INFLUENCE OF THIABENDAZOLE SEED TREATMENT ON THE INTEGRATED CONTROL OF *HETERODERA FILIPJEVI* ON SIX WHEAT GENOTYPES WITH DIFFERENT LEVELS OF GENETIC RESISTANCE UNDER CONTROLLED CONDITIONS

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ABSTRACT

Dababat, A. A, S. R. Pariyar, J. M. Nicol, G. Erginbas-Orakci, M. B. Goll, C. Watrin, E. Duveiller, H. J. Braun, J. A. Cabrera, and R. A. Sikora. 2014. Influence of thiabendazole seed treatment on the integrated control of *Heterodera filipjevi* on six wheat genotypes with different levels of genetic resistance under controlled conditions. Nematropica 44:25-30.

This research investigated the influence of the fungicide, thiabendazole, used as a seed coating on the infection of the cereal cyst nematode, *Heterodera filipjevi*, on wheat, and studied possible interactions of thiabendazole with wheat genotypes of different genetic sources of resistance. Thiabendazole was applied to the three susceptible (S) wheat genotypes: Seri (spring wheat), Bezostaya, and Gerek (winter wheat) and three moderately resistant (MR) winter wheat genotypes F130L 1.12/ATTILA, Katea, and Sonmez at 25, 50, and 100 g ai/100 kg seeds. Thiabendazole caused a significant reduction in cyst number on Seri, Gerek, and Bezostaya when compared with the respective controls. The number of cysts of *H. filipjevi* per root system was significantly reduced on the MR genotype when compared to the S genotype in the absence of fungicide seed treatment. Thiabendazole showed a consistent effect on the MR genotype in terms of cysts forming on the root system. Thiabendazole at 50 g ai/100 kg seeds caused the highest reduction in cyst numbers on both S and MR genotypes over the controls. Thiabendazole treatment did not affect plant shoot height, shoot and root weight, nor root length when compared with untreated controls. Symptoms of phytotoxicity were not observed at any of the thiabendazole treatments. The results demonstrated that fungicide seed treatment can lead to reductions in *H. filipjevi* infection on susceptible genotypes and may increase the effectiveness of moderately resistant genotypes. The mode of action needs further research.

Key words: Heterodera filipjevi, resistant, seed treatment, susceptible, wheat genotype.

RESUMEN

Dababat, A. A, S. R. Pariyar, J. M. Nicol, G. Erginbas-Orakci, M. B. Goll, C. Watrin, E. Duveiller, H. J. Braun, J. A. Cabrera, and R. A. Sikora. 2014. Influencia del tratamiento de semillas con thiabedanzol en el control integrado de *Heterodera filipjevi* sobre seis genotipos de trigo con diferentes niveles de resistencia genética en condiciones controladas. Nematropica 44:25-30.

En este trabajo se investigó la influencia del fungicida, thiabendazol, usado como recubrimiento de semillas, en la infección del nemátodo de los cereales, *Heterodera filipjevi*, en trigo y sus posibles interacciones con genotipos resistentes de trigo de diferentes fuentes. Se aplicó thiabendazol a tres genotipos de trigo susceptibles (S): Seri (trigo de primavera), Bezostaya y Gerek (trigo de invierno) y a tres genotipos de trigo de invierno moderadamente resistente (MR): F130L 1.12/ATTILA, Katea y Sonmez en dosis de 25, 50, y 100 g i.a/100 kg de semillas. El thiabendazol causó una reducción significativa del número de quistes en los genotipos S Seri, Gerek y Bezostaya cuando se compararon con sus respectivos controles. El número de quistes de *H. filipjevi* por sistema radical se redujo significativamente sobre los genotipos MR en comparación con los genotipos S en ausencia de tratamiento de las semillas con fungicida. El thiabendazol mostró un efecto consistente sobre los genotipos MR, en términos de formación de quistes sobre el sistema radical. Thiabendazol a dosis de 50 g i.a/100 kg de semilla causó la mayor reducción del número de quistes sobre ambos genotipos, S y MR en relación a los controles. El tratamiento con thiabendazol no afectó ni a la longitud ni a los pesos de la parte aérea o de las raíces comparadas con los controles no tratados. No se observaron síntomas de fitotoxicidad en ninguno de los tratamientos con Thiabendazol. Estos resultados demostraron que el tratamiento de las semillas con fungicida puede reducir la infección por *H. filipjevi* en genotipos susceptibles y puede incrementar la efectividad de los genotipos moderadamente resistentes. El mecanismo de acción necesita de futuras investigaciones.

Palabras clave: Heterodera filipjevi, resistente, susceptible, tratamiento de semillas, trigo genotipo.

INTRODUCTION

A major obstacle to wheat production in dryland areas is the cereal cyst nematode, *Heterodera filipjevi* (CCN). Ten percent of the worldwide production of cereal is lost because of plant-feeding nematodes (Whitehead, 1998). Cereal cyst nematodes alone can decrease wheat yield and have synergistic negative effects with abiotic factors, such as water stress. In addition, plant-parasitic nematodes, such as *H. filipjevi*, are a major biotic factor limiting cereal production in temperate rainfed growing regions of the world (Dixon, 2009). The CCN group has been documented as causing economic yield loss in rainfed wheat production systems in several parts of the world including North Africa, West Asia, China, India, Australia, the United States, and many countries in Europe where they can occur in mixtures and often form complexes with root-rotting fungi (Nicol *et al.*, 2006). Predominant species are *H. avenae*, *H. filipjevi*, and *H. latipons*, and each species can have several pathotypes. Yield losses ranging from 15-20% in wheat in Pakistan, 40-90% in wheat and 17-77% in barley in Saudi Arabia, and 20% in barley and 23-50% in wheat in Australia have been reported (Nicol, 2002). More recently, yield losses have been recorded up to 50% in commonly grown wheat cultivars in Central Anatolian Plateau (CAP) in Turkey (Nicol *et al*., 2003; Sahin et al., 2009). Several CCN species affect wheat production in North Africa and West Asia and yield losses of about 50% in intolerant cultivars were reported (Smiley *et al*., 1996; Nicol, 2005).

The use of resistant wheat genotypes to control CCN is considered biologically effective, economically acceptable, and environmentally friendly (SP-IPM CGIAR, 2010). Many sources of resistance have been developed against CCN, *H. avenae*, and used successfully in Australia, France, India, and Sweden in field experiments with commercial wheat and barley varieties (Nicol *et al.*, 2009). Preliminary research indicates heterogeneous responses between populations to different resistant genotypes (Nicol and Rivoal, 2008). Currently, resistance has only been identified against the CCN, *H. filipjevi*, in Turkey, and this resistance is not yet present in high-yielding cultivars. Resistance to the other nematodes in the CCN complex is still being sought, and the effectiveness of this resistance depends on its durability and on correct identification of the nematode species and (or) pathotypes (Nicol and Rivoal, 2008).

Thiabendazole is a fungicide and parasiticide first introduced in 1962 (Smith and Reynard, 1992). It is mainly used to control mold, rot, and blight on bananas, carrots, citrus fruits, mushrooms, pome fruits, potatoes, soybeans, and wheat and as a post-harvest treatment (Smith and Reynard, 1992). It has been used against several species of nematodes and helminth species, such as roundworms, in livestock and humans. The actual mode of action of thiabendazole on nematodes is unknown. It most likely affects helminth-specific mitochondrial enzyme fumarate reductase, inhibiting the citric acid cycle, mitochondrial respiration and subsequent production of ATP, ultimately leading to death (Davidse, 1986; Smith and Reynard, 1992; Kamaraj *et al*., 2011). Thiabendazole is a suitable compound for seed treatment and can be applied to seeds at high concentrations. In addition, the amount of active ingredient needed to treat seeds is low, and seed treatment directly reduces the high cost associated with all other application forms. To date, the activity of thiabendazole on *H. filipjevi,* and its use as a seed treatment on wheat, has not been investigated, and it would be useful to determine the sensitivity of *H. filipjevi* to thiabendazole to evaluate its potential for field use. Our objectives were to study the effects of a combination of seed treatment and genetic host resistance to obtain improved CCN control. The specific objectives of the investigations were to determine: i) the efficacy of different rates of thiabendazole used as a seed coating on different wheat genotype to control *H. filipjevi*, ii) the influence of different rates of a thiabendazole seed coating on various plant growth parameters, and iii) existence of thiabendazole phytotoxicity on wheat.

MATERIALS AND METHODS

The research was conducted under controlled conditions at the Transitional Zone Agriculture Research Institute-Eskişehir, Turkey. Plants were grown in a potting mixture [70:29:1 of sand: field soil: organic matter (v/v)]. Sand and field soil were sieved and autoclaved at 110°C for 2 h on two successive days while organic matter was autoclaved at 70°C for 5 h before use. Six wheat genotypes having different resistance reactions to *H. filipjevi* were used (Table 1). Wheat seed was sown singly into tubes, 13-cm long and 3 cm in diameter filled with the sterile potting mixture. Three concentrations of thiabendazole at a rate of 25, 50, and 100 g ai/100 kg seeds were used. Thiabendazole seed treatment was applied as standard seed coating at Syngenta headquarters in Switzerland.

Untreated seeds were used as the control in this study. Each treatment was replicated 10 times and placed in a randomized complete block design (RCB). The experiment was conducted twice.

Soil samples were collected from CAP, Haymana, Ankara (Lat. 39°24´13´´Long. 32°37´14´´) and cysts were extracted by using the Fenwick-Can technique (Fenwick, 1940). Cysts were collected by hand-picking and were surface sterilised with 0.5% NaOCl for 10 min and rinsed several times in autoclaved distilled water. Autoclaved water was added to the cysts and stored at 4°C. Juveniles were obtained by placing the cysts on Baermann funnels at room temperature for 7 d to stimulate hatch. After seed germination, 400 second-stage juveniles (J2) of *H. filipjevi* in one ml of distilled water were inoculated into three, 2-cm deep holes made into the soil around the base of the plant. Plants were grown in a growth chamber at 25 ± 2°C with a 16-hr photoperiod of artificial light and with a relative humidity of 70% (± 5). Plants were top watered whenever needed and fertilised with Nitrophoska Solub/Hakaphos (N: P: K, 8:12:24) at a rate of 1 g/L of water after 28 d of plant growth. The plants were harvested 9 wk after nematode inoculation. Two weeks before harvest, irrigation was stopped to allow the soil to dry for better extraction of cysts. Cysts were extracted by the modified floation method (Coyne *et al.*, 2007). Roots were first washed very carefully with a stream of water to remove surrounding soil. The roots were then washed thoroughly with a strong stream of water to separate white females and mature cysts from the roots. These were collected in a small container. The soil from the tubes was placed in the bucket with water and stirred for 10 s and left for about 30 s to allow the heavy sand and soil debris to settle and then poured through sieves of 850-µm and 250-µm aperture for cyst collection. The process was repeated three times to ensure that all white females and brown cysts were extracted. Cysts were collected from the 250-µm sieve. The total number of cysts was counted under a binocular microscope and recorded. Plant height, root length, and root and shoot fresh weights (g) were collected. The number of cysts on each root system and the number of eggs per 10 randomly selected cysts from each replicate were counted. Data were analysed using Sigma plot 11.0. Statististical analysis was performed using one-way analysis of variance (one-way ANOVA). Different letters indicate significant variations ($P \leq 0.05$, n = 10) and a > b.

Table 1. Wheat genotypes used in this study and their genetic resistance to *Heterodera filipjevi*.

Wheat genotype	Accession Number	Genetic reaction[x] to CCN	Type[y]	Source[z]
F130L1.12/ATTILA	980872	MR	WW	MX-TCI
Katea	950590	MR	WW	BUL
Sonmez	950193	MR	WW	TK
Seri	951027	S	SW	MX
Bezostaya	950189	S	WW	RU
Gerek	950497	S	WW	TK

[x]MR- Moderately Resistant, S- Susceptible.
[y]WW- Winter Wheat, SW-Spring Wheat.
[z]TK-Turkey, RU-Russia, MX-Mexico, BUL-Bulgaria, and TCI-Turkey-CIMMYT–ICARDA; CIMMYT-International Bulgaria and Wheat Improvement Centre, ICARDA-International Centre for Agricultural Research in the Dry Areas.

RESULTS

Effect of Thiabendazole on Heterodera filipjevi *Infection*

The number of *H. filipjevi* cysts per root system was significantly reduced ($P \leq 0.05$) following seed treatment with thiabendazole on the moderately resistant (MR) genotypes when compared to the S genotypes (Fig. 1). Highest reduction in cyst number was recorded in highly susceptible cv. Bezostaya as compared to control. However, thiabendazole seed treatment did not show significant reduction in *H. filipjevi* cyst number on the S genotype, Gerek, but significantly reduced the cyst numbers per root system in the S genotype, Seri, as compared to untreated plants. Treating the MR genotype with thiabendazole had no significant effect on nematode infection in terms of cyst number per root system (Fig. 1). The average number of *H. filipjevi* cysts on the MR genotype F130L and Katea was slightly reduced at the thiabendazole concentration of 25 g ai/ 100 kg seed. However, a significant reduction in cyst number on the S genotypes was recorded at all concentrations whereas the highest cyst number reduction was detected at 50 g ai/100 kg seed in all treatments on both the S and MR genotypes when compared to other concentrations (Fig. 2).

Effect of Thiabendazole on Numbers of Eggs and Juveniles per Cyst

Thiabendazole seed treatment had no significant effects on total number of eggs and J2 per cyst in four

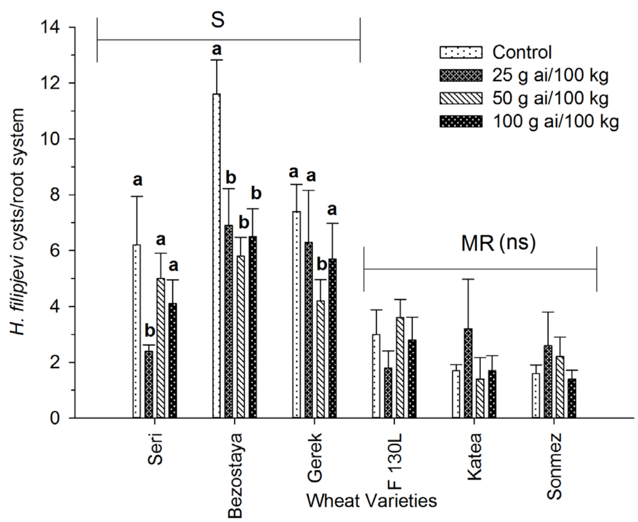

Fig. 1. Influence of thiabendazole on *Heterodera filipjevi* average cyst number on three moderately resistant (MR) and three susceptible (S) genotypes. Bars with different letters are significantly different based on Tukey's HSD test ($P \leq 0.05$; n = 10).

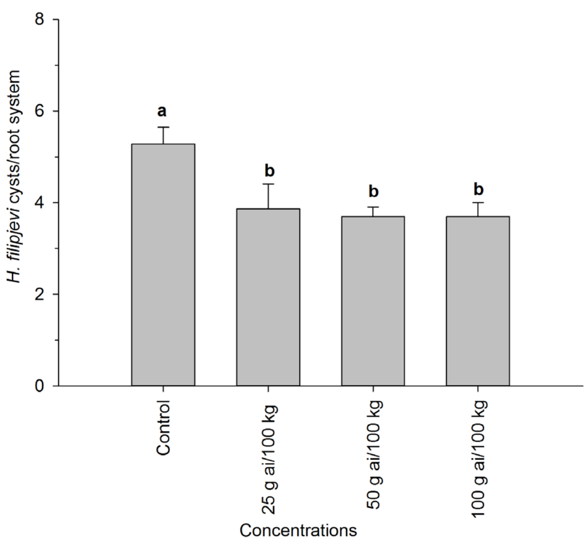

Fig. 2. Average number of *Heterodera filipjevi* cysts across wheat genotype as affected by thiabendazole concentration. Bars with different letters are significantly different based on Tukey's HSD test ($P \leq 0.05$; n = 10).

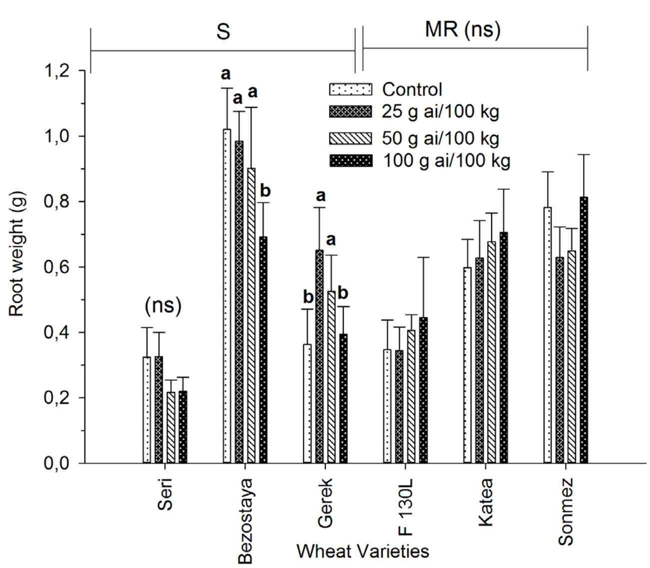

Fig. 3. Influence of thiabendazole on root weight (g) of three moderately resistance (MR) and three susceptible (S) genotypes as affected by fungicide concentration. Bars with different letters are significantly different based on Tukey's HSD test ($P \leq 0.05$; n =10).

of six wheat genotypes as compared to the control (data not presented). However, the total number of eggs and J2 per cyst was significantly reduced in the S genotype, Gerek, and the MR genotype, F 130L, with thiabendazole. Seed treatments with different rates of thiabendazole did not show a consistent effect on nematode reproduction in wheat genotypes as measured by total number of eggs and J2 per cyst.

Effect of Thiabendazole on Plant Growth Parameters

Plant height, shoot weight, and root length were not significantly increased by thiabendazole application. However, the S genotype, Gerek, exhibited a significant increase in root weight at concentrations of 25 g ai/100 kg seed (Fig. 3) while root weight was significantly reduced in Bezostaya at a concentration of 100 g ai/100 kg seed. In Seri, F 130L, Katea, and Sonmez, no root differences were detected.

DISCUSSION

These experiments demonstrated the activity of a thiabendazole seed coating on infection of the CCN on three S and three MR wheat genotypes. Treating the MR genotypes with thiabendazole had a negligible effect on nematode infection at the different concentrations. The MR genotypes used in this experiment are known to have partial resistance to *H. filipjevi* (Dababat, unpubl. data.). This study was the first report of fungicide thiabendazole seed coating interaction with *H. filipjevi*. Thiabendazole concentration at 50 g ai/100 kg seed caused the highest reduction in cyst number in all treatments on the S genotypes. Similar results were reported following seed treatment with the fungicides tebuconazole (Basta®) and epoxiconazole (Opal®) (Anonymous, 2012). In the present study, germination and plant growth were positively affected by thiabendazole seed treatment especially on the S genotypes. The results demonstrate thiabendazole protected the S genotypes by reducing J2 infection, and/or nematode development, leading to reduction in cyst number without causing any phytotoxicity on wheat seedlings at any stage. However, thiabendazole seed treatment did not show a consistent effect on eggs and juveniles per cyst, though a significant reduction in the number of egg and J2 per cyst in S genotype, Gerek, and the MR genotype, F130L, were recorded.

Neither shoot height, shoot weight, nor root length was significantly increased by seed treatment as compared to the control. However, a slight increase in shoot height and shoot weight was detected on thiabendazole-treated seed and MR genotype. Interestingly, the results from the present tests showed that the S genotype, Bezostaya, did not increase in plant height even though root length and root weight were highest among the genotypes infected with *H. filipjevi*. The results indicate that plant growth parameters were correlated with plant genotype rather than with the effects of seed treatments.

The results of these investigations demonstrated that wheat genotypes treated with thiabendazole can protect the plant during the seedling stages from nematode infection and thereby increase sustainable wheat productivity. This is important with locally adapted S varieties grown where CCN exists. Thiabendazole application could be used on these varieties until suitable CCN resistance is bred into the genotype. Fungicides with nematicidal or nematistatic activity could improve yield productivity as a holistic approach until a better genetic-based solution is available.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Food, Agriculture, and Livestock, Republic of Turkey, and the Transitional Zone Agriculture Research Institute-Eskisehir for the technical and logistical support and Syngenta, Switzerland, and the Deutsche Gesellschaftfür Internationale Zusammenarbeit (GIZ) and Advisory Service on Agriculture Research on Development (BEAF) Germany for financial support of this study.

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Received: *31/XII/2012* *Accepted for publication:* *21/I/2014*

Recibido: *Aceptado para publicación:*