inter- and intra-cellular and no specialized structures resembling haustoria could be found. After nineteen hours, the infection hyphae had progressed into the root tissue (Fig. 2) to an extent comparable in length to hyphae from germinating spores on slides after a similar length of time. The spore and original germ tubes appeared, by their staining reaction, completely devoid of protoplasm.

[6]Made up with 125 ml 37% formaldehyde, 1305 ml 95% ethanol, 125 ml glacial acetic acid, and 945 ml distilled water.

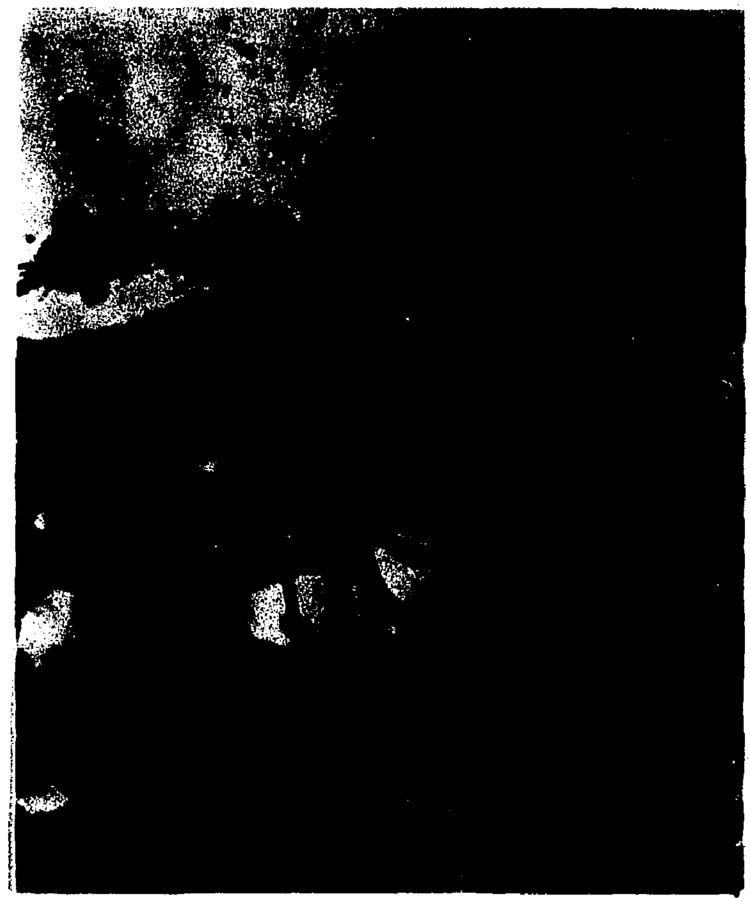

Figure 2. Infection of safflower root by zoospores, showing spores at the surface and infection hyphae penetrating the tissue. Approx. 420 X

Phytotoxic Effects

It seemed possible that the fungus could produce an inhibitor or a toxin that could influence germination of safflower seed. To examine this possibility, seeds were germinated in sterile culture filtrates of the fungus. Five cultures of P. drechsleri were grown on dilute canned tomato juice for nine days. Each culture was passed through sterilizing filters, along with uninoculated tomato juice and a water control. Four ml of each of the filtrates were added to each of nine replications in petri dishes, and 15 surface-sterilized N 10 seeds were placed in each dish. After two days the number of seed germinating in each plate was counted. The results are shown in Table 5. The least germination was found to be the uninoculated medium control. The sterilized culture filtrates appeared to enhance germination. Filtrates from culture No. 10923 produced the least stimulation. This culture grew the slowest, so its inhibition may have been due to less activity by the fungus in the medium, which was inhibitory. The seedlings were allowed to continue growth, and examined periodically. No injury or inhibition to growth could be noted.

Histopathological Effects

To determine the type of injury to safflower by P. drechsleri, tissue was exposed to the fungus in various ways. Safflower stems were collected in the field and the leaves were removed. The stems were surface sterilized with a 1:1000 solution of mercuric chloride and rinsed in sterile water. Discs of stem tissue were prepared by

TABLE 5

The Effect of Culture Filtrate on Germination of N 10 Safflower Seeds

Culture	Number of Seeds Germinating 135 Seeds/Treatment	Per Cent Germination
#3	54	40.0
#4	54	40.0
#5	48	35.5
#6	48	35.5
#10923	34	25.2
Sterile Medium	28	20.7
Sterile Water	34	25.2

cutting, under aseptic conditions, cross sections about 1 cm. thick. These were placed in sterile petri dishes. Small pieces of dialyzing membrane were placed on ten of the discs so that the edge of the membrane extended over the edge of the disc. Millipore sterilizing filters were placed on ten other discs in a similar manner. A second disc of stem tissue was placed on those covered with dialyzing membrane and Millipore filters, and ten were left without any separation. The top disc in each case was inoculated with P. drechsleri. After four days the discs of tissue were examined. In all cases the inoculated discs on top were darkened, decomposed, and shrunken. The lower discs which were not separated from the upper discs appeared the same as the top disc of tissue. The lower discs covered with filters or membranes appeared the same as the uninoculated controls. They were not shrunken and were discolored only in areas where the cut surface was exposed to air. However, the tissue was so softened that a slight pressure would cause collapse.

Tissue Extractions

Five varieties of safflower were grown in the field to supply material for laboratory experiments. Normal cultural practices were followed with the exception of dense planting and thinning. Extractions of both roots and shoots were made following standard methods (29). Roots and shoots were harvested in the field, washed with tap water, and the surfaces allowed to dry. The parts to be extracted were weighed and then placed in plastic bags and slowly frozen to rupture cells. In all of the operations with the exception of testing on the fungus the material was stored below 8 deg C. The plant material was then ground with an equal weight of distilled water in a Waring blender and poured through cheese-cloth to remove large particles. Fifty ml portions of both roots and shoots of each variety were mixed with an equal volume of each of the following: Ether, acetone, and ethanol. To a 50 ml portion, 1 ml concentrated sulfuric acid was added. To another 1 ml 2N potassium hydroxide was added and one was left as a water extract. All but the other extracted samples were then centrifuged at 3000 g and the supernatant liquid poured off. Small portions of the residue were transferred to petri dish cultures of _P. drechsleri_. The cultures were examined for inhibition of growth. Bacterial contamination was common on the residue from the water extraction, but the others were comparatively free from contamination. The ether extracted sample was shaken vigorously in a separatory funnel and allowed to separate. The ether fraction was evaporated to about 2 ml into which filter paper sensi-discs were dipped. The sensi-discs were air dried and tested on agar in petri dishes inoculated with _P. drechsleri_. The supernatant liquid from extractions with volatile solvents were reduced under low pressure to slightly less than 50 ml and centrifuged. The sediment was tested as previously described and the supernatant liquid was tested in peni-cylinders. The potassium hydroxide extraction was neutralized with sulfuric acid and tested. The sulfuric acid extract was neutralized with potassium hydroxide and the resulting precipitate centrifuged. The residue and supernatant liquid were tested as mentioned above.

Diseased plant extracts were made in a similar manner except that the leaves were removed, and the stems were cut to convenient lengths, placed in plastic boxes, sterilized with 5 per cent Clorox rinsed with sterile distilled water. Inoculations with a virulent culture of P. drechsleri were then made. When the stems were about 50 per cent infected, the plant material was ground, extracted, and tested by the same method as was healthy tissue. No inhibition of P. drechsleri could be found in any of the healthy or diseased plant extracts.

An absorption spectrum of the liquid extracts running from 350 to 925 millicrons was found on a Bausch and Lomb Spectronic 20. Readings above 650 were made with an infra-red phototube and filter. No significant variations in the optical density curves among the extracts from different varieties were found within the range of this test.

To test the effect of the diseased plant extract on safflower, the water extracts of N 10 and Gila were passed through sterilizing filters. Two ml of each extract was injected into stems of mature safflower plants in the field. One plant each of five varieties and five replications were arranged in a Latin square design for each of Gila and N 10 extracts utilized. The plants were periodically examined for external symptoms but none were found. After 26 days the plants were harvested, the stems split, and examined. The pith of the stems showed a brown discoloration extending most of their lengths. The discoloration was estimated visually and the results were analyzed. No significant differences in discoloration could be found between extracts of Gila and N 10 in any of the varieties.

Root Growth

It was occasionally observed in the hydroponic tank inoculations that healthy roots extended down between completely killed roots. Several explanations for this come to mind. These could have escaped fungus infection or new growth could have been stimulated by the loss of roots. To find if resistant and susceptible roots had different rates of regrowth, Gila and N 10 were grown in a root box (20). A galvanized sheet metal box 24" x 18", 18" high with a sliding door, in which was installed a fine spray nozzle (Monarch F-110-C 4.680) was used. Holes one-half inch in diameter, and 3 inches apart were drilled in the top. Glass wool was wrapped around the hypocotyls to support the plants and the roots were suspended in the box (Fig. 3). Lawn sprinkler nutrient cartridges were placed in the pipe occasionally and iron citrate and ammonium nitrate were used to reduce chlorosis. The plants grew moderately well but occasionally showed chlorosis. The roots were allowed to grow down into the box and were pruned to six inches below the root stem transition three times during the experiment. The prunings from each of the plants were placed in vials and dried. After 49 days the remaining roots and shoots were harvested and also dried in vials. All the dried plant parts from replications were weighed. The results, shown in Table 6, were analyzed by the paired comparison method. No significant differences were found between Gila and N 10 in the weight of roots pruned, weight of roots remaining, or the total pruned and remaining roots. However, Gila had significantly heavier shoots and N 10 had a significantly higher root: shoot ratio. To find whether pruning had an effect on the growth,

Figure 3. A view of the root box used in the pruning and root:shoot ratio experiments. Week old plants are shown in place.

TABLE 6

Average Dry Weight of Safflower Plant Parts
gm

Pruned Prunings	N 10	Gila
1	.0099	.0052
2	.0095	.0134
3	.0043	.0042
Total	.0237	.0228
Roots Remaining	.0316	.0352
Total Roots	.0553	.0580
Shoots	.0518	.1288**
Root:shoot Ratio	.644	.276*

*Significant, .05 level

**Highly Significant, .01 level

the experiment was repeated. Half of each variety was pruned, the others were allowed to grow. The plants were collected after 46 days, dried, and weighed in the same manner as described above. Results are shown in Table 7. These results were also analysed by the paired comparison method. Significant differences were found between roots, shoots, and root:shoot ratio. Gila had lighter roots and heavier shoots and a lower root:shoot ratio than did N 10.

To determine whether these root-growth characteristics shown in Gila are common to other resistant safflower varieties, a randomized block experiment was set up using five varieties and seven replications. The plants were allowed to grow in the root box unpruned for 40 days. Then they were harvested, dried, and weighed. The results are shown in Table 8. No significant differences were found except between total weight of plants. However, the calculated F value for the root:shoot ratio was 2.7621, with 24 and 4 degrees of freedom. This is slightly below the tabular F value. This may have been due to the use of fewer replications than in the previous experiment in which significance was found between Gila and N 10.

TABLE 7

Average Dry Weight of Safflower Plant Parts
Pruned and Unpruned
gm

Pruned Prunings	N 10	Gila
1	.0108	.0076
2	.0106	.0156
3	.0028	.0015
Total	.0242	.0249
Roots Remaining	.0426	.0383
Total Roots	.0668	.0632**
Shoots	.0965	.1771**
Root:shoot Ratio	.690	.356 *
Unpruned		
Roots	.0605	.0553**
Shoots	.0978	.1924**
Root:shoot Ratio	.619	.262 *
Composite Root:shoot Ratio	.637	.318 *

*Significant, .05 level

**Highly Significant, .01 level

TABLE 8

Average Dry Weight of Different Varieties of Safflower Plant Parts

gm

Varieties	Susceptible	Resistant			
	N 10	Gila	N 8	WO 14	US 10
Roots	.0626	.0677	.0526	.0570	.0715
Shoots	.1004	.1650	.1235	.1177	.2014
Total Roots and Shoots	.1630	.2357	.1761	.1747	.2729
Root:shoot Ratio	.692	.407	.562	.562	.372

DISCUSSION AND CONCLUSIONS

The taxonomy of the genus *Phytophthora* is unavoidably confused, due to the inherent variability in its species. This was evident during this study, particularly with respect to the pathogenicity of the several isolates. *Phytophthora drechsleri* has been accepted as an authentic species by most workers, but its validity has been questioned (24). A modern systematic study is long overdue. The genus as now recognized is composed of highly variable species and the identifications must be viewed with this in mind.

Some difficulties in working with the fungus were encountered, during the work reported here. The lack of sporulation on solid media was overcome by transferring mycelium from liquid media to sterile distilled water to stimulate sporulation. Very poor growth on synthetic and semi-synthetic media was avoided by the use of natural substrata until the essential nutrient requirements were determined. A synthetic medium was necessary to establish the utilizable carbon and nitrogen. After several attempts it was found that in addition to usual ingredients, calcium and thiamine seemed necessary for growth. Calcium may not be absolutely required for growth, as it has been shown to have a protectant action against certain monovalent cations such as hydrogen, sodium, and potassium. An adjustment of various mineral salts could possibly eliminate the calcium requirement (9). Most of the carbon sources tested were utilized, including cellobiose and some pectic materials. These data tend to show the ability of the fungus to hydrolyze cellulose and pectin. Such knowledge is important in a study of parasitism. No growth was found on acidic pectic products as pectic acid, polygalacturonic acid, and galacturonic acid. This was probably due to the depressed pH value of the media containing these acidic pectic materials, as sodium pectate and pectin were utilized. Starch was utilized as an energy source but sucrose and maltose were found to be the best carbon sources. *Phytophthora drechsleri* utilized a wide variety of nitrogen sources, and only nitrite did not support growth at the concentration used. This may have been due to a high oxidation potential of the medium due to the nitrite. Sporulation was depressed when ammonium salts were supplied as the only nitrogen source, but amino acids, proteins, and other inorganic salts supported good sporulation in most cases.

The infection of safflower plants was followed in hydroponic culture. The roots were inoculated with zoospores, sectioned, and the infection followed microscopically. After four and one-half hours the roots were infected. The zoospores had lost their flagella and germinated. The infection hyphae penetrated the root without accessory structures, but were reduced in diameter after penetration. The hyphae were inter- and intra-cellular and no specialized structures resembling haustoria were present. After nineteen hours the hyphae were comparable in length to those germinating in water. The sections were carefully examined for any anatomical resistance reactions (12) but none were found.

Filtrate from tomato juice cultures of *P. drechsleri* was tested for adverse effects on germination and seedling growth in petri dishes. No inhibition of germination or injury to seedlings could be found. The germination was greater in culture filtrates than those from in the uninoculated medium. Discs of safflower tissue inoculated with P. drechsleri were completely decomposed after four days. The tissues which were separated from infected tissue by dialyzing membrane or millipore sterilizing filters was discolored only where cut surfaces were exposed to air. In both cases the tissue was softened, and any pressure would result in maceration of the tissue. This feature, in addition to the utilization of pectinaceous materials, presents evidence for the ability of P. drechsleri to produce pectinase.

Extracts from both roots and shoots from the five varieties of safflower, were tested for any inhibition of growth of P. drechsleri. The extractions were made with ether, acetone, ethanol, base, acid, and water followed by centrifugation. The supernatants and residues were tested without finding any inhibition. Diseased plant material was tested in a similar manner and again no inhibition of growth was found. Mature safflower plants injected with 2 ml of the diseased plant water extract, which had been filtered through sterilizing filters, showed a brown discoloration in the pith after 26 days. No differences in intensity of the reaction between resistant and susceptible plants or resistant and susceptible plant extracts could be found. During these tests neither plasmic nor anatomic characteristics could be found which might account for the resistance of some varieties of safflower.

When grown in a root box in the greenhouse resistant and susceptible varieties of safflower were found to have significantly different root:shoot ratios. The root:shoot ratio of susceptible plants was about double the ratio found for resistant plants. The derivation of resistant varieties by transferring resistance by a series of backcrosses to a susceptible variety tends to support the relationship of a low root:shoot ratio and resistance. More distantly related resistant plants similarly showed a low root:shoot ratio. The association of resistance with a low root:shoot ratio might be accounted for in several ways. A smaller root system exposes less surface area to infection and acts as an escape mechanism. This would explain the lack of immunity to root rot in safflower. Another explanation, if resistance is an energy requiring reaction of the roots a greater energy potential of a proportionately larger shoot supplying a smaller root system would be an advantage to roots exposed to infection.

SUMMARY

Several isolates of *Phytophthora drechsleri* were obtained from infested soil, and along with the American type culture collection isolate, were compared as to pathogenicity. Differences were found between all isolates examined, indicating the existence of physiological strains.

Since *Phytophthora drechsleri* could not be maintained on commonly used synthetic media, a medium containing calcium and thiamine was developed which supported good growth. The ability of the fungus to utilize various carbon and nitrogen sources was examined. It was found that a wide range of carbon sources supported growth and ammonium and nitrate salts, amino acids, and proteins were all utilized as nitrogen sources.

The fungus did not sporulate on solidified agar media. It was found that when mycelium was transferred from a liquid medium to distilled water, sporangia were formed abundantly. Another change of water stimulated the release of zoospores. Attempts to produce oospores by pairing cultures and use of various media were unsuccessful. Germination of sporangia was observed. The zoospores were released and were free-swimming for a short time, then adhered to a surface and germinated by a germ-tube.

The type and progress of infection was followed in plants inoculated with zoospores in aerated hydroponic culture. Germ-tubes penetrated directly and grew through the root tissue without any apparent resistance reaction on the part of the host. Infections were found after four and one-half hours and the hyphae had progressed in growth at a rate comparable to that in vitro. The effect of culture filtrate on seed germination and growth were tested, but no inhibition could be found. The uninoculated medium showed the greatest inhibition to germination. Discs of stem tissue were exposed to infection in various ways. Those inoculated directly were shrunken, darkened, and decomposed. Tissue separated from infected tissue by dialyzing membranes or sterilizing filters appeared normal but was softened and any pressue would macerate the disc. Extracts were made from diseased and healthy safflower plant parts using ether, acetone, ethanol, and water of high, low, and neutral pH. The extracts were examined spectrometrically and were tested for an ability to inhibit P. drechsleri. No inhibition could be found, but when the aqueous diseased plant extract was injected into safflower, it stimulated an extensive brown discoloration.

Root regrowth and root:shoot ratio were measured by growing plants with roots suspended in a box with a fine spray of water. Pruning had little effect on root growth, but N 10 and Gila had significantly different root:shoot ratios. A lower root:shoot ratio was found to be related to resistance.

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