

## Effects of *Pythium arrhenomanes* Infection and Root-Tip Amputation on Wheat Seedling Development

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### ABSTRACT

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An in vitro system was developed for studying the effects of *Pythium* infection on wheat seedlings. Root length, leaf length, dry kernel weight, dry plant weight, and percent water content of the plant were used to quantify the effect of *Pythium arrhenomanes* infection on wheat seedling development at 2-day intervals beginning at the fifth day and continuing up to the 13th day after emergence. *P. arrhenomanes* infection treatments were compared to root-tip amputation, no treatment, and Ridomil seed treatment with and without *P. arrhenomanes*. Ridomil was completely ineffective for controlling *P. arrhenomanes* in these tests. Infection with *P. arrhenomanes* significantly reduced root and leaf length and dry plant

weight accumulation during the observation period. Uninoculated root-tip amputation treatments temporarily reduced root and leaf elongation, and dry plant weight accumulation 5-7 days after emergence. However, with the initiation of lateral root development nearly normal growth was resumed. With *P. arrhenomanes* infection, dry kernel weights remained relatively constant throughout the experimental period in contrast to a rapid decline in treatments without *P. arrhenomanes*. Percent water contents were significantly lower in plants infected with *Pythium* than in uninfected plants.

Observations of *Pythium* damage on wheat seedling development have been limited to the use of naturally or artificially infested soil methods (1,2,6) with damage based on the total plant dry weight (6) or percentage reduction in emergence (1,2). Krassovsky (3) utilized an in vitro method to study the relative importance of seminal and nodal root systems as they relate to wheat seedling growth and development. In this study, we describe an in vitro system for quantifying the effects of *Pythium arrhenomanes* Drechs. on wheat seedling development. Also, data is presented that compares the relative effects of *P. arrhenomanes* infection, Ridomil seed treatment, and root amputation on selected seedling growth parameters: leaf and root elongation, fresh and dry plant weight, dry kernel weight, and percent water accumulation.

### MATERIALS AND METHODS

Observations and measurements of wheat seedling development were made by using the in vitro system illustrated in Fig. 1. The system consisted of an aluminum baking pan (7 × 11 × 21 cm) fitted with doubled soft-wire hangers, and paper towel wicks (four per side with a fold, 2 cm from the top) used to support developing wheat seedlings. Each paper towel wick was trimmed to conform to the dimensions of the pan and the lower corners of each half were stapled together. Forceps were used to place wheat seeds between the two layers of toweling to a depth of 2-3 cm. After setting up several units, water was added to a uniform level initially and adjusted daily as needed. Each unit was incubated in a growth box at 21 ± 2 C with a 12-hr daylength (fluorescent; 280 lux). For the first 2 days, each unit was enclosed in a large plastic bag. This resulted in a more uniform emergence than that observed in open units. One cultivar (TAM W-101; CI 15324, *Triticum aestivum* L.) was used throughout the study. All seeds were surface disinfected (30 sec in ethanol:sodium hypochlorite [5.25%], 1:1, v/v), rinsed in sterile distilled water, air dried for 24 hr in a sterile hood, and stored for later use. In addition, a 150-g sample of the seed was treated with Ridomil (N-[2,6-dimethylphenyl]-N-(methoxyacetyl)-alanine

methyl ester; 0.31 g a.i./kg) (CIBA-Geigy Corp., Greensboro, NC 27409). A treatment unit consisted of one paper hanger supporting eight seeds (later thinned to four seeds), and represented one of four replications.

Treatments were as follows: A—no treatment; B—*Pythium* infection beginning 2 days after planting; C—Ridomil-treated seed

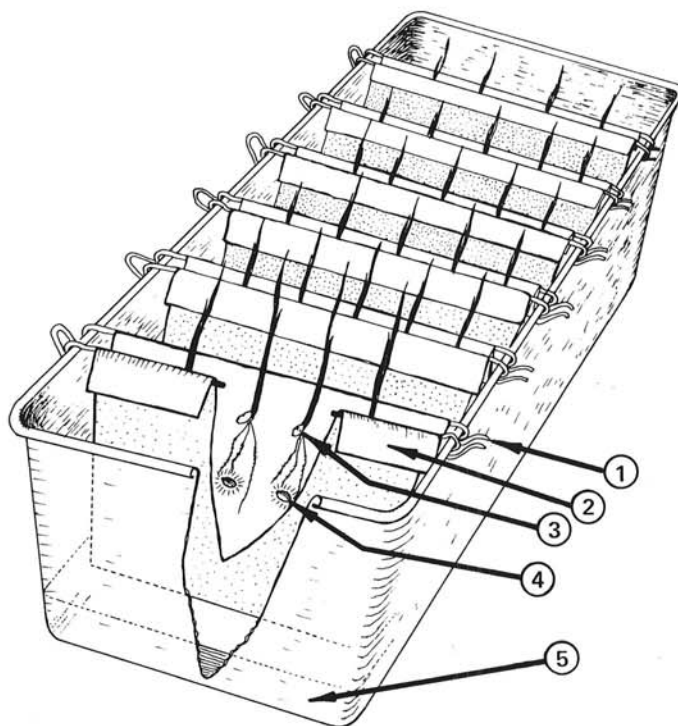


Fig. 1. Unit for observing in vitro wheat seedling development: 1 = "soft-wire" double hanger; 2 = absorbent paper toweling support for seedling development; 3 = wheat seedling; 4 = inoculum or treatment area; and 5 = level of water or nutrient solution.

without *Pythium*; D—Ridomil-treated seed with *Pythium*; and E—root-tip amputation without *Pythium*. With the exception of the Ridomil seed treatment, all treatments were initiated 2 days after planting when the coleoptiles were <0.5 cm in length and roots were 1–2 cm in length. *Pythium* infection was induced by placing a single infested wheat seed 3 cm below each wheat seedling. Inoculum was from 4-day-old cultures of *P. arrhenomanes* grown on 50 ml of autoclaved wheat seed medium (wheat seeds and water [1:1, v/v] autoclaved 20 min at 1.1 atmospheric pressure in 250-ml cotton stoppered flasks). One isolate of *P. arrhenomanes* was used throughout these studies. This isolate was cultured from a field-collected wheat root and it was pathogenic. Root amputation treatments involved removing 1–2 mm of each seminal root tip.

Observations and measurements of treatment effects were begun 5 days after emergence and periodic measurements (at 2-day intervals to the 13th day) were made of root length, leaf length, and fresh and dry kernel weights, and fresh and dry plant weights. Root and leaf lengths were measured from point of attachment to the wheat kernel. Fresh and dry kernel weights were determined as weight of detached wheat kernel. Fresh and dry plant weight was total plant weight minus respective kernel weight. All dry weight measurements were taken by weighing tissues dried in a microwave oven (Model 747, Sears Roebuck and Company, Chicago, IL 60684) with a power setting of 75% of the 625 W capacity for 12 min.

Standard analysis of variance techniques for randomized complete block analysis were used as outlined in Steel and Torrie (5).

## RESULTS AND DISCUSSION

Five days after emergence, the mean root lengths for the wheat seedlings were 2.0 and 4.0 cm in the treatments B and A with and without *Pythium*, respectively (Fig. 2). *Pythium* infection had stopped root elongation and development within 2 days after inoculation.

In contrast, the root-tip amputations (E) resulted in only a temporary reduction in root elongation at 5 and 7 days after emergence. With the onset of lateral root development in the root-tip amputation treatments by the ninth day, the roots resumed elongation and growth. By the 13th day, the root lengths of the amputated roots (E) were significantly greater than those of the *Pythium*-infected roots (B and D) but were also significantly less than those of the treatments (A and C) without *Pythium*.

These results indicate that both *Pythium* infection and root amputation reduce seedling root development and elongation.

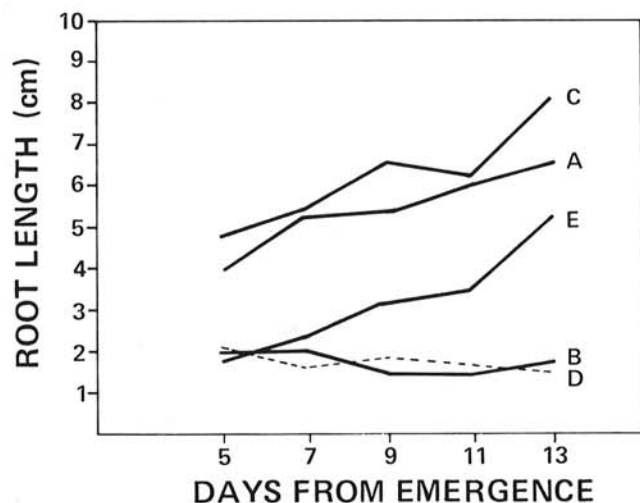


Fig. 2. Root length development in days from emergence for treatments: A = no treatment; B = *Pythium* inoculated beginning 2 days after planting; C = Ridomil-treated seed without *Pythium*; D = Ridomil seed treated with *Pythium*; and E = root-tip amputation treatment. Results shown for one experiment replicated four times with each data point representing the mean of 16 plants. Incubated at 21 C in growth box with 12 hr light at relative humidity of 55–60%.

However, the inability of *Pythium*-infected roots to recover and resume normal growth via lateral root development indicates that *Pythium* damage involves more than the destruction of meristematic tissue in the root cap region.

The Ridomil seed treatment with (D) and without (C) *Pythium* indicated that Ridomil did not prevent *Pythium* damage to the wheat roots (Fig. 2). Similar seed treatment comparisons in

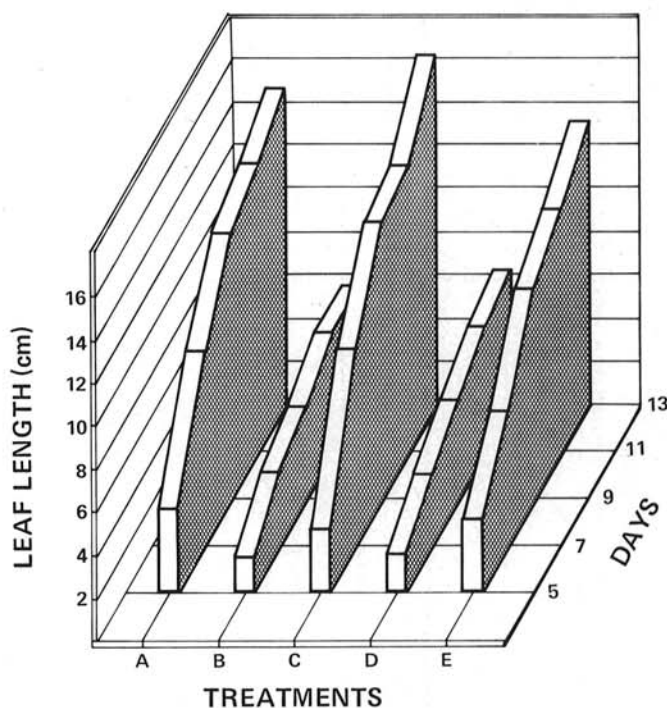


Fig. 3. Leaf length development in days from emergence for treatments: A = no treatment; B = *Pythium* inoculated beginning 2 days after planting; C = Ridomil-treated seed without *Pythium*; D = Ridomil-treated seed with *Pythium*; and E = root-tip amputation treatment. Results shown for one experiment replicated four times with each data point representing the mean of 16 plants. Incubated at 21 C in growth box with 12 hr of light at relative humidity of 55–60%.

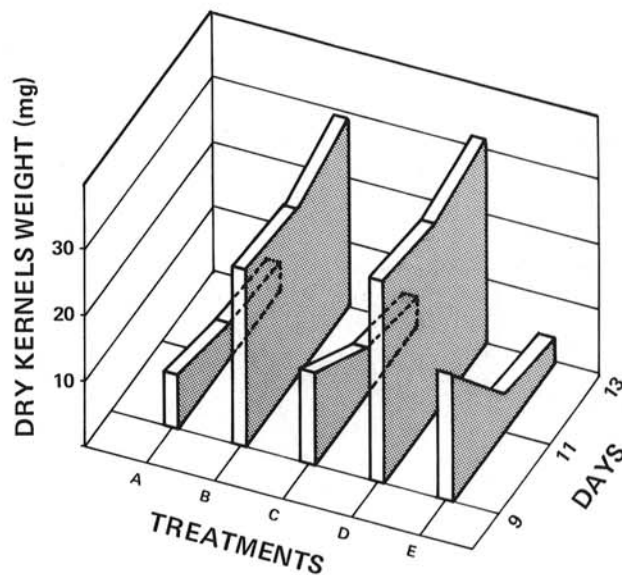


Fig. 4. Dry kernel weight data in days beginning at 9 days after emergence for treatments: A = no treatment; B = *Pythium* inoculated beginning 2 days after planting; C = Ridomil-treated seed without *Pythium*; D = Ridomil-treated seed with *Pythium*; and E = root-tip amputation treatment. Results shown for one experiment replicated four times with each data point representing the mean of 16 plants. Incubated at 21 C in growth box with 12 hr light at relative humidity of 55–60%.

greenhouse soil tests using this isolate, however, indicated that *Pythium* was controlled effectively. Ridomil-treated seed (0.31 g a.i./kg) using the same treated seed source resulted in 80–90% emergence as compared to 1–2% emergence for untreated seed each as compared to uninoculated control emergence (*unpublished*).

Leaf length responses to the various treatments were similar to those of the roots (Fig. 3). With *Pythium* infection (B and D), leaf length was significantly reduced throughout the 5th to 13th day growth period compared to the treatments without *Pythium* (A and C). Similarly, the root-tip amputation treatment (E) for leaf elongation closely paralleled the root growth responses. Also, as with the root length, Ridomil did not change the effect of *Pythium* infection on leaf length.

Without *Pythium* infection (A, C, and E), the dry kernel weights had declined significantly by the 11th day after emergence as compared to the dry kernel weights for the treatments with *Pythium* (B and D) (Fig. 4). Conversely (Fig. 5), the dry plant weights increased significantly in the treatments without *Pythium* (A, C, and E) and contrasted with only slight increases for the

treatments with *Pythium* (B and D). Both the dry kernel weight and the dry plant weight data indicated that the *Pythium*-infected plants were less metabolically active.

Krassovsky (3) found that the seminal roots played an active role in water and salt absorption. She found that the seminal roots have the capacity to absorb almost twice the amount of water per unit of dry weight as do the nodal roots (secondary). Furthermore, Simmonds and Sallans (4) in field root-amputation studies found that the seminal roots were important to the early stages of wheat seedling development and that damage to the seminal roots could ultimately be reflected in terms of decreased grain yield. Although Krassovsky (3) and Simmonds and Sallans (4) were studying and comparing the relative importance of the seminal and nodal root systems from different viewpoints, they both concluded that the seminal roots are important in relation to wheat seedling development. In this study, we wanted to determine the effects that

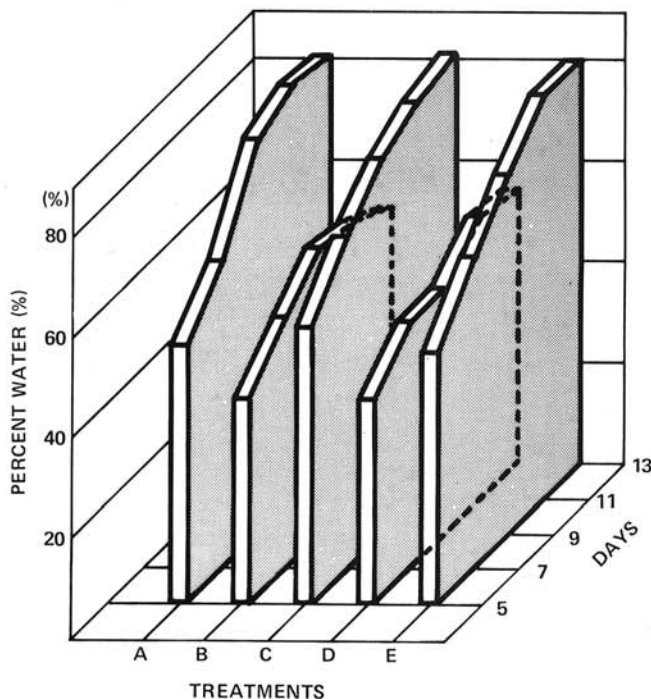
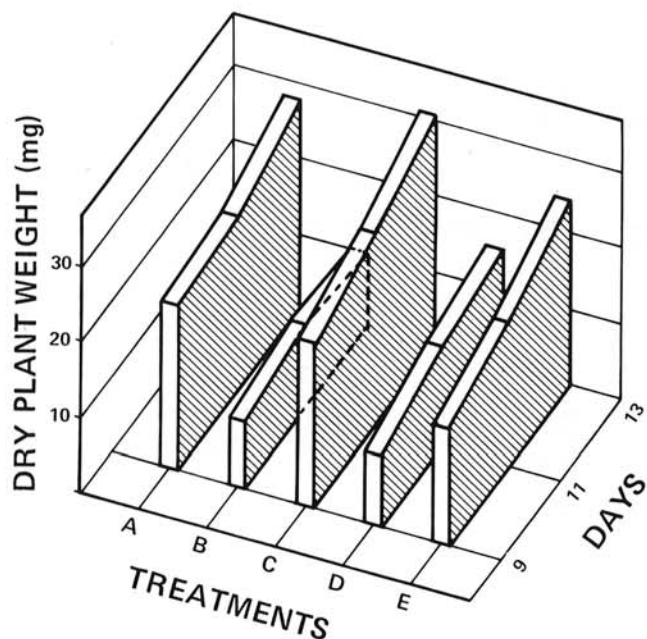


Fig. 5. Dry plant weight in days from emergence beginning at 9 days after emergence for treatments: A = no treatment; B = *Pythium* inoculated beginning 2 days after planting; C = Ridomil-treated seed without *Pythium*; D = Ridomil-treated seed with *Pythium*; and E = root-tip amputation treatment. Results shown for one experiment replicated four times with each data point representing the mean of 16 plants. Incubated at 21 C in growth box with 12 hr of light at relative humidity of 55–60%.

Fig. 6. Percent water data in days from emergence for treatments: A = no treatment; B = *Pythium* inoculated beginning 2 days after planting; C = Ridomil-treated seed without *Pythium*; D = Ridomil-treated seed with *Pythium*; and E = root-tip amputation treatment. Results shown for one experiment replicated four times with each data point representing the mean of 16 plants. Incubated at 21 C in growth box with 12 hr of light at relative humidity of 55–60%.

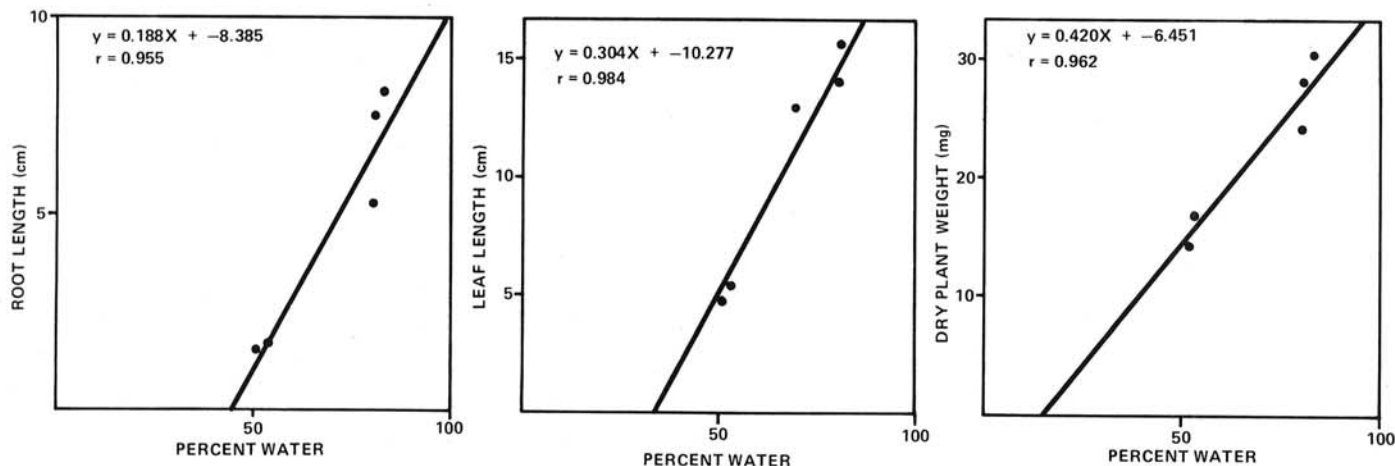


Fig. 7. Linear regressions of percent water in relation to wheat seedling root length, leaf length, and dry plant weight, respectively.

*Pythium* might have on seminal root development and subsequent growth. All of the results indicated that *Pythium* can significantly affect various plant growth parameters and that these effects involve more than a simple destruction of meristematic root-tip tissue. Secondly, the dry kernel weight data indicated that *Pythium* had significantly reduced the level of metabolic activity. Presumably, *Pythium*-infected plants were unable to utilize the available nutrients in the wheat seed endosperm for growth. A partial explanation for reduced metabolic activity is indicated by the effects of *Pythium* on the percent water content of the plants in the various treatments. As shown (Fig. 6), the percent water content was significantly greater for the treatments without *Pythium* (A, C, and E) than for treatments (B and D) with *Pythium*. Furthermore (Fig. 7), the simple linear regression comparisons of percent water individually with root length, leaf length, and dry plant weight parameters showed that percent water was linearly correlated with each.

In conclusion, the in vitro system described here was suitable for

identifying parameters suitable for quantifying wheat seedling growth and development, and for measuring the effects of *Pythium* infection on them.

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