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Comparison of physical, chemical and biological methods of controlling garlic white rot

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Abstract

Treatment of garlic cloves with tebuconazole (at 1 ml of Folicur 25% l^{-1}) achieved a significant reduction in the rate of disease progress and the final incidence of plant death by *Sclerotium cepivorum*: garlic yields were improved. Although soil solarization provided the best control of garlic white rot, bringing soil populations of *S. cepivorum* to negligible levels, similar levels of disease control and garlic yields were achieved when tebuconazole was sprayed to stem bases of plants grown from cloves also treated with tebuconazole. This double treatment almost doubled the yield compared with untreated plants and significantly increased bulb quality under high disease pressure conditions. Soil solarization was also highly effective in a second consecutive crop of garlic, with significant improvements in yield and garlic quality. In contrast, lower levels of disease control were obtained when selected isolates of *Trichoderma harzianum* and *Bacillus subtilis* were applied to the soil and cloves respectively.

Introduction

White rot (WR), caused by *Sclerotium cepivorum* Berk., is one of the most important soilborne diseases of garlic (*Allium sativum* L.) in Spain, mainly in furrow-irrigated fields and in those with frequent monoculture of *Allium* crops or short rotations with other non-host crops. This results in drastic increases of pathogen populations, leading to high disease levels and, therefore, considerable yield losses.

Soil solarization has proved to be satisfactory, bringing soil populations of the pathogen to negligible levels in areas with the appropriate weather conditions (Coley-Smith, 1987; Satour et al., 1989; Basallote-Ureba and Melero-Vara, 1993), and has provided partial control in other areas (Porter and Merriman, 1985; Entwistle, 1990a; Pereira et al., 1996). Consequently, disease incidence was markedly lowered in solarized soil, and garlic and onion yields were improved (Porter and Merriman, 1985; Satour et al., 1989; Basallote-Ureba and Melero-Vara, 1993; Cunha et al., 1993; Basallote-Ureba et al., 1994). Soil fumigants give a good control of WR, but environmental and economic considerations greatly limit its use (Slawson et al., 1989; Entwistle, 1990a; Pérez-Moreno et al., 1996). Suicidal germination of sclerotia in the soil by the application of germination stimulants, such as diallyl disulphide (DADS), during crop-free periods proved efficient in reducing sclerotia of *S. cepivorum*, providing DADS concentrations and environmental conditions are adequate (Coley-Smith and Parfitt, 1986; Crowe et al., 1990; Entwistle, 1990a; Pérez-Moreno et al., 1996).

Fungicidal protection of *Allium* plants can be achieved by sequential applications of dicarboximides such as vinclozolin and iprodione, but these frequently lose effectiveness due to their microbial degradation in the soil (Entwistle and Hawling, 1984; Bugaret et al., 1996). However, good results have been obtained with another dicarboximide, procymidone, when seed or

soil treatments were combined with aerial applications (Porter, 1990; Fullerton et al., 1995) except for the case of southeast Queensland (Jackson et al., 1997). With regard to DMI fungicides, the use of the triazole tebuconazole was very effective when applied to the soil (Dennis, 1997) or to the garlic cloves (Jackson et al., 1997), but it was highly phytotoxic, causing seed and seedling mortality when used as a seed treatment for onion (Fullerton et al., 1995). When treatment of garlic cloves with procymidone or tebuconazole was compared in field experiments, a much better control of WR and the subsequent increase in garlic yield were shown for the tebuconazole-treated cloves (Jackson et al., 1997).

The application of fungal and bacterial antagonists to the soil opens the possibility of disease control without the use of chemicals, and usually provides an environmentally sound control measure. Among the microorganisms reported to provide biocontrol of *S. cepivorum*, one of the most effective seemed to be *Trichoderma* spp. (Abd-El-Moity and Shatla, 1981; De Oliveira et al., 1984; Chet, 1987; Ghaffar, 1988; Abd-El-Moity, 1992; Kay and Stewart, 1994). *Bacillus subtilis* (Ehremberg) Cohn, was also considered an effective biocontrol agent, inhibiting mycelial growth of *S. cepivorum* through antibiosis (Utkhede and Rahe, 1983a,b; Reddy et al., 1992).

Trichoderma harzianum applied to solarized plots improved control of *S. cepivorum* compared with the results achieved with the addition of *B. subtilis*, applied after soil solarization (Pereira et al., 1996).

The effectiveness of the control methods studied varies with environmental conditions, pathogen and type of antagonist. A comparative study of the effects of the above-mentioned methods of control on disease progress and yield quality have not been attempted. Therefore, the objectives of this work were: (i) to compare the effectiveness of different methods of controlling garlic WR under field conditions in southern Spain, using *T. harzianum*, *B. subtilis*, soil solarization and tebuconazole, and (ii) to evaluate their effects on the crop yields and on the quality of bulbs (long-term effect).

Materials and methods

Origins and production of biocontrol agents

An isolate of *T. harzianum* (JR2) obtained from a *S. cepivorum*-infested garlic field in Granada, Spain was used in these experiments. After selection from several antagonists by *in vitro* and field tests (Basallote-Ureba et al., 1995) isolate JR2 was multiplied on wheat bran : peat moss : water (5 : 3 : 5, w), which was incubated at 25 °C in the dark for 3 weeks. Immediately after the incubation period, inoculum was air-dried, then finely milled and distributed in the furrows at the rate of $11\,g\,m^{-1}$ immediately before garlic planting.

The isolate of *B. subtilis* used (AP-365) originated from Brazil and had been selected for its fungal antagonism *in vitro* (Basallote-Ureba et al., unpublished). This bacterial culture was increased in a sterile aqueous medium containing glucose (1%), bacto-peptone (1%) and mineral salts (Backhouse and Stewart, 1989), which was incubated for 8 days under continuous agitation (75 rpm) in an orbital shaker at 24 °C and with 12 h photoperiod. Bacterial suspensions, 10^8 cfu ml^{-1}, were obtained by centrifuging ($10 \times g$) the culture for 10 min, washing and resuspending the pellet twice in sterile water, in order to obtain an extract-free suspension of bacterial cells.

Field experiments

Four field experiments were carried out during the period 1994–97. Two of them were conducted in a field (A) artificially infested by adding sclerotia from infected *Allium* plants and infested soil in 1991, 1992 and 1996. Experiments in field A were conducted in the 1994–95 and 1996–97 seasons, whereas this field was fallow in 1995–96. The other two experiments were carried out in consecutive years (1995–96 and 1996–97) in the same naturally infested plot (field B). Experimental design was set up as complete randomised blocks with five replications in field A and four in field B. Plots were 6 and 45 m^2 in fields A and B, respectively.

Field A, located in the Research Farm of CIFA Córdoba, Spain, was a sandy loam soil, 1.2% organic matter and pH 8.1. The soil of field B, in Granada, Spain, was a silty loam, 1.4% organic matter and pH 8.3.

Treatments in field A (Table 1) were: (1) untreated control, (2) *T. harzianum* added to the furrows at planting time, (3) dipping of garlic cloves in a suspension of *B. subtilis* (10^8 cfu ml^{-1}) 5 min, in 1994–95, or soil solarization conducted from late July to mid September, 1996, in 1996–97, (4) dipping of garlic cloves in tebuconazole (1 ml of Folicur 25% l^{-1} of water) for 5 min, and (5) dipping of garlic cloves in

Table 1. Effect of different soil and clove treatments on the progress of white rot of garlic inoculum density, final incidence of dead plants, and yields in field A, artificially infested by *S. cepivorum*

Year	Treatment[x]	Application	Inoculum density[y] (viable sclerotia/kg soil)		Dead plants[z] (%)	Yield[z] (t/ha)	Weight of bulbs with diam. $\geq$ 45 mm[z] (%)
			Before solarization	At planting			
1994–95	Control		—	—	21.2 a	12.3 a	80.7 a
	T. harzianum	Furrow, planting	—	—	15.9 a	12.7 a	85.4 a
	B. subtilis	Cloves	—	—	15.7 a	13.5 a	87.7 a
	Tebuconazole	Cloves	—	—	3.7 b	14.0 a	78.0 a
	Tebuconazole	Stem base only	—	—	13.8 a	14.0 a	85.3 a
1996–97	Control		6.0 a	4.5 abc	17.2 a	17.8 a	83.3 a
	T. harzianum	Furrow, planting	5.5 a	7.5 ab	12.0 ab	19.5 a	86.1 ab
	Solarization	Soil	4.5 a	0.0 c	0.8 c	24.8 b	85.5 ab
	Tebuconazole	Cloves	2.5 a	9.5 a	4.1 bc	20.8 ab	92.8 c
	Tebuconazole	Cloves + stem base	6.0 a	2.5 bc	1.5 c	24.1 b	89.3 bc

[x]A substrate colonized by isolate JR2 of *T. harzianum* for 3 weeks was added to planting furrows at the rate of 11 g m^{-1}. Garlic cloves were dipped for 5 min in a suspension (10^8 cfu ml^{-1}) of *B. subtilis* or in a solution of 0.25 ml tebuconazole l^{-1}. The application of this fungicide to garlic plants consisted of sequential sprays to garlic stem bases with the same solution of tebuconazole.

[y]Soil samples taken in 1994 corresponded to blocks, therefore data of treatments were not recorded (—). In 1996, five soil samples (0–20 cm depth) were randomly taken from each plot and mixed, viable number of sclerotia of *S. cepivorum* being determined in each of four 100-g aliquots, and values averaged over the replicated plots. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's protected LSD test.

[z]Values are means of five replications. In each column, means for each experiment followed by the same letter are not significantly different at $P \leq 0.01$, according to Fisher's protected LSD test. Percentages of dead plants and weight of bulbs were transformed into arcsin $(\%)^{1/2}$ for analyses of variance.

tebuconazole as in treatment 4 plus spraying garlic stem bases with the same concentration of tebuconazole at 10-day intervals from mid March to late April in 1995, or from late March to mid May in 1997.

The treatments in field B (Table 2) were: (1) untreated control, (2) soil solarization from 1st August to 6th September, 1995, (3) application of *T. harzianum* to the planting furrows, and (4) garlic clove treatment with tebuconazole, as described for experiments in field A. The same treatments were studied in 1996–97, but the plots of treatment (3) of the previous year were solarized (from 4th July to 9th October, 1996) in order to compare it with the long-term effect of soil solarization of 1995 (treatment 2).

Soil of plots to be solarized was thoroughly rotovated and irrigated to reach field capacity in the upper 30–40 cm layer 1–2 days before being covered with a 40-μm-thick transparent polyethylene sheet.

Planting of garlic cultivar Morado de Pedroñeras (12 cloves m^{-1} in rows 50 cm apart) was carried out by late November–early December, depending on the experiments. Mineral fertilization, hand weeding, and irrigation (with sprinklers in field A, and by furrows in field B) were conducted according to standard practices.

Monitoring levels of sclerotia in soil

Density of sclerotia (ID) of *S. cepivorum* was determined in soil samples (0–20 cm depth) taken just before soil solarization, and at planting time in 1995–96 and 1996–97 seasons. In 1994, ten soil samples were taken and bulked from each of the blocks in field A, but treatments were not considered. In 1995 and 1996, five and eight soil samples were bulked from each of the experimental plots in fields A and B respectively. After air-drying and thorough mixing of soil samples, four 100-g aliquots of each sample were analysed for ID determination. Sclerotia were extracted from the soil following a previously described procedure (Crowe et al., 1980), modified by Basallote-Ureba and Melero-Vara (1993).

Analysis of variance was applied to data on ID; in the case of samples taken at planting, transformed $(ID + 0.5)^{1/2}$ values were analysed. Mean values corresponding to the different treatments in each experiment were compared by Fisher's protected LSD test.

Table 2. Effects of different treatments on the progress of white rot of garlic inoculum density, final incidence of dead plants and yields in field B, naturally infested by *S. cepivorum*

Year	Treatment[w]	Application	Inoculum density[x] (viable sclerotia/kg soil)		Dead plants[y] (%)	Yield[z] (t/ha)	Weight of bulbs with diam. $\geq$ 45 mm[z] (%)
			Before solarization	At planting			
1995–96	Control		14.7 a	6.8 a	15.0 a	11.6 a	71.5 a
	Solarization in 1995		19.2 a	0.0 a	1.0 b	14.8 b	76.8 a
	T. harzianum	Furrow	32.7 a	32.5 a	14.7 a	11.5 a	68.6 a
	Tebuconazole	Cloves	30.2 a	29.0 a	5.1 ab	13.9 ab	76.3 a
1996–97	Control		15.6 a	30.0 ab	40.2 a	8.9 a	76.6 a
	Solarization in 1995		13.7 a	5.6 b	8.5 bc	15.0 bc	86.7 b
	Solarization in 1996		20.0 a	0.6 b	0.8 c	19.6 c	92.3 b
	Tebuconazole	Cloves	39.3 a	45.6 a	19.6 ab	10.9 ab	76.6 a

[w]A substrate colonized by isolate JR2 of *T. harzianum* for 3 weeks was added to planting furrows at the rate of 11 g m^{-1}. Garlic cloves were dipped for 5 min in a solution of 0.25 ml tebuconazole l^{-1}. Plots solarized in 1995 were untreated in 1996, whereas those that were amended with *T. harzianum* in 1995 were solarized in 1996; plots planted with tebuconazole-treated cloves were the same for the two years.

[x]Eight soil samples (0–20 cm depth) were randomly taken from each plot and mixed, viable number of sclerotia of *S. cepivorum* being determined in each of four 100-g aliquots, and values averaged over the replicated plots. In each column, means followed by the same letter are not significantly different at $P \leq 0.01$ according to Fisher's protected LSD test.

[y]Values are means of four replications. For the experiments of 1995–96 and 1996–97, means followed by the same letter are not significantly different at $P \leq 0.01$ and $P \leq 0.05$, respectively, according to Fisher's protected LSD test. Percentages of dead plants were transformed into arcsin $(\%)^{1/2}$ for analyses of variance.

[z]Values are means of four replications. Means of each experiment followed by the same letter are not significantly different at $P \leq 0.01$, according to Fisher's protected LSD test. Percentages of weight of bulbs were transformed into arcsin $(\%)^{1/2}$ for analysis of variance.

Evaluation of disease levels and garlic yields

Depending on the experiments, 9–10 sequential observations of the incidence of plant death were made from March until harvest. Angular transformation of final percentages of dead plants was done before conducting the analysis of variance. Mean values for the different treatments in each experiment were compared by Fisher's protected LSD test. Epidemic progress was studied by linear regression of disease incidence with time after planting and by other regression models.

Correlation analyses were performed between ID at planting and final disease for the control and solarized plots of the experiments of 1995–96 and 1996–97.

Garlic yield in the plots was measured, and the effect of the treatments on bulb quality was determined, by assessing the percentage of total yield corresponding to each of five commercial categories established by bulb diameters being $>$ 50, 45–50, 37–44, 30–36 mm, and smaller and discarded bulbs (untrimmed). Analysis of variance of the percentage weight of bulbs in each category was performed after angular transformation of the data. Mean values for the different treatments in each category and experiment were compared using Fisher's protected LSD test. The possibility of a relationship between final disease incidence and total garlic yield was investigated by means of the correlation analyses between these two variables in every plot of the four experiments.

Results

Inoculum density in the plots of the different treatments before initiation of solarization was higher in field B (Table 2) than in field A (Table 1), but there were no significant differences among the treatments in each experiment (Tables 1 and 2). Average densities of viable sclerotia of *S. cepivorum* in the soil at the date of planting, were 11.0 and 4.8 viable sclerotia/kg soil in field A, in 1994 and 1996 respectively, and 17.1 and 20.4 in field B in 1995 and 1996 respectively. Soil solarization reduced the density of viable sclerotia in the upper 20-cm layer of soil to negligible values. Although

the ID in plots with tebuconazole-treated cloves was relatively high in different fields and years (Tables 1 and 2), disease incidence was rather low compared to other treatments for which the ID was at similar levels.

The progress of disease incidence for the different treatments, fields and years is shown in Figure 1, and fitted well a linear regression in most cases. In contrast, other regressions fitted the progress of disease in some treatments but not in others. Therefore, these other regressions were disregarded. Infections by *S. cepivorum* began to kill the garlic plants ca. 3 months after planting, and final disease incidence was under 25%, except for the control plots of field B in the 1996–97 season (Figure 1, Tables 1 and 2). No correlation was observed between the ID at planting and final disease incidence.

Disease progress and garlic yield in an artificially infested soil (field A)

Rate of disease progress in field A, evaluated by the linear regression of the incidence of dead garlic plants with time after planting, was markedly reduced by clove treatments with tebuconazole (Figure 1A). This

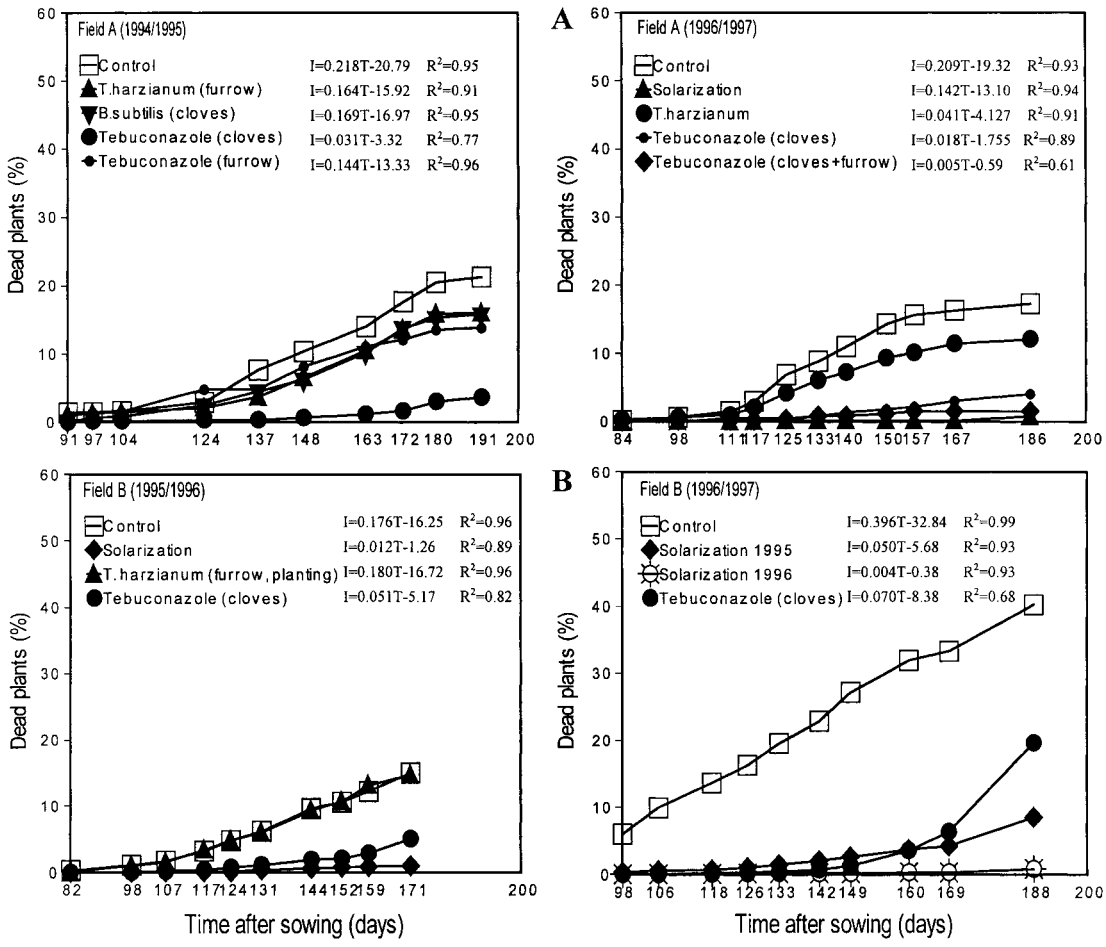

Figure 1. Disease incidence (% dead plants) of garlic infected by *Sclerotium cepivorum* over time (T) after planting cultivar Morado de Pedroñeras in plots receiving different treatments, and linear regression equations for them. (A) Artificially infested field A; (B) naturally infested field B. Two experiments, in two different seasons, as indicated, were conducted in each of the two fields.

reduction was improved when tebuconazole was also applied to stem bases of garlic plants grown from cloves treated with the same fungicide immediately before planting in 1996/1997 (Figure 1A). With soil solarization there was negligible disease, and yields were significantly ($P \leq 0.01$) improved. Similar disease levels and yields were obtained at harvest when tebuconazole was applied both to cloves and stem bases, and equivalent values were also achieved by clove treatment with tebuconazole (Table 1). Application of *B. subtilis* to cloves or addition of *T. harzianum* to the furrows at planting resulted in average disease levels being reduced by ca. 25% of the values for the control plots, but this difference was not statistically significant (Table 1). Correlation coefficients between disease incidence at harvest and garlic yield were -0.505 ($P = 0.011$) for the experiment of 1994–95 and -0.813 ($P < 0.001$) for that of 1996–97.

Yield quality, as assessed by the percentage weight of larger bulbs (≥ 45 mm), was improved ($P \leq 0.01$) only by the tebuconazole treatments in the experiment of 1996/1997 (Table 1).

Disease progress and garlic yield in a naturally infested soil (field B)

In the two experiments of field B, the ID level was negligible following soil solarization and it remained relatively low by the second consecutive planting of garlic in plots solarized in 1995. ID at planting greatly increased from the first to the second season in control and tebuconazole-treated plots (Table 2). Rates of disease progress in field B were notably higher for the control in the second year, whereas garlic yields decreased accordingly (Figure 1B, Table 2). The tebuconazole treatment gave similar results. Since the application of *T. harzianum* to planting furrows in 1995 was not effective in controlling garlic WR, and did not increase garlic yields, plots that received this treatment were solarized in 1996. Therefore, a comparison between recent and previous solarization of soil was made. The second crop of garlic on plots solarized in 1995 had, on average, disease levels ten times higher than those solarized in 1996, but they were not significantly different. Consequently, yields in the latter were 30% higher than for the plots solarized in 1995. Although less satisfactory than soil solarization, treatment of cloves with tebuconazole halved disease levels (Table 2). Correlation coefficients between final disease incidence values and garlic yields were -0.815 and -0.917 for the experiments of 1995–96 and 1996–97 respectively, which indicated highly significant levels ($P < 0.002$).

Percentage of bulb weight corresponding to the two larger commercial category sizes (≥ 45 mm) did not differ significantly among the treatments in 1995/1996 experiment, but it was significantly higher ($P < 0.01$) in the solarized plots (both in 1995 and in 1996) of the 1996/1997 experiment.

Discussion

In agreement with previous indications (Crowe et al., 1980; Entwistle, 1990b), these results suggest the lack of a direct relationship between ID and final disease incidence, except in solarized plots, where a low ID corresponded to a low percentage of dead plants. Three different types of relationship between sclerotial populations of *S. cepivorum* in the soil and the incidence of WR have been reported (Entwistle, 1990b), i.e. (1) sclerotia populations in soil and those needed for infection are both small, (2) soil populations of *S. cepivorum* are large but small densities are required for infection, and (3) large populations are present and needed for infection. Our results seem to fit in the first type, represented by WR of garlic in California. As the climate there, as in southern Spain, is Mediterranean, these conditions seem to be a factor for this type of relationship.

The high variability of disease incidence among the different replications within some treatments (Tables 1 and 2) did not allow statistical significance to be proved. This situation is, however, not uncommon in this pathosystem (Satour et al., 1989). On the other hand, the large increase in disease level in the control plots of field B between the two years of study (Table 2) seems to be primarily related to the increase in sclerotial populations in the soil after one susceptible crop, although the influence of environmental conditions is also possible.

Soil solarization was the most effective treatment for eradicating *S. cepivorum* from infested soil in the different fields and years tested, but this effectiveness was reduced when the second consecutive garlic crop in plots solarized two years before was considered (Table 2). Thus, the results obtained in previous studies in Spain (Basallote-Ureba and Melero-Vara, 1993; Basallote-Ureba et al., 1995) and elsewhere, both on onion and garlic crops (Porter and Merriman, 1985;

Pereira et al., 1996) were confirmed. They suggest that soil suppressiveness is not likely to occur in solarized plots, where disease seems to have a monocyclic nature (Figure 1). In contrast, field experiments in Egypt indicated a more satisfactory long-term effect of soil solarization to control onion WR despite furrow irrigation (Satour et al., 1989), which is determinant of inoculum spread from non-solarized to solarized plots. In addition, a large and consistent improvement of *Allium* yields was associated to soil solarization in Spain (Tables 1 and 2; Basallote-Ureba and Melero-Vara, 1993) and Egypt (Satour et al., 1989), but it was negligible in Victoria, Australia (Porter and Merriman, 1985) probably because marginal conditions for soil solarization prevailed there.

In agreement with recent results (Jackson et al., 1997), treatment of garlic cloves with tebuconazole at the concentration of 1 ml Folicur 25% l^{-1} provided good control of garlic WR, and improvement is possible if it is complemented by sequential spraying of stem bases with the same fungicide (Tables 1 and 2). This suggests that most infections initiate at early stages of the crop, though above-ground symptoms start to show up ca. 3 months after sowing. Effectiveness of the fungicide is not likely to last for that long. Fungicide treatment of garlic cloves was shown to be effective under a wide range of ID and in different years and resulted in subsequent yield increases (Tables 1 and 2), which were, nevertheless, not as large as those reported in southeast Queensland, Australia (Jackson et al., 1997). However, the effectiveness of tebuconazole treatment of garlic cloves in field B seemed to be reduced in the 1996 planting compared to 1995, probably because of the increased ID in the 1996 planting. The protective nature of these treatments was confirmed by the relatively low disease incidence in relation to ID values observed for soil samples taken at planting, and by the increase of ID for the next season in the tebuconazole-treated plots.

The application of *T. harzianum* to the soil, in contrast with other studies (Abd-El-Moity and Shatla, 1981), was less effective, and clove treatment with *B. subtilis* had an effect similar to that of *T. harzianum*. Therefore, the use of these biological control agents seems to be more appropriate as one component of integrated control practices that combines either with chemical treatments or with soil solarization, as suggested by Chet (1987).

In agreement with a previous study on soil solarization (Basallote-Ureba and Melero-Vara, 1993; Basallote-Ureba et al., 1995), bulb quality was improved in solarized plots compared to untreated or tebuconazole-treated plots in the experiment of 1996/1997 of field B (Table 2). Furthermore, estimations of economic value of the crop, taking into account the higher prices of larger bulbs, indicated an additional benefit of soil solarization in this experiment, in which there was a high disease pressure. Improvement of bulb quality was also observed in field A (experiment 1996/1997) for the treatment that combined tebuconazole applications to cloves and stem bases (Table 1).

The integration of the two most efficient methods of control of WR of garlic, i.e. soil solarization and tebuconazole treatment of garlic cloves, is suggested as very satisfactory method under high disease levels. This is of particular interest when a long-term effect of solarization is desired, since clove treatment with tebuconazole would be appropriate under low disease pressure such as in the second year after soil solarization. Thus, a reduction of costs of soil solarization per year would be achieved, making its use more economically feasible.

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