RESEARCH/INVESTIGACIÓN

INTERACTIONS BETWEEN *HETERODERA AVENAE* AND *FUSARIUM CULMORUM* ON YIELD COMPONENTS OF WHEAT, NEMATODE REPRODUCTION AND CROWN ROT SEVERITY

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ABSTRACT

Hassan, Gh. A., Kh. Al-Assas, and T. Abou Al-Fadil. 2012. Interactions between *Heterodera avenae* and *Fusarium culmorum* on yield components of wheat, nematode reproduction and crown rot severity. Nematropica 42:260-266.

The interactions between *Heterodera avenae* and *Fusarium culmorum* on growth and yield components of durum wheat cv. Sham 3, *H. avenae* reproduction and crown rot severity were studied in a plastic house experiment. Grain yield reduction caused by the treatment *H. avenae* alone and *F. culmorum* alone was 12.3 and 25.5% respectively. The simultaneous inoculation of *H. avenae* and *F. culmorum* resulted in 38.4% reduction, indicating an additive effect of yield losses due to the two pathogens. A synergistic interaction was found when the nematode was added one month before the fungus, where the grain yield reduction (43.8%) exceeded the sum of individual loss caused by the nematode and fungus alone. The interaction was antagonistic when the fungus was added one month before the nematode, which caused a reduction in grain yield (33.3%) less than the sum of individual loss caused by the nematode and fungus alone. The interaction of nematode and fungus resulted in decreasing final population densities for the nematode especially when the fungus was added one month before the nematode. The highest fungus infection severity (2.8) and disease index (91.7%) was observed when the nematode was added one month before the fungus.

Key words: Cereal cyst nematodes, root rot fungi, soil borne pathogens, wheat yield loss.

RESUMEN

Hassan, Gh. A., Kh. Al-Assas, and T. Abou Al-Fadil. 2012. Interacción entre *Heterodera avenae* y *Fusarium culmorum* sobre componentes de la producción del trigo, reproducción del nematodo y severidad de la podredumbre del cuello. Nematropica 42:260-266.

Se estudió el efecto de la interacción entre *Heterodera avenae* y *Fusarium culmorum* sobre el crecimiento y componentes de producción del trigo durum cv. Sham 3, la reproducción de *H. avenae* y la severidad de la podredumbre del cuello en un experimento llevado a cabo en un invernadero de plástico. La reducción de la producción de grano causada por los tratamientos *H. avenae* y *F. culmorum* aplicados individualmente fue 12.3 and 25.5% respectivamente. La inoculación simultánea de *H. avenae* y *F. culmorum* resultó en una reducción 38.4% indicando un efecto aditivo sobre las pérdidas de producción debido a los dos patógenos. Se encontró una interacción sinérgica cuando el nematodo se añadió un mes antes que el hongo y la reducción de la producción de grano fue 43.8%, lo cual excedía a la suma individual de las pérdidas causadas por cada uno de los patógenos. La interacción fue de antagonismo cuando el hongo se añadió un mes antes que el nematodo, lo que dio lugar a una reducción de la producción de grano del 33.3%, menor que la suma individual de las pérdidas causada por los patógenos individualmente. La interacción entre el nematodo y el hongo resultó en un descenso de las densidades de la población final del nematodo, especialmente cuando el hongo se añadió un mes antes que el nematodo. La mayor severidad de la infección causada por el hongo (2.8) y el índice de enfermedad (91.7%) se observó cuando el nematodo se añadió un mes antes que el hongo.

Palabras clave: Nematodo formador de quistes de los cereales. Hongos de podredumbre de las raíces, patógenos del suelo, pérdidas de producción de trigo.

INTRODUCTION

Soil borne pathogens, including dryland cereal root rots and cereal nematodes, are a major constraint to cereal production worldwide, particularly where cereals dominate rotations, and sub-optimal growing conditions and or cultural practices are common (Nicol *et al*., 2008). Yield loss caused by these soil borne pathogens has been reviewed and documented in many regions of the world including Europe, America and in particular the more marginal cereal production areas of West Asia, North Africa, Australia and Canada with losses reported between 3-50% (Nicol *et al*., 2008; Tanha Maafi *et al*., 2009). In the case of soil borne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soil borne pathogens has been demonstrated in many crops throughout the world (Back *et al*., 2002). Plant-parasitic nematodes interact with fungi in a variety of ways to cause plant disease complexes. Even some nonplant-parasitic nematodes are able to carry fungal spores internally which not only increases spore mobility but also protects them from fungicides. Plant-parasitic nematodes wound plants in the process of penetration and feeding, opening infection courts for fungal pathogens. Other nematodes modify plant tissue to favor a fungus and thus increase the growth and reproduction of the fungus. Quantitative and qualitative changes in root exudates, which are induced by certain nematodes, stimulate the germination, growth and reproduction of fungal propagules in the rhizosphere. These exudates may also indirectly inhibit components of the rhizosphere microflora which are antagonistic to some plant pathogens. Furthermore, a number of studies have reported breakdown of host resistance during concomitant infections (Bergeson, 1972; Barker and McGawley, 1998; Karlsson, 2006; Back *et al*., 2002). Depending on the species of nematode and fungus, concomitant infections may stimulate or inhibit nematode reproduction (Jorgenson, 1970; Bergeson, 1972; Cook, 1975; Nordmeyer and Sikora, 1983; Sharma and Nene, 1989; Barker and McGawley, 1998; Back *et al*., 2002). Interaction between nematodes and fungi can be synergistic, where association between nematode and pathogen results in plant damage exceeding the sum of individual damage by pest and pathogen. Conversely, where an association between nematode and pathogen results in plant damage less than that expected from the sum of each organism, the interaction may be described as antagonistic. Neutral or additive interaction occurs when the plant damage is similar to the sum of individual damage by pest and pathogen (Barker and McGawley, 1998; Back *et al*., 2002; Karlsson, 2006). Only limited examples of interaction between cyst nematodes and root rot fungi have been documented (Barker and McGawley, 1998). Species of *Rhizoctonia* and *Fusarium* have been reported to have interactions with *Heterodera* (Jorgenson, 1970; Meagher *et al*., 1978).

The cereal cyst nematode, (CCN), *Heterodera avenae* Woll., is distributed worldwide and has been reported as the most damaging nematode on wheat, especially in semi-arid regions, where it can increase drought stress (Rivoal and Cook, 1993; Nicol, 2002). More recently, *H. avenae* has also been reported on wheat and barley in North East Syria, Al-Hassakeh governorate, where soil population densities in some fields can be as high as 85 eggs and second-stage juveniles/g soil, and where yield losses were estimated, in a field infested with 40 eggs and juveniles/g soil, at 50 and 57% in durum and bread wheat, respectively (Hassan *et al*., 2010). Root and crown rot fungi of wheat are one of the most important diseases worldwide, and cause economic yield loss in many parts of the world where cereals are the predominant cropping system and the crop is grown under sub-optimal growing conditions (Tunali, 2008; El-Khalifeh *et al*., 2009; Nicol *et al*., 2010). It is common for a complex of fungi to include one or more species that cause crown rot (*Fusarium* spp.) and common root rot [*Bipolaris sorokiniana* (syns. *Helminthosporium sativum*)] (Nicol *et al*., 2008; Tunali, 2008; Tanha *et al*., 2009). In Syria, severe root rot occurs on wheat in many regions of North East Syria, Al-Hassakeh governorate, where *H. sativum* and *F. culmorum* are the most common root rot fungi (El-Naeb, 2002; El-Khalifeh, 2006; Yousef *et al*., 2006). Yield losses caused by root rot on wheat ranged between 4-33% under Syrian field conditions (El-Khalifeh, 2006), and between 46-79% with susceptible cultivars under plastic house conditions (El-Naeb, 2003).

Since both root rot fungi and cereal cyst nematodes are found together especially under cereal monoculturing (Meagher *et al*, 1978; Van Leur *et al*., 1997; Scholz, 2001), an interaction that increases root damage is possible, and a synergistic interaction might cause a higher yield loss than when the fungus or nematode are found individually. Such information was lacking for *H. avenae* and the root rot fungus *F. culmorum* on wheat. Therefore, the objective of this work was to study the interaction between *H. avenae* and *F. culmorum*, and their effects on yield components of wheat, nematode reproduction and crown rot severity under plastic house conditions.

MATERIALS AND METHODS

Nematode inoculum: A composite soil sample was collected from a field in North East Syria, Al-Hassakeh governorate, naturally infested with *H. avenae*, at the beginning of the growing season 2009/2010. Soil was taken to a depth of 20-30 cm from at least ten points, thoroughly mixed, clods crushed, and air dried. Cysts of *H. avenae* were extracted from 200 g sub-samples with a Fenwick can (Southey, 1986) and separated from soil debris by hand picking. The cysts were then incubated at 10°C in tap water and freshly hatched second-stage juveniles were transferred weekly to fresh water and kept at 4°C until needed. The *H. avenae* inoculum was applied as a suspension containing 10 second-stage juveniles/g soil.

Fungal inoculum: The *F. culmorum* isolate was initially isolated from diseased wheat sub-crown internodes collected from fields in North East Syria, Al-Hassakeh governorate (Al-Masri, pers. Communication). The isolate was cultured on half-strength potato dextrose agar (PDA), containing 100 ppm Streptomycin and 100 ppm Ampicillin, and incubated for 14 days at room temperature and ambient light. The inoculum was obtained by floating the surface of Petri dishes containing sporulating colonies with distilled water and carefully scraping the surface of the plates. The conidial suspension was filtered through two layers of cheesecloth and the density of spores per ml adjusted to obtain 2500 spores/g soil.

Experimental design: The study consisted of six treatments with four replicates. The treatments included: Control (no nematode or fungus), *H. avenae* alone, *F. culmorum* alone, nematode and fungus were added simultaneously (N + F), the nematode was added one month before the fungus (N + F-1 month) and the fungus was added one month before the nematode (F + N-1 month). These treatments were arranged in a randomized complete block design. In December, each pot was filled with 2 kg sterilized soil-sand (1:1), nematode and fungal inoculum was uniformly spread on the soil surface and covered by 1 cm of soil, then five seeds of the cv. Sham 3 of durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.] were placed on the soil surface and an additional 5 cm of soil was added. For the treatments with a month delayed application of nematode or fungus, the inoculum was injected around the plants to a 5 cm soil depth. The pots were placed in the plastic house and kept at 10 ± 2°C for seven weeks, followed by ten weeks at 18 ± 2°C and finally at 24 ± 2°C for six weeks until maturity (23 weeks after sowing), the plants were watered as needed.

Yield, pest and pathogen data: At harvest (23 May 2010), the average plant height, number of tillers per plant, and number of spikes per plant were recorded. All plants of a pot were then harvested to determine grain yield (kg/ha), straw yield (kg/ha) and weight of 1000 kernels (g). Grain yield and weight of 1000 kernels were compared to the control treatment.

To assess the average final population density of *H. avenae* in each treatment, the cysts were extracted from four sub-samples of 200 g soil of each pot with a Fenwick can, then crushed in a tissue grinder in 50 ml of tap water. Averages of eggs and second-stage juveniles per treatment were calculated after microscopic examination of three 1-ml aliquots per sample.

For infection severity caused by *F. culmorum*, the discoloration of subcrown internodes (SCI) of each plant was evaluated based on a 0 to 3 scale (0 = SCI healthy, 1 = 1–25% of SCI brown, 2 = 26–50% of SCI brown and 3 = more than 50% of SCI brown). The average SCI per treatment was calculated (Ledingham *et al*., 1973), and the average disease index (DI %) per treatment was calculated using the equation (Tinline *et al*., 1975):

$$\text{DI \%} = \frac{\Sigma(\text{infection severity} \times \text{number of plant at this severity})}{(\text{Number of total plant} \times 3)}$$

All data were subjected to analysis of variance and means compared with the protected Fisher LSD at the 5% level using GenStat Twelfth Edition.

RESULTS

Yield components: A significant reduction (*P < 0.001*) in number of tillers/plant compared to the control was determined for all treatments except the treatment *H. avenae* alone. The highest reduction was recorded in the treatment *F. culmorum* alone (2.2 tillers/plant), which was not significantly different compared to treatments N + F and F + N-1 month (Table 1). All treatments reduced significantly (*P < 0.001*) the number of spikes/plant and plant height compared to the control. The lowest number of spikes/plant (1.2 spikes/plant) was observed when both nematode and fungus were added simultaneously (N + F) and was significantly different compared with remaining treatments except the treatments *F. culmorum* alone and F + N-1 month (Table 1). The same treatment (N + F), caused the greatest reduction of the plant height (38.4 cm) and was significantly different than all other treatments (Table 1). A significant reduction (*P < 0.001*) in grain yield and weight of 1000 kernels was found for all treatments compared to the control. The greatest reduction was found when the nematode was added one month before the fungus (N + F-1 month), with 44 and 35% reductions in grain yield and weight of 1000 kernels, respectively. These reductions exceeded the sum of individual loss caused by nematode alone and fungus alone and differed significantly compared to all treatments except the treatment N + F. In the treatment N + F, the reductions in grain yield and weight of 1000 kernels were 38 and 31%, respectively, and equated approximately to the additive damage by the nematode and fungus. When the fungus was added one month prior to the nematode (F + N-1 month), the reduction in grain yield and weight of 1000 kernels were 33 and 18%, respectively, which was less than the sum of individual losses caused by the pest and pathogen alone (Table 1). The treatment F + N-1 month caused a significant reduction (*P < 0.001*) in straw yield compared to all treatments except the treatments *F. culmorum* alone and N + F. Furthermore, the reduction in straw yield in the treatments *H. avenae* alone and N + F-1 month was not significantly different compared to the control (Table 1).

Pathogen data: The greatest increase in *H. avenae* population density was in the treatment *H. avenae* alone. The treatments N + F, N + F-1 month and F + N-1 month reduced significantly ($P < 0.001$) the final population density of *H. avenae* compared to the treatment *H. avenae* alone, and the greatest reduction was found when the fungus was added one month before the nematode (F + N-1 month) (Table 1).

All treatments, except treatment *H. avenae* alone, caused a discoloration of subcrown internodes (SCI) of wheat plants, and corresponded to the higher disease index recorded for the same treatments (Table 1). The highest infection severity (2.8) and disease index (91.7%) occurred when the nematode was added one month prior to the fungus (N + F-1 month), and were significantly higher than all other the treatments except the treatment N + F, where no significant difference was detected. However, there were not significant differences between the treatments *F. culmorum* alone and F + N-1 month for the infection severity and consequently for the disease index (Table 1).

DISCUSSION

Our results give additional information on the effects of individual or combined inoculations of two important soil borne pathogens on yield components of durum wheat (cv. Sham 3) and the effect of both pathogens on each other. The reduction of most yield components in the treatments *H. avenae* alone and *F. culmorum* alone compared to the control indicates that both pathogens have potential to decrease growth and yield of wheat, as has been reported previously (Namouchi-Kachouri *et al*., 2009; Hassan *et al*., 2010; Erginbas *et al*., 2010; Nicol *et al*., 2010). Yield losses may be increased by fungal infection after or at the same time as nematode penetration (Abdel-Momen and Starr, 1998; Back *et al*., 2002; Daami-Remadi *et al*., 2009). This effect has been demonstrated specifically for interaction between *Heterodera* spp. and *Fusarium* spp. or other soil borne fungi (Adeniji *et al*., 1975; Meagher *et al*., 1978; Barker and McGawley, 1998). Our study showed that a synergistic interaction was caused by early penetration of the roots by *H. avenae*, followed one month later by an inoculation of *F. culmorum* in the treatment N + F-1 month, and resulted in the highest reduction in grain yield and weight of 1000 kernels. The second-stage juveniles of *H. avena*e root penetration might have predisposed the roots to attack by *F. culmorum*. In addition, the presence of a synergistic interaction was reflected in the highest infection severity and disease index of plants in the same treatment. Similarly, Scholz (2001) reported that the synergistic interaction between *H. latipons* and *Bipolaris sorokiniana* caused increasing grain yield reduction of barley when *H. latipons* was added nine weeks before *B. sorokiniana*. Nordmeyer and Sikora (1983) demonstrated that the synergistic interaction between *H. davert*i and *F. avenaceum* and *F. oxysporum* in *Trifolium subterraneum* was found when the fungus was added prior to the nematode. Endoparasitic nematodes, like *Heterodera* spp., have been frequently reported to form synergistic interactions with soil borne fungi (Sugawara *et al.*, 1997; Karlsson, 2006). Our results also indicated that the interaction between *H. avenae* and *F. culmorum* can be additive. The simultaneous attack of the roots by both pathogens in the treatment N + F resulted in reduction in grain yield and weight of 1000 kernels which was equal to the sum of individual loss caused by the nematode and fungus alone. The biotic stress of the plants of simultaneous infection corresponded to the second highest infection severity of (SCI) and disease index in the treatment N + F. Similarly, Meagher *et al*. (1978) demonstrated

Table 1. Effects of treatments of *H. avenae* and *F. culmorum* on growth and yield components of durum wheat cv. Sham 3, final nematode density and fungal infection severity.[y]

Treatment	Number of tillers/plant	Number of spikes/plant	Plant height (cm)	Grain yield		Weight of 1000 kernels		Straw yield (kg/ha)	Final nematode density[z]	Fungal infection severity (scale 0-3)	Disease index (%)
				(kg/ha)	% reduction	(g)	% reduction				
Control	4.3 d	4 d	56.7 e	2581 e		32 d		3086 c	0 a	0 a	0 a
H. avenae alone	3.7 cd	3.1 c	50.8 d	2263 d	12.3	28.7 c	10.3	2751 bc	45.9 e	0 a	0 a
F. culmorum alone	2.2 a	1.7 ab	45.2 c	1923 c	25.5	25.1 b	21.6	2290 a	0 a	2.1 bc	70 bc
N + F	2.8 ab	1.2 a	38.4 a	1590 ab	38.4	22.2 a	30.6	2447 ab	21.4 c	2.4 cd	78.3 cd
N + F-1 month	3.1 bc	2.2 b	41.3 b	1451 a	43.8	20.8 a	35	2809 c	36.3 d	2.8 d	91.7 d
F + N-1 month	2.6 ab	1.6 ab	47.5 c	1721 b	33.3	26.1 b	18.4	2094 a	7.6 b	1.9 b	61.7 b
$LSD_{0.05}$	0.8	0.6	2.8	140	-	2.009	-	358	1.9	0.43	14.4

[y]Values are means of four replicates. Means in each column followed by the same letter are not significantly different ($P < 0.001$) according to Fisher's protected LSD.

[z] Number of eggs and second-stage juveniles/g soil.

that the interaction between *H. avenae* and *Rhizoctonia solani* caused greater decline in grain yield of wheat in Australia when both nematode and fungus are present. Gröntoft and Jonasson (1991) reported an additive effect in summer rape due to simultaneous infestation of *Verticillium dahliae* and *H. schachtii*. The highest impact on grain yield and weight of 1000 kernels, caused by treatments N + F-1 month or N + F, shows that root injury by *H. avenae* at the beginning experiment exerted a major effect on the plant resistance to disease. However, several studies reported that the more negative effect on plant growth is due to a synergistic interaction or additive effect where the combined effect of the tested pathogens on plant growth was generally more than that caused by each alone (Alam *et al*., 1990; Khan and Hosseini-Najed, 1991; Daami-Remadi *et al*., 2009). The reduction in grain yield and weight of 1000 kernels in the treatment F + N-1 month can be qualified as an antagonistic interaction, where this reduction was less than the cumulative loss caused by individual organisms. This might be related to the differences in plant stage, when the nematode was added. This nematode only penetrates tissues immediately behind the root caps, older segments of roots are immune. In this treatment, the nematode inoculum was placed at the level of the seed; we expected some downward movement of a portion of the nematodes in the pot with watering. Siddiqui and Husain (1991) reported lower yield impact of the disease complex *M. incognita/F. solani* on papaya, when applied later in the plant development. It again shows the major importance of the nematode in development of the complex interaction and the subordinate role of the fungus. However, an antagonistic interaction on sugarbeets between *H. schachtii* and *F. oxysporum* was found by Jorgenson (1970) in a field experiment, when both pathogens were present.

The reduced final density of *H. avenae* in the treatment F + N-1 month compared to all treatments indicates relatively poor development conditions for the nematode, where fungal infection disrupts nematode feeding sites (Back *et al.*, 2002). Plants affected by disease complexes may be more prone to early senescence and death which, in turn, may prevent the nematode from completing their life cycles (Griffin et al., 1993; Walker *et al.*, 1998). Competition of nutrients or root space may be responsible for decline of nematode populations (Ketudat, 1969; Jorgenson, 1970). Ibrahim *et al*. (1997) found that infection of wheat by *Helminthosporium sativum* or *Trichoderma harzianum* reduced the number of *H. avenae* cysts on roots. Similarly, Cook (1975) showed that the final population density of *H. avenae* was lower on roots of barley plants infested by the take-all fungus *Gaeumannomyces graminis*. Furthermore, many previous studies reported decreasing cyst nematode populations in disease complexes (Meagher *et al*, 1978; Sharma and Nene, 1989; Gröntoft and Jonasson, 1991; Scholz, 2001). However, Nordmeyer and Sikora (1983) observed a population increase of the cyst nematode *H. daverti* on *F. avenaceum* and *F. oxysporum* infested roots of *T. subterraneu*m, when the fungus was added before the nematode. Generally earlier symptom appearance and increased disease severity in combined interactions with fungal pathogens and nematodes occurs in synergistic interactions (Daami-Remadi *et al*., 2009). In our study, the treatment N + F-1 month showed highest discoloration of the (SCI) and disease index. Due to nematode activity at the beginning of the treatment, the number of spores in contact with the root may increase which resulted in increasing infection severity caused by the fungus (Scholz, 2001). Scholz (2001) found that infected barley roots by *H. latipons* nine weeks prior to *B. sorokiniana* had the highest discoloration of SCI. While Nordmeyer and Sikora (1983) observed that the disease indices of *T. subterraneum* decreased when *H. daverti* was added one or two weeks prior to addition of *F. avenaceum* or *F. oxysporum*. However, the antagonistic interaction in the treatment F + N-1 month resulted in the least infection severity and disease index.

In conclusion, our study shows that an interaction between *H. avenae* and *F. culmorum* exists under unrestricted water supply. These pathogens are a real threat to wheat production in Syria, and their impact on growth and yield components of durum wheat (cv. Sham 3) depended on the inoculation time of the nematode or fungus. Future pot experiments using cysts and fungal chlamydospores, which are similar to life stages occurring in the field, should be explored. The investigation of the interaction between pest and pathogen under field conditions is also recommended. The interaction of these pathogens on other host and non-host wheat varieties at variable initial densities as well as under drought remains to be investigated.

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