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Evaluation of wheat cultivars for *Anguina tritici* resistance, development and influence of nematode on wheat growth

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Abstract. The effects of *Anguina tritici* on wheat growth and nematode reproduction were studied in a lathe house. One wheat seed and one cockle containing *ca* 16,000 hibernating second stage juveniles (J2) were planted to individual 10-cm-diam clay pots containing sterilized sandy loam soil. Host status was assessed on the basis of development of symptoms, reproduction rate (P_f / P_i) per cockle as well as per plant and P_f / P_i in relation to grain yields. Appearance of cockle disease symptoms was recorded. Eight wheat cultivars including Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711 exhibited twisting and crinkling of leaves. In these plants P_f/ P_i exceeded 1.0 ranking them as susceptible/good host. Six cultivars including Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, and Rawal-87 exhibited no foliar symptoms with a P_f / P_i lower than 1.0 and their ranking was resistant/tolerant/poor host. Reproduction rates could be directly correlated to size of new cockles. Highest *A. tritici* reproduction was 1.77, which occurred on Pak-81 and was associated with cockles of 5-mm in diam. Punjab-85 produced a P_f / P_i of 0.89 with cockles of 3.7 mm-diam. Number of cockles produced on each cultivar was also increased as reproduction rates were increased.

Keywords: *Anguina tritici*, rate of reproduction, symptomatology, wheat cultivars, yield reduction.

INTRODUCTION

Seed gall nematode, *Anguina tritici* (Steinbuch) Chitwood, an important pest of wheat, has been reported from India (Midha and Swarup, 1974), Pakistan (Anwar *et al.*, 1992, Iraq (Al-Beldawi, *et al.*, 1985), Ethiopia, Yugoslavia, Romania and Syria (Kiryanova and Krall, 1963). This nematode feeds as an ectoparasite on young leaves around the growing points and as an endoparasite on the tissues of the inflorescence. Nematode feeding upon young leaves leads to twisted, crinkled and folded leaves. Additionally, tips of infected leaves may become attached to the growing stem. During formation of floral primordia nematodes enter the soft tissues and nematode reproduction occurs at the expense of plant reproduction. Nematode-filled cockles develop instead of normal seeds.

Anguina tritici also vectors *Clavibacter tritici* (*Corynobacterium michiganense* pv. *tritici*), the causal agent of yellow ear rot or "tundu" of wheat. Plants exhibiting symptoms of tundu do not produce cockles or viable wheat that year. Current-year cockles infected with these bacteria are highly toxic to cattle and sheep that feed on them (Bird, 1981; McKay and Ophel, 1993).

Yield losses caused by this nematode vary from 6% to 53% depending upon the resistance level of the wheat cultivars (Khan and Ather, 1998; Paruthi and Bhatti, 1981). These losses cannot be minimized by the use of chemicals due to complexities of nematode biology and the relatively low economic value of wheat. The best way to manage this nematode is through resistant cultivars. Numerous breeders have screened wheat germplasm against *A. tritici*. Selection criteria have been based on foliage and cockle symptoms (Paruthi and Bhatti, 1981; Shahina *et al.*, 1989) or these symptoms plus rates of reproduction (Khan and Ather, 1998; Anwar *et al.*, 1992, 1995, 1996).

The purpose of this study was to quantify: (1) the ability of *A. tritici* to reproduce on various commercial wheat cultivars, (2) the effect of nematode reproduction on grain yield and (3) the effect of nematode reproduction at different initial nematode densities on wheat growth.

MATERIALS AND METHODS

Nematode inoculum

Harvested remains of wheat consisting of wheat cockles, shrivelled grains and weed seeds were collected from flourmills located in an industrial area of Islamabad, Pakistan. Wheat cockles were separated from the mixture and selected for uniformity of size. Twenty cockles were randomly picked to enumerate second stage juveniles (J2) per cockle. There were *ca* 16,000 J2 per cockle based on the average of 20 cockles.

Wheat seed planting and nematode inoculation

Uniform-sized seeds of fourteen wheat cultivars including Blue Silver, Barani-70, Barani-83, Chakwal-86, Faisalabad-83, Faisalabad-85, Kohinoor-83, LU-26, Pak-81, Punjab-85, Punjab-87, Rawal-87, Shalimar-88 and WL-711 were surface sterilized by soaking for 20 minutes in 2% Clorox, rinsed three times in sterile water, and damp-dried. At normal seedbed conditions J2 require 3 to 5 days to emerge from cockles whereas wheat seed takes only 3 days to sprout. Cockles were therefore soaked in distilled water for 24 hours prior to planting to facilitate simultaneous emergence of sprouts and J2. A hole was dug in the center of each 10-cm diam clay pot filled with an autoclaved sandy clay loam soil (45% sand, 25 % silt and 30% clay). One wheat seed and one cockle were planted per pot. Initially sixteen pots were planted for each wheat cultivar. Half were inoculated and the other half served as the non-inoculated check. At third-leaf stage four uniform-sized inoculated plants and four uniform-sized check plants were selected from each wheat cultivar and allowed to grow to maturity. Pots were arranged randomly on a bench in the lath house and rotated twice monthly to compensate lighting differences throughout the growing season, December 1999 to April 2000.

Effect of different initial nematode densities on nematode reproduction and wheat growth

The effect of five initial nematode densities (P_i) on the growth of wheat cv Pak-81 was studied. This cultivar was selected being most susceptible to *A. tritici* from experiment No. 1. Pot size, soil types and planting of seeds were as described above. There were five pots for each nematode P_i. Each pot was inculcated with 0, 1, 2, 3, 4, and 5 cockles. At the termination of the experiment plants were harvested and tops, roots and grain yield were processed for plant growth and number of cockles, their size and J2 per cockle was assessed to compare the reproduction rate at different P_i.

Data collection

Plants were regularly inspected during the growing period to record visible symptoms of nematode damage. At harvest the grain yield per plant, number of cockles per plant, cockle size, and J2 populations were recorded. Cockle size was determined with a caliper. Means for cockle size and J2 per cockle represented an average from 20 cockles.

Data analysis

Analysis of variance was performed by using the statistical software SAS V6.12 (SAS Institute, Cary, NC, USA). Means were compared with Duncan's multiple range test.

Table 1. Symptom expression and yield of selected wheat cultivars infected or non-infected with *Anguina tritici.*

Cultivars tested	Foliar Symptoms	Mean cockle Size (mm)	Grain yield per plant [g]	
			Check	Inoculated
Blue Silver	+	4.3	6.8	4.70*
Barani-70	+	4.8	7.65	4.55*
Barani-83	-	3.6	7.40	6.22
Chakwal-86	+	5.0	7.57	4.62*
Faisalabad-83	-	3.7	6.32	5.45
Faisalabad-85	-	4.0	7.65	5.60
Khoinoor-83	-	3.8	6.87	6.05
LU-26	+	4.2	6.12	4.10*
Pak-81	+	5.4	7.25	3.27*
Punjab-85	-	3.7	7.32	6.07
Punjab-87	+	4.2	7.20	6.40*
Rawal-87	-	3.4	6.47	6.03
Shalimar-88	+	5.2	6.90	5.10*
WL-711	+	4.1	4.77	5.25*

+ = Appearance of symptoms, - = No appearance of symptoms.
* Indicates the significant difference between nematode inoculated and check at P = 0.01 according to Duncan's multiple range test.

RESULTS

Ear cockle disease symptoms

Wheat cultivars responded differently to infection by *A. tritici* (Table 1). On 20-day old seedlings a swelling of the stem at ground level and twisting and crinkling of leaves only became visible on Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88, and WL-711 wheat cultivars. Intensity of symptoms also differed among these eight cultivars, which is obvious due to difference in genetic make-up. On infected plants the tips of emerging leaf blades were frequently arched dorsally back toward the growing stalk tip. *Anguina tritici* inoculations did not produce foliar symptoms on Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, or Rawal-87 wheat cultivars. Yellow ear rot or ear blight known as "tundu" disease in India and Pakistan was observed in a few plants of Blue Silver, Pak-81 and Chakwal-86 only.

Ear emergence was earlier and distinctly different on wheat cultivars exhibiting foliar symptoms compared to check plants or inoculated plants without foliar symptoms. Infected ears were smaller and broader, with or without awns and with more open glumes. It was interesting to observe that cockles produced by cultivars exhibiting foliar symptoms were smaller compared to those produced by cultivars with out foliar symptoms. Meanwhile, numbers of J2 per new cockle were directly proportional to the size of cockle (Table 1, 2).

Grain yield

The yield of fourteen wheat cultivars tested against *A. tritici* is presented in Table 1. There was a significant difference ($P = 0.01$) in grain yield of nematode infected plants of Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711 wheat cultivars compared to their respective non-inoculated check. No significant difference in grain yield was observed between inoculated plants of Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, and Rawal-87 wheat cultivars compared to their checks.

Nematode reproduction

Anguina tritici reproduced on all fourteen wheat cultivars and reproduction was influenced by wheat cultivars. Reproduction rate of J2 per new cockle (P_f / P_i) was greater than 1.0 for Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711 wheat cultivars. Reproduction was lower on Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, and Rawal-87 wheat cultivars leading to P_f/P_i less than 1.0. The greatest P_f/P_i value was 1.77 for Pak-81 and lowest (0.89) for Punjab-85 (Table 2). The production of cockles was variable among cultivars. The greater number of cockles developed in ears of plants of wheat cultivars with disease symptoms than that of plants without symptoms (Table 1). The population of J2 per cockle was influenced by cockle size. The cockles of 4 mm or less contained J2 close to P_i (J2 = 16000) whereas those

Table 2. Rate of *Anguina tritici* reproduction on selected wheat cultivars.

Cultivars tested	Number of J2 per cockle	Number of cockles per plant	Reproduction rate [P_f/P_i] per Cockle	Reproduction rate [P_f/P_i] per Plant
Blue Silver	22200	30	1.39	41.64
Barani-70	25700	12	1.61	19.66
Barani-83	15200	2	0.95	2.28
Chakwal-86	27900	23	1.74	40.89
Faisalabad-83	15300	4	0.95	3.99
Faisalabad-85	17000	6	1.06	6.57
Khoinoor-83	15600	6	0.97	5.82
LU-26	20900	23	1.31	30.78
Pak-81	28300	10	1.77	10.77
Punjab-85	14200	11	0.89	9.98
Punjab-87	17600	8	1.10	8.58
Rawal-87	15100	8	0.94	7.52
Shalimar-88	26700	28	1.67	47.59
WL-711	22600	24	1.41	23.70

Table 3. The influence of *Anguina tritici* initial population densities [P_i] on reproduction rate [P_f/ P_i] and growth of wheat cv Pak-81.

P_i [cockles] per plant	Dry weight [g] Root[1]	Dry weight [g] Shoot	Yield per plant [g]	Cockle size [mm][2]	J2 per cockle[2]	Reproduction rate [P_f/P_i]
0	1.86a	5.68a	4.80a	-	-	-
1	1.54b	4.58b	3.26b	5.50	30, 600	1.91
2	1.16c	4.08c	2.64c	5.10	24, 500	0.76
3	0.86d	3.80d	2.12d	4.80	21, 200	0.44
4	0.64e	3.20e	1.52e	4.50	17, 600	0.27
5	0.44f	2.38f	0.00f	4.00	16, 800	0.21

[1]Means followed by the same letter are not significantly different at P = 0.01 according to Duncan's multiple range tests. [2]Means of 20 cockles.

cockles with a size more than 4 mm contained greater than P_i. The P_f /P_i per plant of *A. tritici* was higher than 1.0 for all cultivars. The wheat cultivars with higher P_f / P_i per cockle than 1.0 had also greater P_f /P_i per plant compared to that of less P_f /P_i than 1.0 (Table 2).

Effect of different initial nematode densities on nematode reproduction and wheat growth

Nematode reproduction and foliage and floral symptom development were greatly influenced by the size of P_i. Reproduction rate was > 1 for P_i of one cockle per plant but < 1 for all other P_i's (Table 3). Shoot and root growth and grain yield were significantly affected by P_i as indicated by control plants (Table 3).

DISCUSSION

Host resistance to endo-migratory root feeding nematodes is assessed using reproduction rates (Anwar and Van Gundy, 1989; Pinochet *et al.*, 1976). For sedentary endoparasites such as the cyst nematode resistance has been based on reproduction and number of cysts (Grant *et al.*, 1996). With root knot nematodes: reproduction, gall indices and egg mass indices have characterized host resistance (Barker *et al.*, 1981). For ectoparasitic nematodes host resistance was based on final soil population levels after two years (McKenry *et al.*, 2001). By contrast, evaluation of host response to shoot feeding nematodes has frequently been based on either foliar and inflorescence symptoms probably because they produce very specific symptoms (Shahina *et al.*, 1989) or final nematode population (P_f) per cockle (Anwar *et al.*, 1995; Khan and Ather, 1998). In this work we have characterized symptoms and quantified P_f / P_i per cockle as well per plant and reproduction in relation to yield decline due to nematode infection. We have compared the response of cultivars to nematode inoculations on the basis of P_f / P_i per plant to P_f / P_i per cockle to evaluate the resistance response of wheat cultivars against *A. tritici* infection.

Differential development of foliar and inflorescence symptoms on wheat cultivars infected by J2 of *A. tritici* grouped them into two categories. The first category includes Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711which exhibited classical ear cockle symptoms (Gupta and Swarp, 1968). This was categorized as a susceptible response. The second category comprising Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, and Rawal-87 was categorized as resistant due to lack of foliar and inflorescence symptoms (Table 1, 4). Rating of cultivars on the development of nematode damage symptoms has been frequently used to evaluate the wheat cultivars to *A. tritici* infection in the Indo-Pak subcontinent (Ilyas and Hussain, 1985; Paruthi and Bhatti, 1981; Shahina *et al.*, 1989).

The response of wheat cultivars to *A. tritici* had been evaluated on P_f per cockle (Anwar *et al.*, 1992, 1995, 1996; Khan and Ather, 1998,). Seinhorst (1967) determined host status by the values of the equilibrium density (E, where P_f = P_i) and the maximum rate of reproduction (the maximum P_f / P_i ratio). Plants are a good host if both values are high, and a poor host if both values are low. These values are influenced by environmental conditions for any plant and nematode combination. High reproduction rates and predicted equilibrium values for *A. tritici* on wheat cultivar Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711 confirm that these are good hosts for this nematode (Table 4). The data on evaluation of wheat cultivars assessed on P_f/ P_i plus equilibrium density values of *A. tritici* is lacking but our observations are in line of that found for root-knot nematode on susceptible cultivars of wheat and soybean (Nardacci and Barker, 1979; Roberts and Van Gundy, 1981) and to observed reproduction rates for a range of endoparasitic and ectoparasitic nematodes reproducing on good hosts (Seinhorst, 1967). Low reproduction rate and low predicted equilibrium density values for

Table 4. Host response designation when wheat foliage is attacked ecto-parasitically and floral primordia endo-parasitically while vectors *Corynobacterium michiganense* pv. *tritici*.

Cultivars tested	Symptom[1] development	Rate of reproduction[2] $[P_f / P_i]$ per Cockle	Rate of reproduction[2] $[P_f / P_i]$ per Plant	Yield reduction[3] per plant	Yield reduction + $[P_f / P_i]^4$ per Cockle	Yield reduction + $[P_f / P_i]^4$ per Plant
Blue Silver	S	GH	GH	S	S	S
Barani-70	S	GH	GH	S	S	S
Barani-83	R	PH	GH	R	R	T
Chakwal-86	S	GH	GH	S	S	S
Faisalabad-83	R	PH	GH	R	R	T
Faisalabad-85	R	PH	GH	R	R	T
Khoinoor-83	R	PH	GH	R	R	T
LU-26	S	GH	GH	S	S	S
Pak-81	S	GH	GH	S	S	S
Punjab-85	R	PH	GH	R	R	T
Punjab-87	S	GH	GH	S	S	S
Rawal-87	R	PH	GH	R	R	T
Shalimar-88	S	GH	GH	S	S	S
WL-711	S	GH	GH	S	S	S

GH = Good host; PH = Poor Host; R = Resistant; S = Susceptible; T = Tolerant.
[1,3]Anwar *et al.* 1995, 1996; [2] Seinhorst, 1967; [4]Oostenbrink, 1956).

A. tritici on wheat cultivars Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, Rawal-87 confirm that these are poor hosts (Table 4). The low rates of *A. tritici* on wheat are comparable to *A. tritici* on resistant barley (Bhatti *et al.*, 1978; Dhawan and Swarup, 1979).

The evaluation of cultivars to nematode inoculations on P_f / P_i per cockle does not provide realistic approach as it lacks P_f / P_i per plant, a standard procedure, used for other crop-nematode combinations (Roberts and Van Gundy, 1981, Seinhorst, 1967). We calculated P_f / P_i per plant (P_F = number of cockle per plant X J2 per cockle / P_i) and compared with P_f /P_i per cockle, which rendered all the wheat cultivars as good host (Table 4).

Complete assessment of plant response to nematode infection should be based on nematode reproduction and damage caused by nematodes (Jones, 1956; Oostenbrink, 1956). Resistant plants restrict or prevent nematode multiplication and yield well. This categorization takes into account nematode and plant influences. Susceptible plants become damaged and nematodes reproduce abundantly upon them. High reproduction of *A. tritici* and resultant yield decline on Blue Silver, Barani-70, Chakwal-86, LU-26, Pak-81, Punjab-87, Shalimar-88 and WL-711 rank them as susceptible. Low reproduction by *A. tritici* (per cockle) and good yield of wheat cultivars Barani-83, Faisalabad-83, Faisalabad-85, Kohinoor-83, Punjab-85, Rawal-87 rank them as resistant. The rating of these cultivars on the combination of yield decline plus P_f / P_i per plant changed the above classification and designated all the resistant cultivars to tolerant due to high P_f / P_i and no significant yield loss on six resistant wheat cultivars (Table 4).

The multiplicity of interactions between *A. tritici* and its favoured host can serve to test designations such as resistance, susceptibility and tolerance. This nematode feeds as an ectoparasite on foliage but becomes endoparasitic on reproductive juvenile tissues that eventually form kernels of grain. Feeding by J2 on foliage can result in twisting, crinkling and complete inversion of photosynthetic surface area. Infections to the foliage are also known to trigger biochemical changes such as reduced carbohydrate pool and considerable quantitative and qualitative changes in protein metabolism (Pathak *et al.*, 1983). Reduced plant vitality can also translate into reduced population development by the nematode and reduced cockle size (Table 1, 2). Feeding by this nematode can also serve to vector plant-damaging bacterial infections, however that aspect was not a component of this study (Bird, 1981). Once J2 moult within individual grain kernels a "sink effect" for nutrients can also occur (Gupta and Swarup, 1968). Heads emerging from infected plants are shorter in length and the grains are undersized leading to overall yield reductions.

The contemporary definition for resistance is none to very little reproduction by the pest on that host (Seinhorst, 1967). Table 1 indicates that each of the fourteen wheat cultivars provided suitable substrate for nematode reproduction. The cockles of these cultivars will perpetuate this nematode in India and Pakistan to cause disease in the next year. All fourteen cultivars must be judged as susceptible to *A. tritici*. Some researchers have evaluated resistance based on the P_i from one cockle compared to the P_f counted from one cockle but data in Table 4 show the error of that methodology.

The contemporary definition for plant tolerance implies that even in the presence of high nematode population levels growth and yield reductions to the plant are minimal. In this study there were six wheat cultivars that yielded the same whether infected with one cockle or none (Table 1). At higher P_i levels some of these "tolerant" plants may have become damaged, however, none of the six expressed foliar symptoms. This supports the notion that these six are susceptible but exhibit a type of non-feeding mechanism associated with their leaves. Without leaf distortions these cultivars yield as well as non-infected plants so by definition six cultivars were susceptible but tolerant. By comparison the other eight cultivars displayed foliar symptoms, produced smaller sized cockles, but also tended to produce fewer J2 within each new cockle.

Five of these six cultivars also tended to produce less grain per plant so we characterize them as susceptible but intolerant of the nematode. Punjab 87 was one of the six cultivars at variance because it produced an abundance of new nematodes, expressed foliar damage associated with J2 feeding but also produced high yield. At higher P_i levels the tolerance of this susceptible plant may be lost.

These data suggest that wheat breeders/agronomists have selected Punjab 87 because of its tolerance to external and internal feeding by *A. tritici*. Six additional cultivars have been selected for the lack of foliar symptoms expressed in association with ectoparasitic feeding by J2 of *A. tritici*. Based on the biology of this nematode, resistance might be predicted to occur by finding anti-feeding mechanisms that function at the site of kernel development. This mechanism would be best used in combination with mechanisms that reduce foliar feeding. If foliar feeding truly is reduced by these six cultivars, there should also be a reduction in the incidence of "Tundu" appearing on the foliage. Stoppage of entry of J2 into developing wheat kernels and Tundu appearance in the heads might also disappear.

Our study concludes that reproduction rates using P_f / P_i per cockle indicated resistance was present in some cultivars. However, when P_f / P_i was based on total cockles produced per plant none of the cultivars was resistant. On the basis of symptom development, half the cultivars did not express a twisting and crinkling of leaves. Expression of yellow ear rot, referred to as "TUNDU" in India and Pakistan, occurred among few cultivars only. On the basis of yield differences between inoculated and non-inoculated about half the cultivars produced significantly less yield in the presence of the nematodes. Reproduction rates could be directly correlated to size of new cockles. These findings demonstrate the ability of *A. tritici* to express differing symptoms on different wheat cultivars. Some wheat cultivars have been designated as resistant because they did not exhibit leaf crinkling and twisting, a symptom initiated by J2 feeding. Others have received this designation because of greater tolerance to "tundu" or to nematode feeding. Some cultivars have received a resistance designation because calculations were based on nematodes per cockle rather than nematodes per plant. None of these cultivars is truly resistant to *A. tritici* and future efforts in breeding/selection should focus on nematode reproduction rates rather than measurements of plant symptoms, nematode feeding or yield.

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