PYTHIUM (BROWNING) ROOT ROT AND POSSIBLE INVOLVEMENT OF TOXINS IN DISEASE

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INTRODUCTION

Pythium (browning) root rot can be caused by as many as 12 Pythium species. The disease is characterized by brown appearance of young diseased plants that generally occur in patches. Several of the causal pathogens are known to produce toxic metabolites (in vitro) that cause root symptoms which resemble that of the pathogen infection. This indicates that there may be a possible involvement of toxins in disease caused by these pathogenic species.

The objectives of this investigation were: 1) to identify pathogenic Pythium species isolated from field-grown wheat plants, 2) to determine in vitro toxin production by these pathogenic isolates and 3) to partially characterize the toxin produced by one of the pathogenic isolates.

This thesis is written in a format that will facilitate submission to the journal of Plant Disease or Phytopathology. Approval for presenting the thesis in this manner is based upon the Graduate College's policy of accepting a thesis written in manuscript form, and is subject to the Graduate College's approval of the major professor's request for a waiver of the standard format.

CHAPTER II

PYTHIUM (BROWNING) ROOT ROT AND POSSIBLE INVOLVEMENT OF TOXINS IN DISEASE.

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ABSTRACT

Two Pythium species, isolated from the roots of field grown wheat plants and found to be pathogenic to wheat seedlings, were identified as being P. arrhenomanes (Pa) Drechs. (isolates #320 and 323), and P. irregulare (Pi) Buis. Cell-free culture filtrates of both species grown in glucose glutamic acid liquid medium, caused severe inhibition of root elongation, as well as significant reductions in fresh and dry root weights of wheat seedlings (cv. TAM-101) after 3 days of exposure. Culture filtrates of Pa also caused significant inhibition of root hair formation, as well as browning, but had no effect on shoot growth (after 3 days af exposure). The Pa filtrates greatly lost their efficacy after autoclaving. Culture filtrates of Pi on the other hand, did not cause any inhibition of root hair formation nor any browning, but retained their efficacy after autoclaving, indicating that these toxins were biochemically different. Pi culture filtrates also caused significant inhibition of shoot growth after 3 days. Pa (isolate #320) was found to produce a toxin(s) which was heterogeneous. One part being heat stable and of M.W. greater than 5000, and the other being thermolabile, and of M.W. less than 5000. Both parts had to be combined in order to get similar toxicity effects as the raw culture filtrate. The culture filtrate (isolate #320) was found to retain its toxic activity after freezing.

INTRODUCTION

Pythium (browning) root rot caused by a number of Pythium species affects all small grain crops as well as forage grasses (18). Browning root rot of wheat (Triticum aestivum L.) was first investigated by vanterpool and Co-workers in Canada in the early 1930's (16). They called the disease "browning" root rot because of the scorched, brown appearance of young diseased plants that occurred in patches on the Canadian prairies. Since then the disease has been observed throughout the Great Plains as well as other regions of the U.S.A, in Australia, England, and in India (4, 12, 20, 22). Diseased seedlings have restricted root growth with brown, water-soaked lesions on their seminal and crown roots. Restriction of root growth leads to leaf die-back, stunting, poor tillering, delayed maturation, and poor yields. Individual diseased plants have been found to yield 20 to 80% less than normal plants, so that reduction in yield of a whole field may be as high as 10 bushels per acre (18). Further loss is usually incurred by a reduction in grain quality. Disease tends to be most severe in wet compacted soils, particularly those that are deficient in phosphorus.

Production of toxins presumably involved in disease development has been studied for a number of Pythium species (1, 2, 3, 5, 7, 10, 11, 15, 17, 26). Vanterpool (17) provided evidence that P. arrhenomanes Drechs., a root rot pathogen of wheat and other cereals, produced a toxin that caused darkening of wheat kernels as well as browning of the roots of young wheat seedlings. The toxin was found to be heat stable (autoclaved),

and its production dependent on the substrate on which the fungus grew. Csinos et al (2, 3) showed that non-viable mycelium (ground and lyophilized) of P. myriotylum Drechs. contained a heat-stable toxin which inhibited root growth of tomato seedlings, and in severe cases the toxin caused browning of both roots and hypocotyls and killed the seedlings. Blok (1) using P. irregulare Buisman, P. paroecandrum Drechs. and P. sylvaticum Campbell and Hendrix, found the auxin IAA in culture filtrates of all three species. Roots of flax or wheat exposed to cell-free culture filtrates from living mycelium started to swell in the region behind the root tip as a result of lateral growth of cells, and often produced large numbers of root hairs. Root elongation was also found to be inhibited. Although IAA auxin was present in the culture filtrates, some other heat-stable substance was thought to be involved, because IAA alone did not cause prolonged inhibition of root growth, whereas the culture filtrate did. Sadik et al (10) isolated and partially characterized an extracellular phytotoxin produced by P. aphanidermatum (Edson) Fitzp., when grown in Czapek-Dox broth. The toxic metabolite was found to be non-dialyzable and lost its biological activity on acid hydrolysis. It was further characterized as a heterogeneous glycopeptide having a molecular weight of 20-40 x 10^3.

The objectives of this study were: a) to identify field isolates of Pythium species causing browning root rot, and b) to determine the pathogenicity of these isolates to wheat seedlings, and c) to test their cell-free culture filtrates for presence of toxin(s), through the use of a standard bioassay technique, and d) to partially characterize the toxin produced by one of the pathogenic isolates.

MATERIALS AND METHODS

Isolation and identification

Field grown hard red winter wheat plants (cv. TAM-101, Amigo, Danne, Sage, in Stillwater, OK) with browned or rotted roots were collected in the Fall of 1985. Diseased roots were first washed with tap water, and then detached from the shoot and placed on top of moist sterilized sand contained in large glass tubes (27 mm dia.). Several wheat kernels (cv. TAM-101, CI 15324) were then placed on these roots after which the tubes were watered, then covered with more sand and incubated at room temperature (25 ± 2 C). Newly developed roots became infected by the existing causal pathogens within 3-4 days of incubation. Roots from infected seedlings were separated and washed with tap water, and then plated directly on corn meal agar (1/5 strength) and incubated at 15 ± 1C. After 24 hr, organisms (mainly *Pythium* species) growing out of the roots were isolated and transferred onto corn meal agar plates and grown for a week (at 15 ± 1 C) for identification.

To induce zoosporangia formation, 1-2 cm pieces of dried wheat leaves were boiled for 10 min. in sterile water, then placed in test tubes that contained water (autoclaved deionized / filtered pond water; 2 : 1; v / v), and 3-4 culture discs (3 mm) from a *Pythium* species grown on corn meal agar for 5-7 days at 15 ± 1 C. Test tubes were then incubated at 20 C for 24 hr, after which the leaves were transferred to fresh sterile petri plates and incubated at 20 C for 2-3 days or until sporangia were produced.

For the purpose of identification, keys by Waterhouse (23) and Middleton (6) were used. The more recent *Pythium* monograph by Plaats-Niterink (21) however, was also referred to later.

Pathogenicity tests

Identified species were tested for pathogenicity to wheat seedlings (cv. TAM-101, CI 15324) following the technique of Singleton and Ziv (13).

Testing isolates for toxin production

Growth medium for toxin assay

Cultures of Pythium species were grown in 250 ml flasks containing 100 ml of a glucose glutamic acid liquid medium modified from Csinos (2), that contained 19.5 g glucose, 7.25 mg glutamic acid, 1.0 g KH_2PO_4 , 0.5 g $MgSO_4 \cdot 7H_2O$ and 2.0 mg thiamine HCl per liter. The PH was adjusted to 6.0 with KOH and the medium autoclaved (1.1 kg/cm^2) for 20 minutes. Pythium species grown on corn meal agar at 15 $\pm$ 1 C for 5-7 days were transferred (one culture disc, 5 mm, per flask) to the medium and grown for different periods of time (4-21 days) at room temperature (25 $\pm$ 2 C) with 12 hr of light (2700 lux.) and 12 hr of darkness.

Filtrate extraction

Culture filtrates were passed through a single coarse filter paper (Grade 201, Reeve Angel, Whatman Inc. Clifton, N.J.), then passed through a bacterial filter (0.2 µm, Acrodisc, Gelman Sciences Inc. Ann Arbor, Michigan 48106), before being used for the bioassay. Autoclaved uninoculated medium and sterile deionized water served as controls.

Bioassay procedure

Surface-sterilized wheat seeds (cv. TAM, W-101, CI 15324, ethanol: Clorox [5.25%], 1:1 v/v for 1 min 20 sec. in sonicator) were germinated on nutrient agar plates at 30 C for 40 hr before being transplanted into the growth system. Sterile seedlings with uniform root length (i.e. 1 ± 0.2 cm) were selected for the bioassay. The bioassay was performed as follows: Each seedling was transferred to a glass test tube (18 X 150 mm) containing 9 glass beads (size 6 mm) at the bottom to support seedling growth. One milliliter of the culture filtrate or a control was added to each test tube prior to transfer of the seedlings. Each test tube represented one replication.

Growth measurement and data analysis

After 48 hr of incubation (12 / 12; light / dark; 27 microeinst. m^{-2} sec^{-1}), seedlings while still in test tubes were observed for inhibition of root hair formation in all treatments with the aid of a stereoscope, and measurements of the distance from the root tip to the first visible root hair(s), as well as the length of the longest root hair at 5 mm distance from the root tip were made. A day or two later, plants were harvested for measuring root and shoot length (cm), as well as fresh and dry root and shoot weights. Data in all experiments was based on 8 replications per each treatment, and each experiment was repeated at least twice. Standard analysis of variance for randomized complete block analysis were used as outlined in Steel and Torrie (14).

Effect of P. arrhenomanes infection as compared to that of its culture filtrate

A number of seedlings were inoculated by placing surface sterilized germinated seeds (2-day-old) on culture plates (isolate # 320, 3-day-old, on corn meal agar) for 10 hr at 15 ± 1 C, and then transferred, at the same time with non-inoculated seedlings, to test tubes containing sterile medium, as well as to those containing sterile deionized water. Test tubes were then capped and incubated for 4 days at room temperature (25 ± 2 C) under 12 hr of light (27 microeinst, m^{-2} $sec.^{-1}$) and 12 hr of darkness.

Dosage of crude toxin

Twenty-day-old culture filtrate of P. arrhenomanes Drechs. (isolate # 320) was diluted (with sterile medium) to various concentrations (i.e 1X, 0.75X, 0.5X, 0.1X). Bioassay of all concentrations of toxin on wheat seedlings (cv. TAM-101) was performed, and a dosage response curve of toxin concentration against root length plotted, and a linear correlation established. Seedlings were incubated for 4 days at room temperature (25 ± 2 C) prior to taking measurements.

Thermostability of toxins

To determine if the toxic metabolites produced by the identified species are heat stable, culture filtrates were autoclaved for 10 min., prior to bioassay on both the autoclaved as well as the non autoclaved culture filtrates.

In another experiment, culture filtrates of P. arrhenomanes (isolate

320) were stored at -15 C for a week. Frozen filtrates were thawed at 25 ± 2 C and bioassayed. All experiments were performed at least twice.

Partial characterization of toxin

Molecular weight assessment

The approximate molecular weight of the toxin produced by *P. arrhenomanes* Drechs. (isolate # 320), was determined by ultrafiltration (Diaflo Ultrafilters, Amicon Corp. Lexington, Mass.) with 5000 M.W. cut-off, and bioassay was done with each of the 5000+ and 5000- M.W. portions of the filtrate as previously described. The retentates on the filters were reconstituted in sterile deionized water (same volume as the original culture filtrate) prior to the bioassay. Bioassay on the similar molecular weight portions of the autoclaved medium served as controls. After 48 hr of incubation, seedlings were observed for inhibition of root hair formation and root elongation, as well as signs of root discoloration (browning) in each of the treatments.

Reconstitution experiments

In separate experiments the 5000 M.W. retentate of the culture filtrate was reconstituted in both sterile deionized water, as well as in its own filtrate (5000- M.W. portion; that has passed through the filter) and bioassay was again performed on each portion, including observations on inhibition of root hair formation and other symptoms characteristic of the raw toxin. Crude cell-free culture filtrate was also bioassayed as well as the autoclaved uninoculated medium serving as controls.

RESULTS

Pythium species

The majority of *Pythium* species (90%) isolated from the roots of the wheat cultivar TAM-101, showed similar morphological characteristics. They produced lobulate sporangia in water, had several antheridia (more than 4 in most cases) associated with each oogonium, and their average oogonia size was 30 μ. Two isolates representative of this group that were collected at two different times were identified as being *P. arrhenomanes* Drechs. (isolates # 320 and 323) (table 1). Isolations from the roots of the three other wheat varieties (i.e Amigo, Danne, and Sage) all yielded species with much smaller oogonia (avg. size 18 u) of irregular contour. They did not produce sporangia under the specified conditions described, and there were only 1-2 antheridia associated with each oogonium in most cases. An isolate representative of this group was identified as being *P. irregulare* Buis. (table 1). All of the identified species were found to be pathogenic to wheat seedlings, as they all inhibited root elongation and caused severe root browning.

Effect of *P. arrhenomanes* culture filtrates on seedling development

Cell-free culture filtrates (13 or 18-day-old) of *P. arrhenomanes* (isolates # 320 and 323, respectively) caused significant inhibition of root hair formation, as well as a general root browning of wheat seedlings after 2-3 days of exposure (Fig. 1). Roots in contact with the culture filtrates were generally devoid of root hairs, or produced very short and dispersed ones as compared to those grown in the uninoculated control medium. Both isolates caused similar effects, however, isolate

320 after 13 days of growth in the medium, while the other (# 323) after 18 days.

Distance from the root tip to the first root hair was found to be significantly greater for those seedlings grown in culture filtrates of both isolates, with root hair length being shorter, as compared to those grown in the medium or water (Table 2). There were no significant differences between water and medium treatments on the basis of root hair formation.

Autoclaving the culture filtrates reduced their inhibitory effects to a great extent (Table 2). This was indicated by absence of root browning, and shortening of the distance from the root tip to the first root hair of seedlings grown in the autoclaved culture filtrates. However, inhibitory effects were not removed completely as observed, since root hairs were still not as densely formed as they were in the uninoculated medium (Table 2).

Culture filtrates (isolate # 320) also caused significant inhibition of root elongation as well as reduction of fresh and dry root weights after 3 days of incubation. However, there were no significant effects on shoot length, nor fresh and dry shoot weights after this same period of exposure (Table 3).

Autoclaving the culture filtrates again reduced their inhibitory effects significantly, particularly in terms of inhibition of root elongation, but did not remove it completely, since fresh and dry root weights of seedlings grown in the autoclaved filtrates were significantly less than those of seedlings grown in the uninoculated medium (Table 3).

Seedlings grown in water generally developed longer roots and shoots as compared to those grown in the medium. However, they generally had

significantly smaller dry root weights, while shoot weights did not differ significantly (Table 3). This trend was observed during the course of this study.

Effect of P. irregulare culture filtrates on seedling development

P. irregulare culture filtrates in comparison to those of P. arrhenomanes (isolate # 323), caused severe inhibition of both root and shoot growth, but did not cause any root browning, nor any inhibition of root hair formation (Table 4). Culture filtrates of both species significantly inhibited root elongation and greatly reduced fresh and dry root weights as compared to the uninoculated medium. However, P. arrhenomanes filtrate slightly stimulated shoot elongation, whereas that of P. irregulare significantly reduced shoot length, although it did not affect fresh and dry shoot weights significantly (Table 4). Seedlings grown in P. irregulare culture filtrates often developed swellings behind the root tips, and large number of root hairs, some very long, were formed directly behind the root tip.

Autoclaving had no effect on the activity of the culture filtrates of P. irregulare, while it significantly reduced the inhibitory effect of the P. arrhenomanes culture filtrates (Table 5). Root length as well as fresh root weights of seedlings grown in the autoclaved filtrates of P. irregulare remained significantly smaller than that of seedlings grown in the medium. However, no significant differences ($P = 0.01$) in root development was observed between seedlings grown in the autoclaved P. arrhenomanes culture filtrates and those grown in the medium (Table 5).

Effect of P. arrhenomanes infection as compared to its culture filtrate

The effect of P. arrhenomanes (isolate # 320) infection on wheat seedlings was comparable with that of its culture filtrate; both significantly reduced root length, as well as fresh and dry root weights as compared to the uninoculated medium (Table 6). However, Pythium-infected seedlings had significantly smaller shoots that weighed much less than those grown in the medium for 4 days. The culture filtrates did not cause any inhibition of shoot growth after this same period of exposure (Table 6). There were no significant differences in seedling development between infected seedlings grown in water, and those grown in the medium.

Culture filtrate activity as related to culture age

Toxicity was detected in the culture filtrates of P. arrhenomanes (isolate # 320) after 7 days of growth of the pathogen in the medium, and was greatest after 13 days (Table 7). Filtrates from 7-day-old cultures caused only slight inhibition of root hair formation, with no browning. Severe root browning, as well as significant inhibition of root hair formation resulted from filtrates of 13-day-old cultures.

Effect of freezing on culture filtrate activity

The toxic metabolite produced by P. arrhenomanes (isolate # 320) was found to retain its activity after freezing, making it possible to store the filtrate frozen for later use. Symptoms caused by the frozen-thawed culture filtrates on wheat seedlings, were similar to those caused by the untreated culture filtrates.

Dosage response curve of the crude toxin

Filtrate of P. arrhenomanes (isolate # 320) from 20-day-old culture was diluted with medium to obtain concentrations of 0.1, 0.5, 0.75 and 1 (full strength). A high negative linear correlation between the culture filtrate concentration and wheat seedling root length was obtained (Fig. 2). The slope of the regression line gives a measure of root growth inhibition between various filtrate concentrations.

Heterogeneity of toxin(s)

P. arrhenomanes (isolate # 320) was found to produce a toxin or toxins that was heterogeneous, one part of M.W. less than 5000, and the other of M.W. greater than 5000(Table 8). Each portion caused inhibition of root hair formation, as well as some inhibition of root elongation as compared to the medium, but neither alone caused any browning, nor their extent of root growth inhibition was as great as that of the raw culture filtrate (Table 8). Both components were needed to cause browning of the roots, as well as the same amount of root growth inhibition as caused by the raw culture filtrate.

Autoclaving the 5000- M.W. portion of the culture filtrate removed its inhibitory effect almost completely, but had no effect on the 5000+ M.W. portions activity, since it still caused significant inhibition of root hair formation (Table 8). There were no significant differences between the effect of the whole medium and its 5000- or the 5000+ M.W. components on root development.

DISCUSSION

The results presented in this paper, show that all three identified Pythium isolates(i.e. P. arrhenomanes, isolates # 320 and 323, P. irregulare) produced substances toxic to wheat seedling growth and development.

Both isolates of P. arrhenomanes (# 320 and 323) produced toxic substances in the growing medium after 13 and 18 days of incubation respectively. Toxicity resulted in inhibition of root hair development and root elongation, as well as general browning of root tissue of 2-3 day old wheat seedlings grown in their cell-free culture filtrates.

Vanterpool (17) first described such effects of toxic metabolites on wheat seedling development in 1933 for P. arrhenomanes Drechs. as causing stunting and severe browning of the roots, as well as shoot growth inhibition and wilting of young seedlings. He grew the fungus for 30 days on a wheat barley medium under less than defined conditions. Such differences in growth conditions could account for certain symptoms such as wilting, he observed, that were not seen in our study. Further, he did not study the effect of the culture filtrates on root hair development, nor made any measurements of root or shoot weights. He also reported that the toxic metabolite produced by his isolate was heat stable, where as we have provided evidence that the toxin produced by our isolates loses its efficacy significantly after autoclaving, although not completely.

Culture filtrates of P. irregulare like those of P. arrhenomanes caused severe root growth inhibition in terms of stunting, but in a very different way. They did not cause any browning of root tissue, and in contrast to P. arrhenomanes filtrates, greatly stimulated root hair formation and elongation in a swollen area immediately behind the root tip.

This conforms to reports by Blok (1) of IAA auxin production by P. irregulare, which is known to cause increased lateral cell growth, and therefore swelling of tissues.

In contrast to P. arrhenomanes culture filtrates, autoclaving had no effect on the activity of the culture filtrates of P. irregulare, again indicating biochemical differences in the nature of the toxin(s) produced by the two species.

Only a few data are available about auxin production by Pythium species. Yoshii et al (24) showed P. debaryanum producing IAA auxin on potato dextrose broth. Ronsdorf (9) found that P. mamillatum and P. intermedium produced auxin in a synthetic medium.

Martin (7) found a non-specific toxin produced by P. irregulare which was transported through the plant and caused leaf necrosis and wilting of several plant species. Such symptoms were not observed in our study, which could have been due to differences in methods of obtaining cell-free culture filtrates, and the bioassay procedures.

The present investigation also provides evidence that P. arrhenomanes (isolate # 320) produces a toxin that is heterogeneous. One component being thermolabile and smaller than 5000 in molecular weight, while the other being heat stable and greater than 5000 in molecular weight. Similar toxicity effects as caused by the raw culture filtrate, was produced only when the two portions were combined.

These findings agree with Sadik et al (10) , where they presented evidence for P. aphanidermatum producing a toxin which was found to be a heterogeneous glycopeptide with a molecular weight of 20-40 X 10^3, although they did not show it to be composed of two parts as has been demonstrated in this study.

Stimulation of root hair formation by growth regulating substances

produced by some Pythium species might serve an advantage to the pathogens towards infection, as hypothesized by Blok (1), since many Pythium species cause infection through root hairs (8). However, it is uncertain whether inhibition of root hair formation caused by toxin(s) produced by P. arrhenomanes has some relation with the attack of the roots by the pathogen.

Although characterization of a number of toxins produced by several fungal or bacterial pathogens has been done, and their role in disease determined (25), there are no reports of extensive characterization of any toxin produced by Pythium species. Results presented in this report are highly indicative of possible involvement of toxins in disease caused by several Pythium species, therefore, further studies are needed toward a more extensive characterization of toxins produced by these pathogenic species, and attempts must be made to elucidate the role of these toxins in disease, as well as their mode of action. From such studies, we will have a better understanding of pathogenesis and host-parasite interactions in diseases caused by Pythium species, and this will lead toward better control measures against these pathogens.

LITERATURE CITED

1. Blok, I. 1973. A growth regulating substance produced by Pythium sylvaticum. Neth. J. Pl. Path. 79: 266-276.

2. Csinos, A. S., and Hendrix, J.W. 1978. Parasitic and non parasitic pathogenesis of tomato plants by Pythium myriotylum. Can. J. Bot. 56: 2334-2339.

3. Csinos, A. S. 1979. Non parasitic pathogenesis of germinating tamato by Pythium myriotylum. Can. J. Bot. 57: 2059-2063.

4. Chamswarng, C., and Cook, R. J. 1985. Identification and comparative pathogenicity of Pythium species from wheat roots and wheat-field soils in the Pacific North West. Phytopathology, 75: 821-827.

5. Janardhanan, K. K., and Husain, A. 1974. Production of a toxic metabolite and pectolytic enzymes by Pythium butleri. Mycopathol. Mycol. Appl. 52: 325-330.

6. Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club. 20: 1-171.

7. Martin, P. 1964. Untersuchungen uber ein phytopathogenes toxin von Pythium irregulare. Phytopathol. Z. 50: 235-249.

8. Nemec, S. 1972. Histopathology of Pythium infected strawberry roots. Can. J. Bot. 50: 1091-1096.

9. Ronsdorf, L. 1935. Verleichende Untersuchungen uber die Wirkung verschiedener Wuchsstoffe auf das Wachstum einiger Pilze. Arch. Mikrobiol. 6: 309-325.

10. Sadik, E. A., Payak, M. W. and Mehta, S. L. 1982. Isolation and partial characterization of an extracellular phytotoxin produced by Pythium aphanidermatum, a stalk rot pathogen of maize. Zeit. Pflanz. Und Pflanz. 89: 266-275.

11. Sadik, E. A., Payak, M. W. and Mehta, S. L. 1983. Some biochemical aspects of host pathogen interactions in Pythium stalk rot of maize. Acta Phytopath. Acad. Scien. Hung. 18: 261-269.

12. Sharma, O. P. and Jain, A. C. 1967. Root rot of wheat in Madhya Pradesh. Indian Phytopathol. 20: 267-269.

13. Singleton, L. L. and Ziv, O. 1981. Effects of Pythium arrhenomanes infection and root-tip amputation on wheat seedling development. Phytopathol. 71: 316-319.

14. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. 481 pp.

15. Takahashi, M. 1951. Studies on the toxic effect of the nutrient solutions used by Pythium ultimum on various plants. Forch. Pflkr. 4: 138-144.

16. Vanterpool, T. C. and Truscott, J. H. L. 1932. Studies on browning root rot of cereals. Can. J. Res. 6: 68-93.

17. Vanterpool, T. C. 1933. Toxin formation by species of Pythium parasitic on wheat. Proc. Worlds Grain Exhibition and Conference, Regina, Saskatchwan. Vol. 2. pp. 294-298.

18. Vanterpool, T. C. 1940. Studies on browning root rot of cereals. Can. J. Res. 18: 240-257.

19. Vanterpool, T. C. 1940. Present knowledge of browning root rot of wheat with special reference to its control. Sci. Agri. 20: 735-749.

20. Vanterpool, T. C. and Sprague, R. A. 1942. Pythium arrhenomanes on cereals and grasses in the northern Great Plains. Phytopathol. 32: 327-328.

21. Van Der Plaats-Niterink, A. J. 1981. Monograph of the genus Pythium Centralbureau voor Schimmelcultures, Baarn. Studies in Mycol. No. 21.

22. Waller, J. M. 1979. Observations on Pythium root rot of wheat and barley. Pl. Pathol. 28: 17-24.

23. Waterhouse, G. M. 1967. Key to Pythium Pringsheim. Commonw. Mycol. Inst. Mycol. Paper No. 109.

24. Yoshii, K., and Hagedorn, D. J. 1971. Production of tryptophol and indolacetic acid in culture by Pythium debaryanum. Phytopathol. 61: 918-919.

25. Yoder, O. C. 1980. Toxins in pathogenesis. Ann. Rev. Phytopathol. 18: 103-129.

26. Ziv, O. and Singleton, L. L. 1981. The effect of Pythium arrhenomanes infection and cultural filtrates on wheat seedling growth (Abstr.). Phytopathol. 71: 267.

TABLE 1.

Identified species	Morphological characteristics
Pythium arrhenomanes (isolate #320 and 323)	Oogonia subspherical to spherical, smooth, average diameter 30µ. Oospores both plerotic and aplerotic, average diameter 30µ. Sporangia inflated, lobulate, forming complexes of elements up to 20µ or more in diameter. Zoospores 20 to 50 or more in a vesicle borne on an evacuation tube of variable length. Antheridia of diclinous origin, generally from 4 to 8 per oogonium. Optimum growth temperature 25-30 C.
Pythium irregulare	Oogonia spherical, average diameter = 18µ, usually intercalary, of irregular contour. Oospores aplerotic, average diameter = 15µ. Sporangia of various shapes, but mostly spherical, terminal or interclary, average diameter = 18µ. Zoospores rarely produced. Antheridia typically monoclinous, 1 to 2 per oogonium; however up to 4 antheridia per oogonium could be observed.

Fig. 1. Inhibition of root (20X magnification) hair formation by the culture filtrate of P. arrhenomanes (isolate #320). (Top) Roots grown in medium, (bottom) roots grown in culture filtrate.

TABLE 2. Effect of culture filtrate from two isolates of Pythium arrhenomanes on root hair formation of wheat seedlings.

Treatment	Dist. (mm) from the root tip to the first visible root hair [1]	Longest root hair (mm) at 5 mm from the root tip [1]
Medium	2.8	0.23
Water	3.1	0.25
Culture filtrate [2] (isolate #320)	14.6 **	0 **
Culture filtrate [3] (isolate #323)	15.5 **	0 **
Autoclaved filtrate (isolate #320)	3.6	0.16
Autoclaved filtrate (isolate #323)	3.9	0.17
LSD (P = 0.01)	3.0	0.08

Averages were determined from 5 replications representing each treatment

[1] Data were collected after two days of incubation.

[2] 13 days old culture filtrate.

[3] 18 days old culture filtrate.

** Denotes significant difference from medium at P = 0.01

TABLE 3. Effect of culture filtrate of P. arrhenomanes on wheat seedling development.

Treatment	Longest root length (cm)	Shoot Length (cm)	Fresh root weight (mg)	Fresh shoot weight (mg)	Dry root weight (mg)	Dry shoot weight (mg)
Medium	4.1	4.9	30.2	35.0	5.2	7.1
Water	5.4	5.2	27.4	42.8	3.9 **	6.9
Filtrate [1] (isolate #320)	2.0 **	4.6	11.8 **	36.0	2.6 **	5.8
Autoclaved filtrate (isolate #320)	4.5	5.1	19.8 **	34.0	4.0 **	6.5
LSD (P = 0.01)	1.4	0.80	9.1	10.1	0.57	1.3

Averages were determined from 5 replications representing each treatment.

[1] 13 days old culture filtrate.

[2] Measurements taken after 3 days of incubation.

** Denotes significant difference from medium at P = 0.01

TABLE 4. Effect of culture filtrates of two *Pythium* species on wheat seedling development.

Treatment	Longest[1] root length (cm)	Shoot Length (cm)	Fresh root weight (mg)	Fresh shoot weight (mg)	Dry root weight (mg)	Dry shoot weight (mg)
Medium	4.3	4.6	27.0	35.2	2.4	4.5
Water	5.6 **	6.1 **	27.4	45.0 **	1.4 **	4.5
Culture filtrate[2] *P. arrhenomanes* (isolate #323)	3.6 *	5.5 **	18.2 **	41.8 **	1.4 **	5.1
Culture filtrate[2] *P. irregulare*	2.1 **	3.2 **	19.8 **	33.4	1.3 **	4.1
LSD (P = 0.01)	0.92	0.74	5.0	6.0	0.73	1.0

Averages were determined from 5 replications representing each treatment.

[1] Measurements taken after 3 days of incubation.

[2] 18 days old culture filtrate.

** Denotes significant difference from medium at P = 0.01

* Denotes significant difference from medium at P = 0.05

TABLE 5. Effect of autoclaved culture filtrates of two *Pythium* species on wheat seedling development.

Treatment	Longest root length[2] (cm)	Fresh root weight (mg)	Dry root weight (mg)
Medium	4.5	30.0	5.2
Water	6 **	31.2	5.2
Autoclaved filtrate[1] *P. arrhenomanes* (isolate #323)	4.9	26.0	5.5
Autoclaved filtrate[1] *P. irregulare*	1.2 **	15.6 **	4.7
LSD (P = 0.01)	0.79	5.5	0.89

Averages were determined from 5 replications representing each treatment.

[1] 18 days old culture filtrate.

[2] Measurements taken after 3 days of incubation.

** Denotes significant difference from medium at P = 0.01

TABLE 6. Effect of culture filtrate of *P. arrhenomanes* in comparison to infection by the pathogen on wheat seedling development.

Treatment	Longest root length[1] (cm)	Shoot length (cm)	Fresh root weight (mg)	Fresh shoot weight (mg)	Dry root weight (mg)	Dry shoot weight (mg)
Medium	6.2	8.3	50	58.4	6.2	9.3
Water	7.4 **	8.7	41.8	66.2	3.3 **	8.2
Culture filtrate[2] (isolate #320)	3.5 **	8.9	24 **	64.6	3.6 **	9.2
Pythium-infected in medium	3.6 **	6.5 **	25.6 **	46.4 **	3.1 **	7.5 **
Pythium-infected in water	4.2 **	6.3 **	26.2 **	47.4 **	3.1 **	7.3 **
LSD (P = 0.01)	1.0	0.75	11.2	9.1	0.86	1.2

Averages were determined from 5 replications representing each treatment.

[1] Measurements taken after 4 days of incubation.

[2] 20 days old culture filtrate.

** Denotes significant difference from medium at P = 0.01

TABLE 7. Effect of culture filtrate age of P. arrhenomanes (isolate #320) on root hair formation of wheat seedlings.

Culture filtrate age (days)	Dist. (mm) from the root tip to the first visible root hair [1]	Longest root hair (mm) at 5 mm from the root tip
0[2]	2.8	0.23
4	2.7	0.22
7	3.9	0.15
13	16.2	0

Averages were determined from 5 replications representing each treatment

[1] Measurements taken after two days of incubation.

[2] Uninoculated medium.

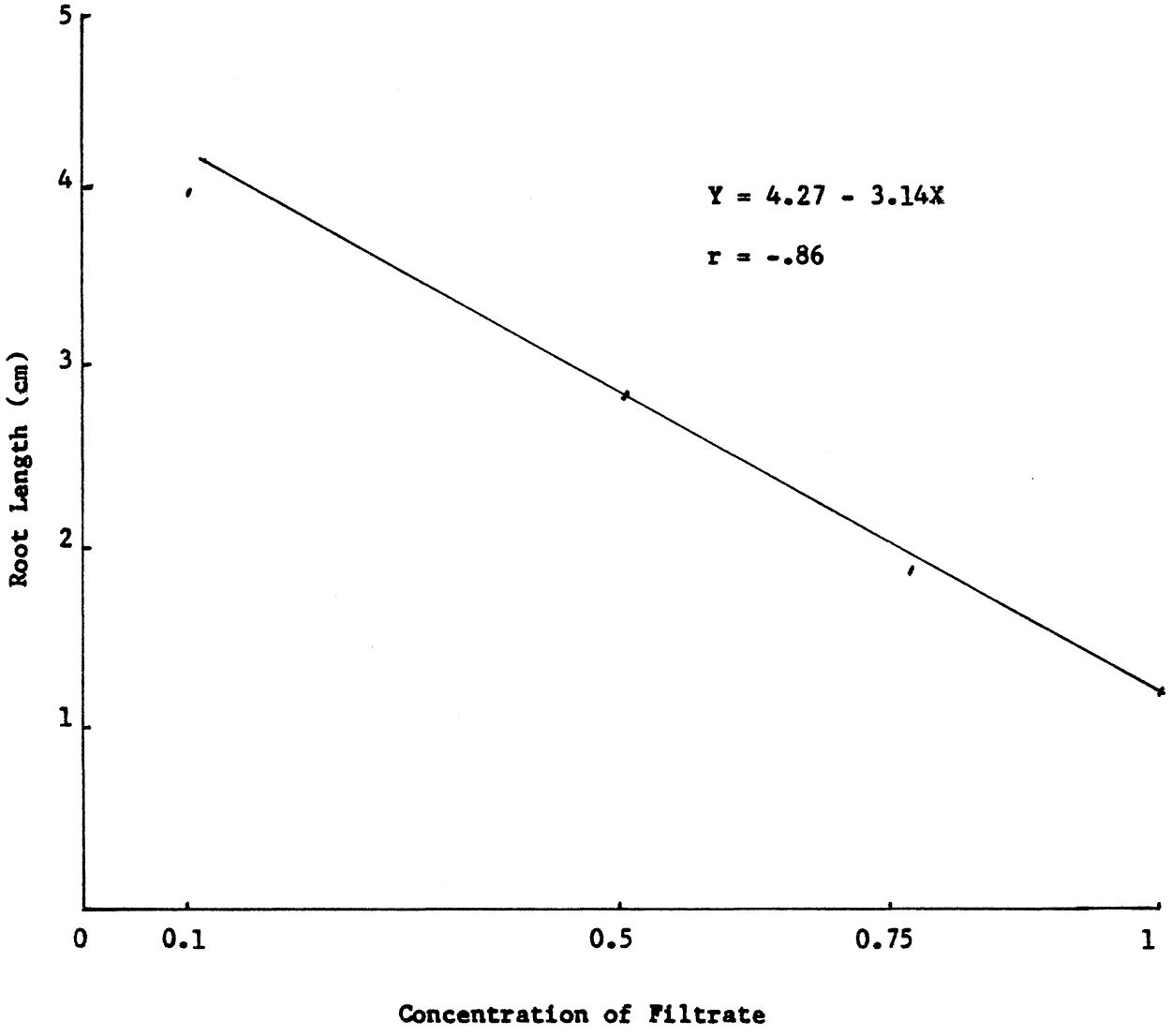

Fig. 2. Linear regression between culture filtrate (20-day-old) concentration of P. arrhenomanes (isolate #320) and wheat root length after 3 days of incubation.

TABLE 8. Effect of the 5000+ M.W and 5000- M.W portions of the culture filtrate of P. arrhenomanes (isolate #320) on root hair formation and root elongation of wheat seedlings.

Treatment	Distance (mm) from the root tip to the first visible root hair [1]	Longest root hair (mm) at 5 mm from the root tip	Longest root length (cm)	Root color
Medium	3.0	0.22	3.4	White
Water	2.7	0.28	3.3	White
Raw culture filtrate	11.2	0	1.3	Brown
Medium (5000+)	2.9	0.24	3.5	White
Medium (5000-)	2.7	0.32	3.7	White
Culture filtrate (5000+)[2]	14.5	0	2.6	White
Culture filtrate (5000-)	7.1	0.04	2.2	White
Culture filtrate (5000+ and 5000- reconstituted)	13.0	0	1.4	Brown
Autoclaved culture filtrate (5000+)	16.2	0	3.2	White
Autoclaved culture filtrate (5000-)	3.3	0.26	2.5	White

Averages were determined from 5 replications representing each treatment.

[1] Measurements taken after two days of incubation.

[2] 20-day-old culture filtrate.

VITA

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