Mycorrhiza (1996) 6:253–257

ORIGINAL PAPER

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The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum* Berk.) under field conditions

Accepted: 8 January 1996

Abstract A field experiment was carried out to determine the effects of the inoculation of onion (*Allium cepa* L.) with *Glomus* sp. Zac-19 on the development of onion white rot (*Sclerotium cepivorum* Berk.) and on onion production. Mycorrhization delayed onion white rot epidemics by 2 weeks and provided a significant protection against the disease for 11 weeks after onion transplanting, as compared with nonmycorrhizal controls. Mycorrhizal plants showed an increase of 22% in yield, regardless of the presence of the white rot pathogen.

Key words *Allium cepa* · Biological control · *Glomus* sp. · Arbuscular mycorrhizae · *Sclerotium cepivorum* · White rot

Introduction

Onion (*Allium cepa* L.) and garlic (*A. sativum* L.) are among the four most important vegetables in Mexico. Onion is grown all across the country (Zavaleta-Mejía 1990): in 1990 45 499 ha were cropped with onion and the total annual production was 770 643 ton (SARH 1990). In recent years, white rot caused by the fungus *Sclerotium cepivorum* Berk. has become very important in the main production areas of garlic and onion in Mexico (Zavaleta-Mejía 1990); the disease has been detected in the states of Guanajuato, Puebla, Tlaxcala and Morelos. In Guanajuato state, losses of up to 100% have been reported (Pérez et al. 1993). To date, attempts to control the fungus (e.g. fungicides, crop rotation and solarization have not been effective (Zavaleta-Mejía et al. 1991). The propagules of *S. cepivorum* have the capacity to survive in soil in the absence of *Allium* for more than 10 years in the form of dormant sclerotia (Coley-Smith 1959).

Arbuscular mycorrhizae (AM) increase nutrient uptake by roots and thus the growth of many plants (Harley and Smith 1983; Stribley 1990), and mycorrhization has been proposed as an alternative for the management of soil-borne pathogens (Schenck 1981; Dehne 1982, 1987; Barea et al. 1984; Campbell 1987; Hornby 1990; Perrin 1990; Reid 1990). Mycorrhizal plants are often less colonized by pathogens and show reduced disease incidence. The present work was carried out to study the effect of inoculating onion with *Glomus* sp. Zac-19 on the incidence of onion white rot and on the production of onion bulbs.

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Materials and methods

A field experiment was established in the Montecillo Field Station in the state of México. The soil in the experimental plot was a sandy-clay-loam, pH 8.1, 0.19% N, 26 ppm P, 3.5 mEq/100 g K, 3.83% organic matter and 0.38 mho/cm cation exchange.

The six treatments tested (Table 1) were replicated six times in a randomized complete block design. The soil was fumigated with methyl bromide with an excess of 40.31 g m^{-2}. One soil sample was taken from each of the nonfumigated plots in order to know the initial inoculum level of viable sclerotia of *S. cepivorum* and to inoculate to the same level in the fumigated plots (0.15 viable sclerotia per g soil). Sclerotia for inoculation of the fumigated plots were obtained in the laboratory from onion bulbs inoculated 45 days previously with mycelia of *S. cepivorum* and incubated in a moist chamber.

Table 1 Treatments tested to study interactions between the arbuscular mycorrhizal (AM) fungus *Glomus* sp. Zac-19 and *Sclerotium cepivorum* (*Nat* natural, *Artif* artificial, *FS* fumigated soil, *NFS* nonfumigated soil, *MR* mycorrhiza inoculated in the onion germinating trays, *MS* mycorrhiza inoculated in the field around the roots at onion transplanting, *SP* artificial soil infestation with the pathogen, *NP* natural infestation of soil by the pathogen)

Treatment	Soil fumigation	Inoculation			
		Glomus sp. Zac-19		*Sclerotium cepivorum*	
		Germinating trays	Field	Nat	Artif
1. FS	+	−	−	−	−
2. FS + MR	+	+	−	−	−
3. NFS (NP)	−	−	−	+	−
4. NFS (NP) + MR	−	+	−	+	−
5. FS + SP + MS	+	−	+	−	+
6. FS + SP + MR	+	+	−	−	+

Seeds of onion (cv. Hybrid Early Supreme) were grown in germinating trays. The mycorrhizal inoculum of Glomus sp. Zac-19, which was originally isolated from bean plants in the state of Zacatecas and since cultured in lucerne plants, consisted of 12 g soil, containing 1 spore per g soil and 0.05 g lucerne roots with 90.6% colonization at the moment of inoculation. For the treatments in which onion plants were colonized by *Glomus* prior to transplanting, the mycorrhizal inoculum was applied in the germinating trays; for inoculation at planting, the inoculum was applied around the roots of the onion seedlings. Seedlings previously inoculated with *Glomus* [treatments FS + MR; FS + SP + MR and NFS(NP) + MR], had an average root colonization of 76.4% when they were transplanted in the field.

The soil was fertilized with urea and superphosphate to a level of 80–40–00 (N-P-K) 15 days after transplanting. Disease incidence (% plants killed by white rot) was monitored each week during the whole season once the first dead plants were observed. Other variables considered were percent colonization of onion roots by mycorrhizal fungi and the initial and final numbers of sclerotia and mycorrhizal spores per g soil. Sclerotia were removed from soil by wet sieving (Crowe et al. 1980) and mycorrhizal spores by a centrifugal-flotation technique (Jenkins 1964). Data were subjected to analysis of variance and, when this indicated significant differences between treatments, Tukey's test was used to compare treatment means.

The onion bulbs were harvested and weighed and a comparison between treatments was carried out by orthogonal contrasts (Lee 1975). Cumulative disease progress curves (one for each treatment) were obtained from the cumulative proportion of dead plants over all dates of assessment. The rate of apparent infection was estimated through transformation of values according to the monomolecular, Gompertz and logistic models and subsequent linear regression analysis (Madden and Campbell 1990). None of these models gave an appropriate description of the epidemics, so for comparative purposes the area under disease progress curve (AUDPC) was used as a descriptor of the epidemics (Shaner and Finney 1977). AUDPC is calculated from the values of the disease intensity integrated between two times using the midpoint rule (trapezoidal integration method). The AUDPC has the units percent-day or proportion days (Campbell and Madden 1990).

Results

The effects of *Glomus* sp. Zac-19 on the incidence of onion white rot are shown in Table 2 and Fig. 1. The first dead plants appeared 7 weeks after transplanting in the treatment without soil fumigation and with the natural inoculum [NFS(NP)]. One week later, dead plants were found in treatments with artificial infestation (FS + SP + MS and FS + SP + MR) and by week 10 dead onion plants appeared in the nonfumigated plus mycorrhiza treatment [NFS(NP) + MR]. As expected, no dead plants were observed throughout the crop sea-

Table 2 Effect of the AM fungus *Glomus* sp. Zac-19 on the percentage of plants killed by onion white rot (*S. cepivorum* Berk.). Abbreviations as in Table 1. Each value is the mean of six replicates. In each row, values followed by the same letter do not differ significantly (Tukey $\alpha = 0.05$)

Weeks after planting	Treatment					
	FS	FS + MR	NFS (NP)	NFS (NP) + MR	FS + SP + MS	FS + SP + MR
7	0.0 A	0.0 A	1.2 ± 2.1 A	0.0 ± 0.0 A	0.0 ± 0.0 A	0.0 ± 0.0 A
8	0.0 A	0.0 A	2.4 ± 3.1 A	0.0 ± 0.0 A	0.8 ± 1.9 A	0.4 ± 1.0 A
9	0.0 B	0.0 B	5.0 ± 3.2 A	0.4 ± 0.9 AB	4.5 ± 4.7 AB	1.2 ± 2.0 AB
10	0.0 C	0.0 C	6.6 ± 5.0 AB	0.8 ± 1.2 C	10.4 ± 4.3 A	4.9 ± 2.1 BC
11	0.0 C	0.0 C	13.0 ± 4.8 B	4.6 ± 1.0 C	31.1 ± 5.3 A	14.0 ± 3.5 B
12	0.0 D	0.0 D	16.5 ± 7.0 BC	7.5 ± 4.4 CD	40.7 ± 9.1 A	23.9 ± 5.9 B
13	0.0 D	0.0 D	19.9 ± 6.8 BC	13.0 ± 5.8 CD	48.0 ± 12.4 A	31.6 ± 9.1 B
14	0.0 D	0.0 D	23.0 ± 5.9 BC	15.6 ± 3.3 C	54.0 ± 15.9 A	36.4 ± 9.5 B
15	0.0 C	0.0 C	28.5 ± 7.9 B	20.3 ± 8.7 B	58.8 ± 16.3 A	47.0 ± 5.8 A
16	0.0 D	0.0 D	35.8 ± 11.5 BC	32.6 ± 15.8 C	63.1 ± 15.0 A	53.3 ± 6.7 AB
17	0.0 D	0.0 D	38.3 ± 13.4 BC	34.7 ± 17.0 C	64.4 ± 16.0 A	56.7 ± 6.6 AB
18	0.0 B	0.0 B	61.3 ± 14.3 A	58.0 ± 19.5 A	73.6 ± 12.3 A	69.3 ± 7.5 A

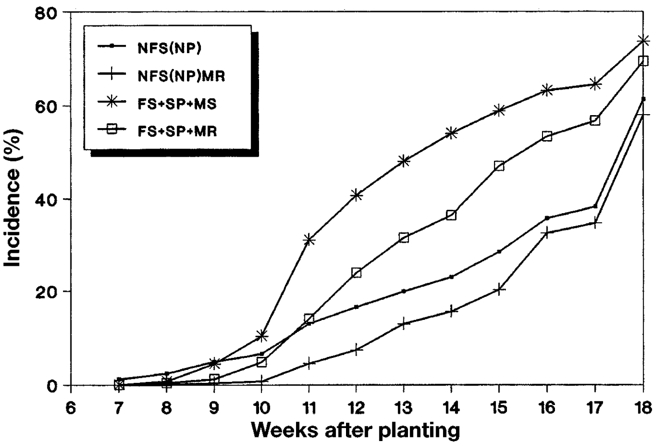

Fig. 1 Effect of mycorrhization with *Glomus* sp. Zac-19 on the incidence of onion white rot (*FS* fumigated soil, *NFS* nonfumigated soil, *MR* mycorrhiza inoculated in the onion germinating trays, *MS* mycorrhiza inoculated in the field around the roots at onion transplanting, *SP* artificial soil infestation with the pathogen, *NP* natural infestation of soil by the pathogen)

son in the fumigated (FS) and fumigated plus mycorrhiza (FS+MR) treatments.

In the treatment with natural infestation and mycorrhization of plants before transplanting [NFS(NP)+MR], there was a delay of 2 weeks in the first appearance of dead plants compared with the treatment without mycorrhization [NFS(NP)] (Fig. 1). Disease incidence during the following 6 weeks was significantly lower (Table 2 and Fig. 1) up to week 16, when both treatments showed equal levels of disease. The greatest incidence of disease was recorded in treatments with artificial infestation; however, it was clear that the incidence was reduced to the greatest extent in onion plants colonized by mycorrhizal fungi prior to transplanting (FS+SP+MR) (Fig. 1 and Table 2). When mycorrhizal inoculum was added to the field at the same time as *S. cepivorum* sclerotia, the effect was not as great.

An analysis of AUDPC (Campbell and Madden 1990) was performed to determine if there were significant differences among the treatments (Table 3). The AUDPC was significantly lower ($\alpha=0.05$) in those treatments where onion plants were already mycorrhizal at the time of planting, regardless of the soil infestation method. Production of onion bulbs in fumigated soil (FS) and fumigated soil plus mycorrhizal plants before transplanting (FS+MR) was significantly higher than in the presence of *S. cepivorum*. The orthogonal contrast analysis indicated significant differences ($\alpha=0.05$) in production between the FS and FS+MR treatments, suggesting that inoculation with *Glomus* caused a significant increase of 22.4% in onion weight (Table 4). It should be pointed out that no significant differences in onion production were detected between treatments with and without mycorrhization of plants before planting in natural infested soil [NFS(NP)+MR and NFS(NP)], but that the treatment NFS(NP)+MR showed an increase of 21.7% in onion weight when compared with the treatment without *Glomus*.

Plants inoculated with *Glomus* previous to planting in the field had 76.6% colonization. The highest percent root colonization (85.9%), 6 weeks after transplanting (Table 5), was found in treatment FS+MR. No significant differences were detected between the other treatments. The treatment without *Glomus* [NFS(NP)] showed the lowest percent mycorrhizal colonization, but indigenous mycorrhizal fungi were able to colonize the roots by 58.5%. Treatments with artificial pathogen inoculation (FS+SP+MS and FS+SP+MR) had similar percent colonization levels in the middle and at the end of the crop season; howev-

Table 3 Estimated area under disease progress curves (AUDPC) for each treatment. Values followed by the same letter are not significantly different (SNK $\alpha=0.05$). Abbreviations as in Table 1

Treatment	AUDPC (% days)
NFS (NP)	1621.4 CB
NFS (NP)+MR	1188.3 C
FS+SP+MS	2966.3 A
FS+SP+MR	2199.9 B

Table 4 Effect of mycorrhization with *Glomus* sp. Zac-19 on healthy bulb production of onion. Each value is the mean of six replicates. Pairs of treatments with the same letter by column are not significantly different (orthogonal contrasts $\alpha=0.05$). Abbreviations as in Table 1

Treatment	Healthy bulbs	
	Weight (kg)	Number
Pair 1		
FS	8.78±1.14 A	33.7±1.79 A
FS+MR	10.75±1.18 B	37.0±1.82 B
Pair 2		
NFS (NP)	3.08±1.27 A	13.0±6.21 A
NFS (NP)+MR	3.75±2.05 A	14.5±7.67 A
Pair 3		
FS+SP+MS	2.30±1.26 A	8.3±4.10 A
FS+SP+MR	2.66±1.28 A	9.0±9.16 A

Table 5 Mycorrhizal colonization. Each value is the mean of six replicates. Treatments with the same letter by column are not significantly different (Tukey $\alpha=0.05$). Abbreviations as in Table 1

Treatment	Colonization	
	2.5 Months after transplanting	Harvest
FS	0.00± 0.00 A	0.00± 0.00 A
FS+MR	85.87± 4.84 B	75.97±11.38 B
NFS (NP)	51.77± 9.73 C	58.55±10.66 B
NFS (NP)+MR	64.71±10.18 C	70.51± 7.78 B
FS+SP+MS	54.80± 2.76 C	54.46±19.93 B
FS+SP+MR	57.08±13.22 C	68.29±10.38 B

Table 6 Initial and final inocula of mycorrhizal spores and sclerotia. Each value is the mean of six replicates. Figures with the same letter in each column are not significantly different (Tukey $\alpha=0.05$). Abbreviations as in Table 1

Treatment	Inoculum (per g soil)			
	Initial		At harvest	
	Spores	Sclerotia	Spores	Sclerotia
FS	0.00	0.00[a]	2.07 ± 0.01 F	0.66 ± 0.01 E
FS + MR	—	0.00[a]	3.79 ± 0.01 D	0.74 ± 0.03 D
NFS (NP)	3.70 ± 0.03	0.15 ± 0.03	4.16 ± 0.01 B	1.20 ± 0.03 B
NFS (NP) + MR	—	0.15 ± 0.03	4.98 ± 0.02 A	1.14 ± 0.02 C
FS + SP + MS	—	0.15 ± 0.03[b]	2.75 ± 0.01 E	1.16 ± 0.03 C
FS + SP + MR	—	0.15 ± 0.03[b]	3.98 ± 0.02 C	1.26 ± 0.02 A

[a] Considered as zero since the sclerotia found were not viable
[b] Was inoculated with the same level of inoculum present in the nonfumigated plots

er, in the treatment in which mycorrhizal onion plants were transplanted (FS + SP + MR), disease appearance was delayed (Fig. 1). A significant positive correlation (86%) was found between onion yield and mycorrhizal colonization. Therefore the increases in onion yield may, in part, be explained by the colonization of onion roots by *Glomus*.

The initial and final numbers of mycorrhizal spores and sclerotia from all treatments are shown in Table 6. Both number of spores and sclerotia increased by the end of the experiment, and the highest spore numbers were found in treatments with nonfumigated soil. In treatments with fumigated soil, there were higher spore levels when onion plants were inoculated before planting in the field. The number of sclerotia also increased with time, and both artificial and natural infestation led to high sclerotia levels.

Discussion

The fact that the first dead plants were observed in the treatment with natural pathogen infestation with or without *Glomus* may indicate that the dormancy of sclerotia was broken more quickly in the natural than in the artificial infestation. Some authors (Coley-Smith 1959; Coley-Smith and Cooke 1971) report that it is necessary to bury the sclerotia of *S. cepivorum* in soil for periods of 4–8 weeks to break dormancy. This may explain why the first diseased plants in this study were detected 8 weeks after planting (1 week after diseased plants were present in the treatment with a natural inoculum). Disease incidence was significantly lower in treatments with *Glomus* during the first 11 weeks after planting. This practice, added to other control treatments such as application of fungicides, could help to delay the disease indexes. In the treatment in which *Glomus* and *S. cepivorum* were inoculated at the same time, mycorrhization had no effect and disease incidence was high, possibly as a result of low colonization by *Glomus* during the first weeks after planting. Under such conditions, the pathogen likely had free access to the root cells.

These results imply a limited protection of onion against *S. cepivorum* by *Glomus*; however, as also indicated by Perrin (1990), the relationship was beneficial for the crop only when the mycorrhizal fungi colonized the roots before the pathogen, since simultaneous infection by both microorganisms showed no effect. Furthermore, as reported also by Zengjia and Xiangdong (1991), inoculation with mycorrhizal fungi before planting conferred more complete protection against plant pathogens. Our results agree with those of Safir (1968), who found a positive effect of AM fungi colonization on the damage caused by *Pyrenochaeta terrestris* in onion. These present observations that mycorrhizal plants suffer less colonization by pathogens and that disease incidence is reduced with the addition of *Glomus* confirms what has been found and suggested by other researchers (Dehne 1982; Ilag et al. 1987). We observed a decrease in the incidence of *S. cepivorum* disease, from 92 to 28.7% (natural infestation) and from 52.8 to 11.9% (artificial infestation). Such differences can be economically meaningful in commercial plots of onion. Significant increases in onion production with AM-inoculated plants have been previously reported (Villalobos 1993; Jaen and Ferrera-Cerrato 1987).

In our experiment, the increase in production of onion bulbs was the same in the absence (22.4%) and in the presence of the pathogen (21.7%) in natural infestation when transplanted plants were previously mycorrhized. The delay in disease incidence is, therefore, likely to be more than just a direct effect of *Glomus* on the pathogen; physiological alterations in the onion plant may in turn affect the relationship between host and pathogen. The protection obtained with *Glomus* sp. Zac-19 in the onion variety used in this experiment could have practical application in the cultivation of baby onion ("cambray" onion), which is subjected to a similar cultivation system but is harvested 10 weeks after planting. Therefore, it may be possible to grow onions free of disease during the first 11 weeks after planting.

Benefits in onion production may be related to percent root colonization and spore levels in the soil, since the treatments with higher colonization and higher numbers of spores were those in which the incidence of the disease was lowest (at least during the first 11 weeks) and plant production was highest. Therefore, for the particular conditions in which this experiment was performed, *Glomus* sp. Zac-19 had a positive effect on plant development, which in turn negatively affected the development of onion white rot.

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