

Studies on the interaction between *Heterodera daveri*, *Fusarium avenaceum* and *F. oxysporum* on *Trifolium subterraneum*

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SUMMARY

The interaction between *Heterodera daveri*, *Fusarium avenaceum* and *F. oxysporum* on two clones of subterranean clover was studied. Simultaneous inoculation of *H. daveri* with *F. avenaceum* or *F. oxysporum* did not reduce the yield of the two clones of subterranean clover more than the additive losses caused by inoculation of each pathogen separately. When the nematode was inoculated one or two weeks after either fungus, plant dry weight was reduced synergistically. Nematode penetration and development was generally increased when the nematode was inoculated after the fungus. Increasing the interval between *F. avenaceum* and *H. daveri* inoculations decreased the number of cysts produced. Leaf reddening, a symptom often observed under field conditions, was developed only on plants inoculated with both *H. daveri* and *F. avenaceum*. The root-rot-tolerant ecotype 48G formed lesions after penetration of *H. daveri*.

RÉSUMÉ

*Études sur l'interaction entre Heterodera daveri,
Fusarium avenaceum et F. oxysporum sur Trifolium subterraneum*

L'interaction entre *Heterodera daveri*, *Fusarium avenaceum* et *F. oxysporum* sur deux clones de trèfle souterrain a été étudiée. L'inoculation simultanée n'a pas réduit le rendement des deux clones de trèfle souterrain plus que les pertes additives causées par des inoculations de chaque pathogène faites séparément. Quand les nématodes ont été inoculés une ou deux semaines après l'un ou l'autre champignon, le poids sec de la plante a été réduit synergiquement. La pénétration et le développement du nématode ont été généralement élevés quand le nématode a été inoculé après le champignon. L'augmentation de l'intervalle entre les inoculations de *F. avenaceum* et *H. daveri* a diminué le nombre de kystes formés. Le rougissement de la feuille, un symptôme fréquent dans les pâturages n'a été observé que sur les plantes inoculées avec *H. daveri* and *F. avenaceum*. L'écotype "48G" tolérant à la pourriture des racines a formé des lésions après la pénétration de *H. daveri*.

Australian clones of subterranean clover (*Trifolium subterraneum* L.) were introduced into Northwest Tunisia in 1967-69 as an alternative fodder legume; large field trials were started by Jaritz (1972). Two years later the first signs of clover decline were observed. The symptoms included root-rot, which often occurred in connection with an abnormal alteration of the root as well as stunting and discolor-

ceum (Corda ex Fr.) Sacc. from diseased plants. Nordmeyer, Sikora and Jaritz (1978) found that *Pythium irregulare* Buisson was also associated with the root-rot complex. The symptoms observed in the field could not be reproduced by inoculations of the individual pathogens under controlled conditions (Nordmeyer, 1979). These results indicated that combinations of the organisms might be the cause of

Materials and methods

H. daverli isolated from *T. subterraneum* from Northwest Tunisia was maintained in the greenhouse on *T. subterraneum* cv. Clare. *F. avenaceum* and *F. oxysporum* were isolated from diseased plants in Tunisia and kept as permanent cultures in soil tubes (Miller, 1945).

Seeds of *T. subterraneum* were submerged in 95 % alcohol for 10 seconds, rinsed in water, immersed in 0.5 % NaOCl for 15 minutes and rinsed in sterile water. The seeds were germinated on moist filter paper in Petri dishes. The seedlings were planted in 250 cm³ plastic pots, ten per pot, containing steam pasteurized sand, which was screened to a particle size of 0.5 mm and smaller. The pots were fertilized weekly with a 0.5 N Hoagland's solution. Each experiment was run for four weeks at 20° and 80 %

fungus (10⁶ macroconidia/25 cm³ sand) were inoculated alone and in the following combinations : nematodes and fungus inoculated simultaneously (NFs) ; fungus inoculated one or two weeks before nematodes (FN1, FN2) ; nematodes inoculated one or two weeks before fungus (NF1, NF2). The number of cysts, plant dry weight and disease index were recorded. Treatments were replicated five times.

PREDISPOSITION OF *T. subterraneum* TO NEMATODE PENETRATION AND DEVELOPMENT AFTER *F. avenaceum* INFECTION

Seedlings (cv. Clare) were inoculated in a seed tray with *F. avenaceum* (10⁶ macroconidia/25 cm³ sand). Five days later the seedlings were removed from the sand, the roots washed and ten plants were trans-

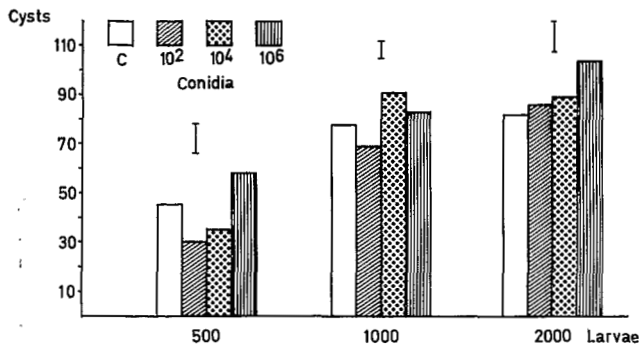
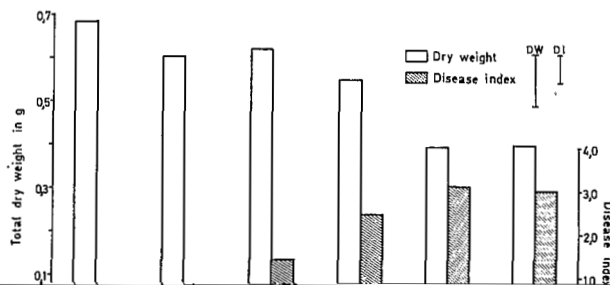


Fig. 1. Influence of different inoculum densities of *F. oxysporum* on the cyst production of *H. daverti* on *T. subterraneum* cv. Clare. The bars represent the LSD_{0.05}.

niles were combined with 10² and 10⁴ macroconidia. No effects were detected in the other nematode-fungal combinations.

INFLUENCE OF DIFFERENT INOCULATION SEQUENCES OF *H. daverti* AND *F. oxysporum* ON CULTIVAR CLARE

Separate inoculations of the fungus and the nematode reduced yields (dry weight) by 15 % and 17 %, respectively (Fig. 2). No reduction in yield over the additive effect of the individual pathogens occurred when the nematode was inoculated one or two weeks prior to the fungus. The respective disease indices were about the same as for the nematode alone, but less than with the fungus alone (Fig. 2).



A 40 % reduction in yield was obtained when both organisms were inoculated simultaneously, but this was not significantly different from the 32 % cumulative loss caused by the individual organisms. Even though the disease index of the simultaneous inoculation was significantly greater than the one of the fungus alone, it did not reach the cumulative value of the individual inoculations.

Inoculations of the nematode one and two weeks after the fungus resulted in a 50 % yield loss, which was greater than the additive losses from treatments of the nematode and fungus alone. The respective disease indices were the same as for the fungus alone.

There were significant increases in cyst number when the nematode and the fungus were inoculated simultaneously (NFs) and when the nematode was inoculated one and two weeks after the fungus, FN1 and FN2 (Tab. 1). When the nematode was inoculated one week after the fungus (FN1) the number of cysts was significantly greater than in any of the other treatments.

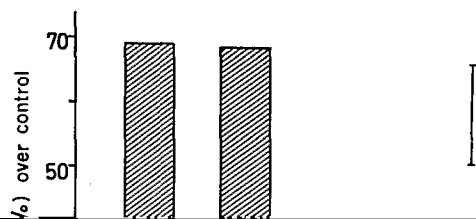
Table 1

Influence of different inoculation sequences of *F. oxysporum* on the cyst production of *H. daverti* on *T. subterraneum* cv. Clare (Abbrev. see text. LSD_{0.05} = 21.5.

Treatment	Cysts/pot
N	108
NF1	102
NF2	110
NFs	130
FN1	200
FN2	130

PREDISPOSITION OF *T. subterraneum* TO NEMATODE PENETRATION AND DEVELOPMENT AFTER *F. avenaceum* INFECTION

Plants inoculated with *H. daverti* five and seven



fungus reduced the yield significantly when compared with the control. Inoculations of nematodes one and two weeks after the fungus resulted in a 40 % yield reduction. The disease indices of these combined inoculations (FN1 and FN2) were significantly greater than the added indices of the individual pathogens.

The number of cysts per gram of root was greatest

Discussion

Yield losses may be increased by fungal infection after or at the same time as nematode penetration (Dittmann, 1963 ; Powell, 1963). This effect has been demonstrated specifically for interactions between *Heterodera* spp. and *Fusarium* spp. by Capus (1917) and Powell (1971), as well as for *Heterodera* spp. and other soil-borne fungi (Adeniji *et al.*, 1975 ; Corbett & Hide, 1971 ; Harrison, 1971 ; Meagher & Chambers,

increased damage due to the fungus. The yield reductions might be related to increased penetration and development of the nematode in the presence of the fungus. This is shown specifically in the third experiment where plants inoculated with *F. avenaceum* allowed more nematode penetration and development than the respective control plants. This effect has also been demonstrated when plants were pretreated with *F. avenaceum* culture filtrates that increased the rate of nematode penetration (Nordmeyer & Sikora, 1982). Inoculating susceptible seed-

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