

Reaction of Wheat Varieties to Infection by *Pythium arrhenomanes* or its Toxic Metabolite (s)

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ABSTRACT

Eight wheat varieties some of which were earlier reported to be resistant to *Pythium arrhenomanes*, were inoculated with *P. arrhenomanes*. Inoculation was performed by placing 2-day-old seedlings on the edge of a *P. arrhenomanes* culture on corn meal **agar** for 3 h at 25°C. Infected seedlings were then transferred to test tubes (9 mm dia.) containing glass beads and 1 ml of sterile water, and incubated for 4 days at 25°C. Non-inoculated seedlings were exposed to the toxic metabolite(s) from *P. arrhenomanes* by placing them in test tubes containing 1 ml of a 25-day-old culture filtrate of *P. arrhenomanes*. Non-inoculated seedlings grown in test tubes containing sterile water served as controls. Measurements were taken on root and shoot length, as well as fresh and dry root and shoot weights. There was an indication that it may be possible to use the toxic metabolite(s) in screening for resistance to *P. arrhenomanes*. Although all of the varieties appeared to be susceptible, there were some differences among them in their reaction to fungal infection or exposure to the toxic metabolite(s).

Keywords: *Pythium arrhenomanes* , Toxic metabolite(s) , Wheat , Resistance.

INTRODUCTION

Pythium arrhenomanes Drechs., along with a number of other *Pythium* species, cause a disease of wheat and other cereals commonly known as browning, or *pythium* root rot [8,9]. Characteristic disease symptoms are severe root browning and stunting, reduced number of tillers, and poor growth and grain yields [9]. It was reported that this pathogen produced a toxic metabolite(s) in vitro, which induced disease symptoms (i.e. root browning and stunting) on wheat [4,5,6]. These findings have suggested the possible involvement of toxic metabolite(s) in disease development, and have raised the possibility of using these metabolite(s) in screening wheat plants for resistance to *Pythium* root rot, caused by *P.*

arrhenomanes.

Varietal resistance to *Pythium* root rot has been used as a practical control measure in sugarcane [2] however, this has not been the case for wheat. None of the commercial varieties of wheat are considered immune, nor highly resistant to *pythium* root rot [1]. However, differences in yields among varieties have been observed in the past [1,3, 8]. Vanterpool [8] found that Thatcher and Apex thrived better than Marquis in adjacent fields. In another study, Kilpatrick [3] reported three wheat varieties (i.e. Kaw, Denton-D, and Denton-E) to be resistant to *P. arrhenomanes*, as well as to a few other root rotting *Pythium* species in emergence studies.

Plant emergence has been used as a criteria for determining resistance to root rot, however

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results based on plant emergence alone may not be very accurate. Perhaps another more reliable criterion would be quantitative evaluation of various growth parameters of infected plants after emergence. Also, it may be possible to use toxic metabolite(s) produced by *P. arrhenomanes*, or other *Pythium* species, to screen wheat plants for resistance to the pathogen(s). Previous reports indicated that the effects of toxic metabolite(s) were similar to those of infection by *P. arrhenomanes*, but in these studies only one variety (i.e. TAM-101) was used [4,5,6]. TAM-101 was reported to be susceptible to *P. arrhenomanes* infection, as well as to its toxic metabolite(s), as both caused significant reductions in root length, as well as fresh and dry root weights, when compared to ..controls. The purpose of this investigation is to compare performances of eight wheat varieties, some of which have earlier been reported to be resistant to *P. arrhenomanes* [3].

MATERIALS AND METHODS

Seeds from wheat varieties Kaw (CI 12871), Crockett (CI 12702), Denton-D (CI 12701), Denton-E (CI 12703), Wichita (CI 11952), Hope (CI 13133) and Kenya (CI 12880) were increased initially in the greenhouse prior to the experiments. These seeds, along with those of TAM-101 (CI 15324), were surface sterilized [80 sec. in ethanol: sodium hypochlorite (5.25%) 1:1, V/V in a cleaning sonicator for TAM-101, 35 sec. for all other varieties]. They were then rinsed three times with sterile water, placed on nutrient agar plates (crease side down, 20-25 seeds/plate) and incubated in the dark (48 h; 30+1°C for the variety TAM-101, 60 h; 30+1°C for varieties Kaw, Denton-E and Crockett, 72 h; 30+1°C for varieties Hope, Wichita, Denton-D and Kenya).

Seedlings free of bacterial and fungal contaminants with seminal roots 1+0.2 cm in length were placed on the edge (root tip in contact with culture) of a two-day-old *P. arrhenomanes* culture (corn meal agar, CMA)

for 3 h at 25 + 1°C in darkness. Seedlings were then transferred to test tubes (18x150 mm) containing nine glass beads (6 mm), and 1 ml of sterile water.

To assess the effects of the toxic metabolite(s), seedlings were placed on non-inoculated CMA plates for 3 h (at 25 +TC), then transferred to test tubes containing 1 ml of a 25-day-old cellfree culture filtrate of *P. arrhenomanes* grown in a glucose glutamic acid liquid medium [6]. The procedure for obtaining cell-free filtrates has been previously described [6]. Non-inoculated seedlings (placed on sterile CMA plates for 3 h) transferred to test tubes containing sterile water served as controls. Seedlings were incubated [25+2°C; 12/12, light (27 *ft* Einst., m² sec.⁻¹) /dark] for four days after which shoot length, longest root length, fresh and dry shoot and root weights were measured. For dry weight measurements, roots and shoots were incubated at 50+2°C for 48 h. Measurements were taken on five seedlings (i.e. five reps.) per each variety and treatment in a randomized complete block design with experiments being replicated twice.

RESULTS AND DISCUSSION

Seven wheat varieties were selected based on the work of Kilpatrick [3] on resistance to *P. arrhenomanes*. Three of these varieties including Kaw, Denton-D and Denton-E, were found to be resistant to *P. arrhenomanes* in emergence studies [3], and the four other varieties selected were among the parents of these resistant ones. In the present study, these varieties were compared to the variety TAM-101, Comparison was based on differences in various growth parameters, four days after inoculation with *P. arrhenomanes*, or exposure to its culture filtrate.

Lengths and fresh weights of both roots and shoots of all the eight varieties inoculated with *P. arrhenomanes* or exposed to its culture filtrate were significantly reduced when compared to water controls (Figs. 1-4).

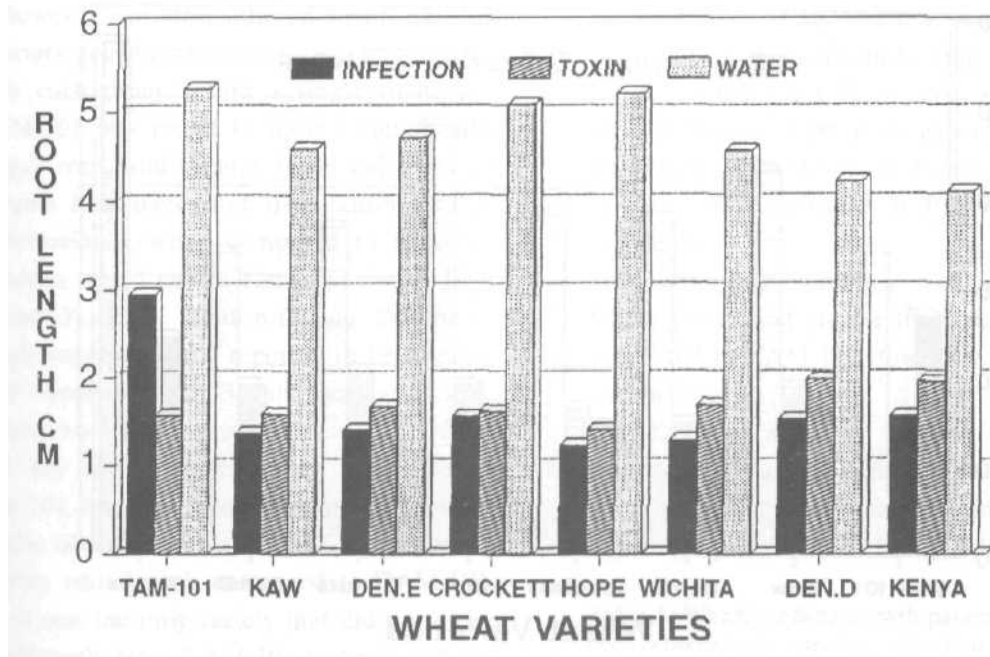


Figure 1. Root lengths of eight wheat varieties after four days inoculation with *P. arrhenomanes*, exposure to its culture filtrate and growth in water.

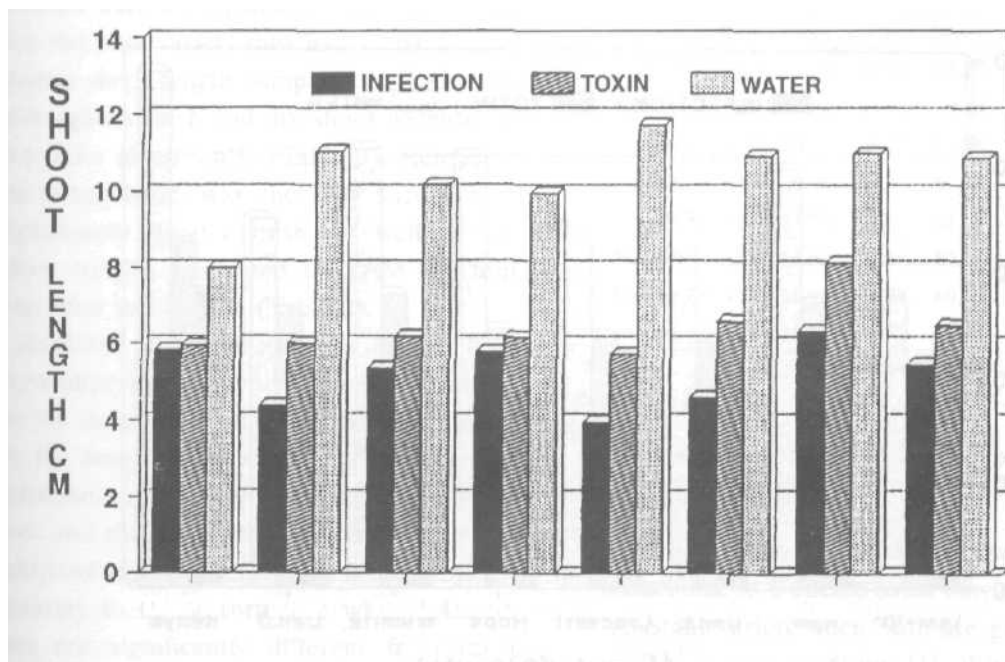


Figure 2. Shoot lengths of eight wheat varieties after four days inoculation with *P. arrhenomanes*, exposure to its culture filtrate and growth in water.

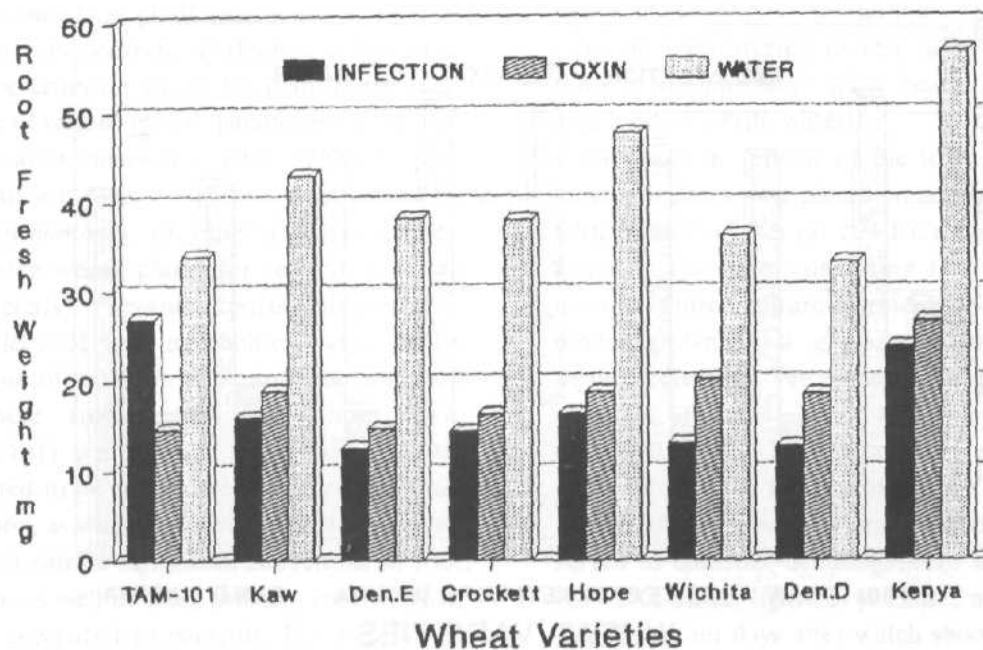


Figure 3. Fresh root weights of eight wheat varieties after four days inoculation with *P. arrhenomanes*, exposure to its culture filtrate, and growth in water.

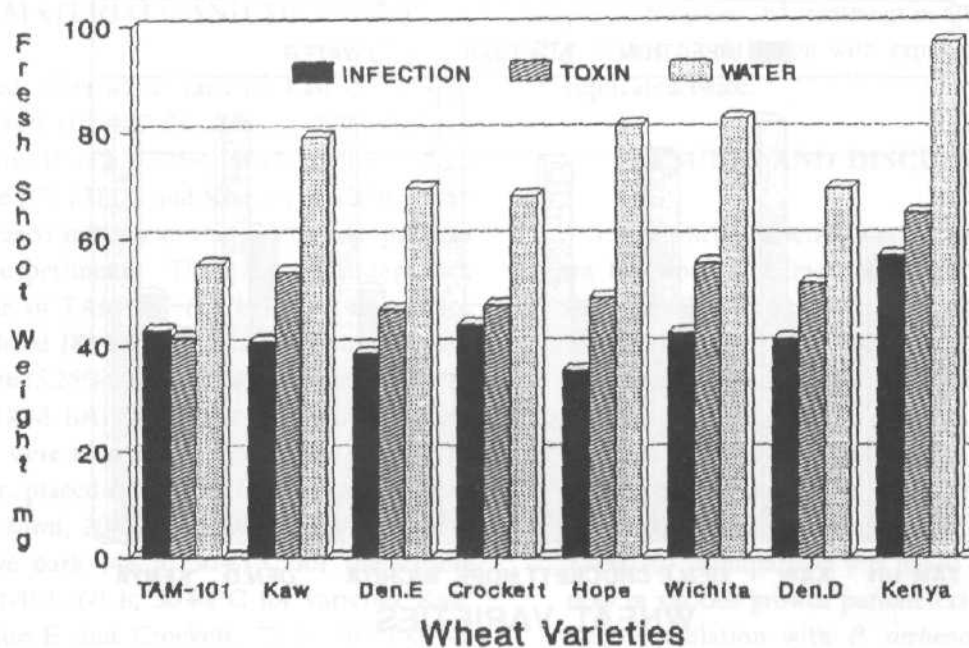


Figure 4. Fresh shoot weights of eight wheat varieties after four days inoculation with *P. arrhenomanes*, exposure to its culture filtrate, and growth in water.

However, varieties differed significantly in various growth parameters, when compared with each other within a single treatment. TAM-101 was found to have a significantly longer root, and greater fresh and dry root weights four days after inoculation with *P. arrhenomanes*, when compared to all other varieties tested except Kenya (Table 2, Figs. 1 and 3). Kaw, Denton-D and Denton-E, which were previously reported to be resistant to *P. arrhenomanes* [3], all had significantly shorter root lengths, as well as smaller fresh and dry root weights, when compared to TAM-101, four days after inoculation (Table 2). Kenya, like other varieties, had a significantly shorter root length compared to TAM-101, but it was the only variety that did not differ significantly from TAM-101 in fresh and dry root weights four days after inoculation (Table 2).

Shoot growth parameters among infected varieties were not significantly different. Hope was the only variety that had a significantly shorter shoot length compared to TAM-101, although its fresh and dry shoot weights did not differ significantly (Table 2). Kenya, on the other hand, was the only variety with significantly greater fresh, as well as dry shoot weights compared to TAM-101 four days after inoculation (Table 2).

Reaction of wheat varieties to the toxic metabolite(s) of *P. arrhenomanes* was similar, for the most part, to their reaction to infection by the fungus. The toxic metabolite(s), like infection, caused significant reductions in the root and shoot growth of all varieties, when compared to the controls (Figs. 1-4). TAM-101, contrary to its performance when infected, was not significantly different from most other varieties in root or shoot growth, four days after exposure to filtrates of *P. arrhenomanes* (Table 3). However, three varieties (i.e. Wichita, Denton-D and Kenya) were significantly different in certain growth parameters from TAM-101. Wichita had signifi-

cantly greater fresh and dry shoot weights, Denton-D had significantly longer root and shoot lengths, while Kenya had significantly greater fresh and dry root, as well as shoot, weights than TAM-101, four days after exposure to culture filtrates of *P. arrhenomanes* (Table 3).

In water, all varieties had significantly longer shoots and greater fresh shoot weights compared to TAM-101, four days after incubation (Table 1, Figs. 2 and 4). Kenya, Wichita, Hope and Kaw also had significantly greater dry shoot weights than TAM-101, while the three other varieties did not differ significantly from TAM-101 (Table 1).

Table 1. Wheat seedling growth parameter differences between eight varieties, after four days growth in sterile water.

Variety	Growth Parameter ^a					
	Length (cm)		Fresh Weight (mg)		Dry Weight (mg)	
	Rmit	Shoot	Root	Shoot	Root	Shoot
TAM-101	5.26	7.15	33.98	55.25	2.25	7.06
Kaw	4.59	11.05	43.02	79.15	2.25	8.94
Denton-D	4.71	10.17	38.21	69.38	2.80	8.39
Crockett	5.08	9.94	38.0	67.89	2.10	6.90
Hope	5.20	11.73	47.67	81.26	2.83	8.65
Wichita	4.56	10.92	36.18	82.52	2.22	8.80
Denton-E	4.22	10.98	33.24	69.29	1.96	8.27
Kenya	4.10	10.87	56.79	96.79	3.29	11.03
LSD	1.01	1.81	9.95	12.48	0.73	1.58

(P=0.01)

^a a Growth parameter means are averages determined from 10 replications for each variety.

It has been earlier shown that a variety susceptible to a disease could outyield a disease resistant variety when both are grown under a disease free condition [1]. With regard to such findings, the performance of TAM-101 in water (although it is not shown to be resistant but performs better in root growth) when compared to other varieties may be explained. Kenya, the only variety with significantly shorter roots in water has

Table 2. Wheat seedling growth parameter differences among eight varieties, after four days inoculation with *P. arrhenomanes*.

Variety	Growth Parameter ^a					
	Length (cm)		Fresh Weight (g)		Dry Weight	
	Root	Shoot	Root	Shoot	Root	Shoot
TAM-101	2.95	5.82	26.98	42.79	1.84	5.64
Kaw	1.36	4.38	15.90	40.77	0.86	6.04
Denton-E	1.40	5.33	12.57	38.48	0.96	5.42
Crockett	1.56	5.76	14.40	43.77	0.89	6.0
Hope	1.23	3.94	16.39	35.23	0.55	4.70
Wichita	1.29	4.59	13.01	42.18	0.60	6.07
Denton-D	1.52	6.32	12.74	41.11	1.0	6.14
Kenya	1.56	5.44	23.74	56.70	1.48	8.16
LSD	0.39	1.62	6.23	9.92	0.49	1.64

(P=0.01)

^a Growth parameter means are averages determined from 10 replication for each variety.

Table 3. Wheat seedling growth parameter differences between eight varieties, after four days exposure to culture filtrate of *P. arrhenomanes*.

Variety	Growth Parameter ^a					
	Length (cm)		Fresh Weight (mg)		Dry Weight (mg)	
	Root	Shoot	Root	Shoot	Root	Shoot
TAM-101	1.57	5.93	46.3	41.47	1.43	5.87
KJW	1.58	5.98	18.93	53.81	1.62	7.24
Denton-E	1.67	6.10	14.70	46.57	1.79	6.54
Crockett	1.62	6.13	16.37	47.36	1.45	6.24
Hope	1.41	5.71	18.79	48.80	1.55	6.16
Wichita	1.69	6.57	20.16	55.34	1.81	8.06
Denton-D	1.98	8.12	15.59	51.53	1.56	7.29
Kenya	1.95	8.4	26.60	46.9	2.65	9.03
LSD	0.38	1.74	7.14	12.74	0.92	1.95

(P=0.01)

^a Growth parameter means are averages determined from 10 replications for each variety

significantly greater fresh and dry root weights, when compared to TAM-101 (Table 1). Root growth parameters among all other varieties grown in water, except Hope, with regard to fresh root weights, were not

significantly different from those of TAM-101 (Table 1).

Results from this study indicate that the variety TAM-101, which was a standard susceptible variety used in previous studies with *P. arrhenomanes* [4, 5, 6], performs significantly better in root growth than all other varieties except Kenya, when infected with *P. arrhenomanes*. This was the case despite the fact that TAM-101, like other varieties tested, was significantly reduced in root and shoot growth as a result of infection or exposure to the toxic metabolite(s) of *P. arrhenomanes*. All three varieties (i.e. Raw, Denton-D and Denton-E), which were previously reported to be resistant to *P. arrhenomanes*, performed significantly poorer in root growth than TAM-101 after inoculation with *P. arrhenomanes*. This indicates that determination of resistance based on emergence [3] alone could be misleading, and measurements of various growth parameters of inoculated plants could be a more precise method of determining resistance. Among all varieties tested, Kenya was the only one which consistently performed as well, or better than TAM-101 for all three treatments. All varieties tested reacted similarly to both infection by *P. arrhenomanes*, or exposure to its toxic metabolite(s). This indicated that such metabolite(s) may have a role in disease development once a host is infected by the pathogen. Also, there may be possibilities for using this metabolite(s) in screening for resistance to *P. arrhenomanes*.

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عکس‌العمل‌های واریته‌های گندم به آلودگی توسط *Pythium arrhenomanes* یا متابولیت‌های سمی آن

چکیده

هشت واریته گندم که اغلب آنها سابقاً به *Pythium arrhenomanes* مقاوم گزارش شده بودند با همین قارچ تلقیح شدند. تلقیح با قرار دادن گیاهچه‌های دو روزه گندم در لبه کشت *P. arrhenomanes* برای ۳ ساعت و ۲۵°C صورت گرفت. گیاهچه‌های آلوده سپس به لوله‌های آزمایشی حاوی مهره‌های ریز شیشه‌ای و یک سانتیمتر مکعب آب استریل منتقل شده و برای ۴ روز در ۲۵°C نگهداری شدند. گیاهچه‌های سالم با قرار گرفتن در لوله‌های آزمایشی حاوی یک سانتیمتر مکعب عصاره کشت مایع ۲۵ روزه *P. arrhenomanes* در معرض متابولیت‌های سمی این قارچ قرار گرفتند. پس از اندازه‌گیری طول و وزن خشک و وزن تازه ریشه و ساقه‌های تمامی واریته‌ها، با اینکه ظاهراً همگی به پاتوژن و متابولیت‌های سمی آن حساس بودند، تفاوتی از نظر عکس‌العمل به دو عامل فوق در بین واریته‌ها مشاهده شد. همچنین ملاحظه گردید که استفاده از متابولیت‌های سمی این قارچ در بررسی مقاومت واریته‌های گندم به *P. arrhenomanes* امکان‌پذیر می‌باشد.