AgroLife Scientific Journal - Volume 2, Number 2, 2013
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

SEED GALL NEMATODE *Anguina tritici* IN BULGARIA: NEMATODE IMPACT ON WHEAT GROWTH AND GRAIN YIELD

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Abstract

Seed gal nematode (Anguina tritici Stein.) has not been reported as a parasite of wheat and barley in Bulgaria for more than thirteen years. However, in 2012 it has been detected in two locations of southeastern Bulgaria. Regarding this, the aim of the present study was to focus on the nematode pathogenicity and its impact on wheat plants. In a laboratory experiments were tasted five varieties of wheat against A. tritici. None of them showed to be resistant to the nematode. Field experiment were carried out in infested farmer fields during the 2012/2013 growing season to evaluate the effect of seed gall nematode on grain yield. A. tritici severity corresponded both with a decrease in number of grain spike and with an increase of number of distorted and died spike. Nematode pathogenicity potential corresponded also with a decrease of grain weight spike and its affect on yield.

Key words*: seed gall nematode, pathogenicity, Triticum aesitvum, susceptibility, grain yield.*

INTRODUCTION

Seed gall nematode was the first plant parasitic nematode to be observed and described. The species was reported by Turbevill Needham in 1743. This is thought to be the first recorded microscopic observation in which the observer associated a pathogenic organism as the causal agent of a plant disease (Lehman, 1979). *Anguina tritici* juveniles feed ectoparasitically on the tissues of growing points of the leaves. Later, the juveniles penetrate the flower buds at the time of flower bud initiation and start to feed endoparasitically. Finally, the nematodes convert wheat grains into galls, caused enormous yield loss (Evans et al., 1993). In India, the annual loss caused by *A. tritici* ranges from 1 to 9% representing financial loss of about 70 million rupees (Kaushal, 1998). In Turkey, the infection rates ranged from 1.5 to 55.2% (Elmali, 2002).

Seed gall nematode has been found in major wheat growing regions of the world and is easily disseminated in seed. Recent surveys of *A. tritici* have not provided any evidence that nematodes are still occurring in most of the countries where previously has been found (Nicol and Rivoal, 2008). The species is still occurring in several countries of Africa (Nicol and Rivoal, 2008), India (Kausar and Khan, 2009), Iran (Bonjar et al., 2004) and Turkey (Imren, 2007).

In Bulgaria, the first documented study on *A. tritici* was from surveys conducted in the mid twenties of the last century (Stoyanov, 1980). Over the next seven decades the nematode has been reported several times from different parts of the country (Gateva, 1999). Since the beginning of this century, damages causing by seed gall nematode have not been observed in any wheat producing regions of the country. In 2012 seed gall nematode has been detected in two wheat fields of the southeastern Bulgaria.

Ever since *A. tritici* was reported as a parasite of cereal crops, its morphological, biological and ecological features have been studied (Southey, 1972; Gokte and Swarup, 1987; Evans et al., 1993). Numerous issues have described the pathogenicity of this plant parasitic species and various aspects of its control strategies (Peterson et al., 1986; Galper et al., 1991; Singh et al., 2012). Despite all mentioned so far, information about seed gall nematode impact on the wheat plants growth and grain yield in Bulgaria is lacking.

Therefore, the objective of the present study was to conduct a survey to determine the effect of *A. tritici* on the growth of several wheat varieties under laboratory conditions. The study also was attempted to evaluate the damages of the nematode on wheat plants under field conditions.

MATERIALS AND METHODS

Pots experiment

The experiment was carried out during 26th of June till 10th of October 2012. Fifty plastic pots of 15 cm diameter were filled with 1000 ml soil mixed with compost : manure (3:1). Then pots were autoclaved in order to sterilize the soil and make it free from any kind of pathogen. Certified seeds of five varieties of wheat, *Triticum aestivum* L. (Table 1) were pre-soaked in water for four hours separately and then five seeds of each variety were sown in each pot with ten replicates for each variety. Fifteen days after sowing five pots of each variety were inoculated with about 10000 second stage juveniles of *A. tritici*. The juveniles were obtained from the wheat galls collected from damaged plants at the end of precious growing season. The wheat galls were soaked in sterile distilled water for 24 hours and the inoculum was prepared by crushing the water soaked cokled seeds. Five non-inoculated pots of each variety served as control. After the inoculation the pots were placed in growth chamber which was programmed with 16°C day/12°C temperature regime, 12 hr day/night cycle and 70% relative humidity. During the growing period of three months the plants were regularly inspected for visible symptoms of nematode damages. At maturity, the plants were uprooted and the length of root and shoot, the number of leaves, tillers and grains/plant and the number of galls/plant were recorded. The number of second stage juveniles per gall was counted as well. Mean value of each group of five plants were treated as a replicate.

Field experiment

Study was carried out in farmer fields nearby, in Yambol province during 2012/2013 wheat growing season where *A. tritici* infestation was first identified in the spring of 2012. The sowing was done during 15-20 October which period is the most appropriate sowing time for the region. Seeds of wheat variety Enola were sown by drill in total area of 16 ha at rate of 550 seeds/m^2. Fertilizers at the rates of N_{200}, P_{150}, K_{150} kg per ha were applied at sowing and stem elongation stages of crop growth, respectively. A broad leaf herbicide was applied at four leaves stage of weeds. All other agronomical practices for a healthy crop production were taken up. Crop was harvested on 27th of June 2013 by combine harvester.

Experimental twin plots (1+1=2 m^2 each) located 100 m apart were randomly selected at four different places in the field at maturity stage. Total number of spikes, number of distorted and totally dried spikes with empty spikelets (nematode damaged) in which plot were counted. Selected plots were harvested by hand and threshed for measuring of grain weight/spike.

Analyses of variance were carried out using SPAS (Statistical package for agricultural scientists, available online at http://www.hau.ernet.in/link/spas.htm).

RESULTS AND DISCUSSIONS

Data summarized in Table 1 show that length of root and shoot and number of leaves, tillers and grains per plant of varieties Aglika, Crystal, Albena and Diamond were significantly reduced in inoculated pots compared to the control. There was no significant reduction in root and shoot length of the Enola plants compared to the control. The highest number of seed galls was occurred on the varieties Enola and Aglika followed by Albena and Diamond. The largest number of second stage juveniles per gall was counted in both Aglika and Crystal varieties followed by Enola, Albena and Diamond.

The results presented in Table 1 show that two of the most grown wheat varieties in Bulgaria - Enola and Aglika can be considered as susceptible to *A. tritici*. This is important to note because in 2013/2014 growing season approximately 18% of wheat areas have been sown by grains of variety Enola (Annual agricultural statistic, 2013).

Table 1. Response of wheat varieties to the seed gall nematode *Anguina tritici*

Variety	Treatment	Length (cm)		Number/plant			Galls/plant	Juveniles/grain
		Root	Shoot	Leaf	Tiller	Grain		
Aglika	Control	13.5	72.4	6	1.74	25	-	-
	Inoculated	11.6	66.3	6	1.38	24	24	10,000
Enola	Control	12.9	69.8	6	1.62	33	-	-
	Inoculated	11.5	67.2	5	1.44	27	27	7,500
Crystal	Control	13.0	73.0	7	1.72	24	-	-
	Inoculated	11.2	64.6	6	1.24	20	17	10,000
Albena	Control	11.8	62.0	7	1.32	30	-	-
	Inoculated	10.9	60.8	7	1.21	22	20	6,000
Diamond	Control	13.6	75.7	6	1.68	29	-	-
	Inoculated	12,0	66.4	5	1.30	19	19	5,000
LSD at 5%		1.42	3.19	0.38	0.31	2.08		

Total number of spikes (m^{-1}), the number of *A. tritici* damaged spikes (m^{-1}) and yield (kg/ha^{-1}) based on means of twin plots are presented in Table 2. The numbers of spikes (m^{-1}) were almost homogenous for the four plots and ranged from 437 to 456 spikes (m^{-1}) per plot. All the four plots except plot 4 have a quite similar number of distorted and died spikes varied from 38 to 59 and from 14 to 22 spikes (m^{-1}), respectively. The infestation ratio excluding the rates of healthy looking spikes was 13.5 ([(44.75+15.5)/446]×100) in wheat variety Enola.

Table 2. Yield obtained from healthy and infested by *Anguina tritici* wheat plants grown in 4 randomly sampled unit area in the infected field

No. of twin plots	NoS1[1]	NoS2[2]	NoS3[3]	NoS4[4]	Y[5] (kg ha^{-}
1	456	53	17	336	4300
2	441	59	22	360	3980
3	450	38	14	398	4220
4	437	29	9	399	4040
Mean	446	44,75	15,5	385,75	4130,5
±SD	±38.57	±3.25	±0.94	±10.72	±41.3
		SW2[6]	**SW3[7]**	**SW4[8]**	
Mean		0.681	0.00	2.053	
±SD		0.04		0.07	

[1]NoS1: average no. of spike (m^{-2}), [2]NoS2: average no. of distorted spikes (m^{-2}), [3]NoS3: average no. of dried spikes (m^{-2}), [4]NoS4: average no. of healthy looking spikes (m^{-2}), [5]Y: yield obtained from combine harvester, [6]SW2: distorted spike weight (g^{-1}), [7]SW3: dried spike weight (g^{-1}), [8]SW4: healthy looking spike weight (g^{-1})

The weight of distorted spikes (m^{-1}) was three times lower than the weight of healthy looking spikes (m^{-1}). Similarly Ozberk et al. (2011) reported 2.79 (g^{-1}) average spike weight of healthy looking spikes (m^{-1}) (variety Fuatbay 2000) and 0.55 (g^{-1}) of distorted spikes.

It is interesting to note that affected by *A. tritici* plant showed visible basal swelling at the second half of April. Afterwards wrinkling, crinkling and twisting of leaves were observed (Figure 1). These symptoms were attributed to ectoparasitic feeding of the nematodes. Damaged spikes had tinner and deformed ear head, with broken and distorted awns. Totally dried spikes contained only galls, without any kernels.

Figure 1. Distorted wheat plants with twisted and crinkled leaves due to *Anguina tritici* development

In the present research average grain yield at the end of growing season was 4,130.5 (kg/ha^{1}). It is quite lower than potential yield of the variety Enola, which is more than 6,500.0 (kg/ha^{-1}). This significant difference between actual and potential grain yield probably due to the seed gall nematode infestation. *A. tritici* infestation is a certain but not the only reason for the observed difference. According to many research workers and farmers, the region (Yambol province) is not very appropriate for growing of common wheat (*Triticum aestivum* L.) (Delibaltova and Ivanova, 2006). Probably because of these two reasons the grain yield was lower than expected.

The galls were green at the beginning and later become comparatively brown and black, full of second stage juveniles at maturity (Figure 2 and Figure 3).

Figure 2. Healthy wheat grains and grains converted into galls

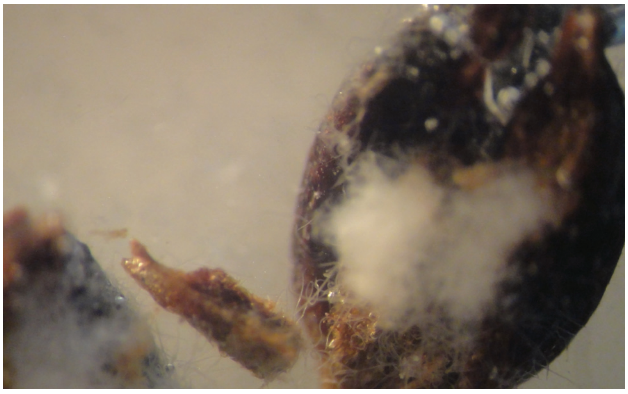

Figure 3. "Wool" of *Anguina tritici* second stage juveniles in gall seeds

Recently in Bulgaria cereal crop production is the major and well developed branch of national agriculture. It is encouraging by government giving subsidy to farmers. Despite that some producers still using uncertified seed and do not take any seed hygienic measures. As a result plant pathologist and representatives of quarantine services are very much aware of the presence of the most important plant parasitic nematodes on wheat, such as *Pratylenchus* spp., *Heterodera avenae* and *A. tritici* in southeastern Bulgaria.

The negative effect of seed gall nematode on grain yield should not be neglected and farmers have to be encouraged to monitor carefully *A. tritici* appearance and to take appropriate measures to control it.

CONCLUSIONS

Plants of all five wheat varieties screened in this study can be invaded by seed gall nematode.

Differences among evaluated varieties allow choosing the variety Enola as a variety with the best plant growth parameters and using it in the further field experiments.

The results of field survey indicated that *A. tritici* infestation ratio on the wheat plants reached a level of 13.5. Due to this grain yield was substantially reduced.

The results of this study suggest that is extremely important to farmers not only to follow the crop rotation with non host plants but also to use certified seeds and to monitor permanently the cereal fields.

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