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Research Papers

Efficacy of chemical and biological spray seed treatments in preventing garlic dry rot

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Summary. Garlic dry rot caused by *Fusarium proliferatum* is an emerging postharvest disease that has resulted in severe economic losses, necessitating design and implementation of efficient disease control strategies. Sanitation of planting cloves is critical for preventing garlic dry rot. This study evaluated the efficacy of commercial chemicals and biocontrol agents, applied at planting as spray treatments, for reducing disease severity and the occurrence of *Fusarium* spp. in garlic, from the field stage then through 9 months of postharvest storage. Tebuconazole was the most effective for reducing disease severity, giving 26.5% reduction of basal plate rots and 44% reduction of bulb rots, and 33.4% reduction in visible symptoms on cloves relative to the untreated controls. Comparable results were obtained by applying *B. subtilis* and *S. griseoviridis*. However, none of the active ingredients tested in this study reduced the incidence of *F. oxysporum* and *F. proliferatum* on basal plates, although tebuconazole reduced the postharvest incidence of *F. proliferatum* on cloves by nearly 50%. Incidence of *F. proliferatum* increased by 37% in bulbs transferred from storage to room temperature (25°C) for 15 days, simulating storage in consumers' homes. These results demonstrate that spray seed clove treatments have inhibitory effects on postharvest garlic dry rot, although further research is required to determine the persistence of these treatments during prolonged storage, especially without low temperatures.

Keywords. *Allium sativum*, *Fusarium* spp., fungicide, biocontrol agent, disease management, garlic dry rot.

INTRODUCTION

Garlic (*Allium sativum*) is an important horticultural crop grown in temperate regions (Lopez-Bellido *et al.*, 2016). Garlic produced in Europe accounts for 3.4% of annual global production (FAO-STAT, 2018), and is of high quality, making a significant contribution to local economies (Spagnoli, 2014).

In northern Italy, garlic production starts with planting in mid-October to early November, with harvest in July of the following year. Bulbs develop during springtime starting from the end of April. After harvest, garlic bulbs are sun-dried for 15 to 30 d; some are selected as planting material for subsequent production, while others are stored for up to 9 to 10 months in cold chambers at -4°C until they are delivered to market. Consumers purchase

and store garlic at room temperature until consumption, which can be for 10 to 20 d or longer.

Postharvest decay of garlic, in which bulbs are partially or completely empty, was first reported by consumers. Upon close observation, centrally depressed polygonal brown spots were observed under clove sheaths during the drying process, with white mycelia visible in severe cases. These symptoms were mostly recorded postharvest and resulted in yield losses of up to 30% (Tonti *et al.*, 2012). In 2002, garlic dry rot was first described as an emerging global disease caused by *Fusarium proliferatum* (Dugan *et al.*, 2003), which was later confirmed by several authors in different geographic areas (Stankovic *et al.*, 2007; Palmero *et al.*, 2010; Sankar and Babu, 2012; Tonti *et al.*, 2012; Salvalaggio and Ridao, 2013; Quesada-Ocampo *et al.*, 2014; Leyronas *et al.*, 2018). In the field, *Fusarium* spp. cause symptoms on garlic roots or remain latent between leaves and cloves (Stankovic *et al.*, 2007; Mondani *et al.*, 2021b). In the only systematic studies to date carried out in Italy, the two main species isolated from asymptomatic and symptomatic cloves at field and postharvest stages were *F. proliferatum* and *F. oxysporum*, with *F. proliferatum* also confirmed as a fumonisin producer (Mondani *et al.*, 2021a, 2021b), in agreement with Seefelder *et al.* (2002). These studies confirmed the relevance of infected seed cloves for dry rot outbreak, even if it is primarily a postharvest disease.

A study conducted in North America showed that the incidence of bulbs infected with *F. proliferatum* at harvest ranged from 25% to 50%, and that up to 77% of cloves were symptomatic when peeled after 9 to 16 months of storage, even if they appeared healthy and firm at the time of harvest (Dugan *et al.*, 2019).

As garlic is propagated vegetatively, selection and use of healthy seed cloves is critical for reducing fungus dissemination and dry rot severity. There are no reports of appropriate methods for selecting healthy garlic seed cloves on industrial scale, or on control strategies to prevent/reduce *Fusarium* incidence along the garlic production chain. Some studies have evaluated the efficacy of chemical or biological treatments against *Fusarium* spp. in garlic. The efficacy of the benomyl in wounded bulbs was previously demonstrated (Dugan *et al.*, 2007), but this fungicide has since been withdrawn in Europe. Due to the EU Directive on Sustainable Use of Pesticides (2009/128/EC), the number of approved chemical active ingredients has decreased, and this trend is expected to continue. It is therefore important that alternative ingredients and control methods are considered, especially those with greater sustainability than pesticides (Lamichhane *et al.*, 2016; 2020).

Gálvez Patón *et al.* (2017), in Spain, evaluated the commercial fungicides Cabrio® Duo (dimetomorph + pyraclostrobin), Luna® Experience (fluopyram + tebuconazole, and Flint® Max (tebuconazole + trifloxystrobin), with promising *in vitro* results. In these experiments, *F. proliferatum* was grown on potato dextrose agar (PDA) plates containing low amounts of the fungicides. However, the same products applied in the field as foliage sprays during crop development (May and June) failed to prevent postharvest garlic rot,

Thermotherapy at 50°C has been reported to greatly decrease the viability of *F. proliferatum* conidia grown *in vitro*, but the treatment has yet to be tested on garlic seed cloves (Palmero Llamas *et al.*, 2013). Other studies have also reported that *Fusarium* spp. was controlled *in vitro* with biocontrol agents (BCAs) (Ju *et al.*, 2013; Evangelista-Martínez, 2014; Ghanbarzadeh *et al.*, 2014; Samsudin and Magan, 2016), but these agents have not been tested on garlic crops in the field. *Trichoderma* spp. and *Bacillus subtilis* were the most promising BCAs, with laboratory trials (Mondani *et al.*, 2021c) demonstrating their efficacy against *Fusarium* spp. isolated from garlic.

Garlic seed clove treatments have not been tested, but this disease management strategy may ensure protection in early stages of plant development and improve yield quality and postharvest product preservability. Attention has been paid to this approach, although detailed knowledge is lacking (Lamichhane *et al.*, 2016; Pedrini *et al.*, 2016; Hitaj *et al.*, 2020). The lack of efficacy of foliar applied fungicides suggests that intervention on propagation material could prevent pathogen activity from early crop growth stages, particularly as *Fusarium* spp. have been confirmed in seed cloves.

The aim of the present study was to assess the efficacy of chemical and biological active ingredients, applied as spray pre-planting seed clove treatments, for preserving garlic bulbs during crop growth and postharvest storage. Disease incidence and severity, caused by *Fusarium* spp., in garlic bulb basal plates and tissues, were used as measures of the effectiveness of the seed clove treatments.

MATERIALS AND METHODS

Media

Water agar (WA) was prepared by dissolving 20 g of agar (2%; Oxoid) in 1 L of double-distilled water. PDA was prepared by mixing 15 g of agar (2%; Oxoid) and 10 g of dextrose with 1 L of potato broth (200 g of potato per litre of water).

Table 1. Commercial plant protection products (chemicals or biocontrol agents) used in field trials.

Product	Active ingredients	Manufacturer	Product label use rates
<i>Chemicals</i>			
MYSTIC 430SC	Tebuconazole, 250 g L ⁻¹	Nufarm Italia, Bologna, Italy	580 mL ha ⁻¹
BUMPER P	Prochloraz, 400 g L ⁻¹ /l + propiconazole 90 g L ⁻¹ /l	ADAMA Italia, Grassobio, Italy	1100 mL ha ⁻¹
<i>Biocontrol agents</i>			
Serenade Max	<i>Bacillus subtilis</i> (5.13 × 10 ¹⁰ CFU g ⁻¹)	Bayer Crop Science, Milan, Italy	2500 g in 500 L, as seed clove treatment
Mycostop	<i>Streptomyces griseoviridis</i> K61 (5 × 10 ⁸ CFU g ⁻¹)	Verdera Oy, Kurjenkellontie, Finland	10 g in 100 L, as seed clove treatment
Remedier	<i>Trichoderma harzianum</i> + <i>T. gamsii</i> (3 × 10 ⁷ CFU g ⁻¹)	Isagro, Milan, Italy	10 g L ⁻¹ as seed clove treatment + 2500 g ha ⁻¹ as soil treatment
Lifstrong Vam L	<i>Mycorrhiza</i> (100 spores g ⁻¹) + <i>B. subtilis</i> , <i>Pseudomonas fluorescens</i> , <i>Azospirillum lipoferum</i> (3 × 10 ⁸ CFU g ⁻¹) + <i>Trichoderma</i> (1 × 10 ⁸ CFU g ⁻¹)	Fertilidea Srl, Pompei, Italy	2 to 4 L ha ⁻¹

Seed treatments, field location, and experimental design

The experiment field was located in San Pietro in Cerro, Piacenza (northern Italy; 45.01 N, 9.94 E). The research was conducted over two consecutive garlic growing seasons (2017–2018 and 2018–2019). Six commercial plant protection products were evaluated, including two chemicals and four BCAs. These had been shown to be effective in preliminary *in vitro* experiments (Mondani *et al.*, 2021c). All the products were used at the doses indicated on the product labels (Table 1). At the end of October each growing season, spray seed clove treatments were applied through the planting machinery (four row bulb planter; JJ Broch), by wetting the cloves and spraying the products into planning furrows. The trials were each arranged in a strip plot design with four replicates, with each strip covering an area of 810 m², with interrow distance of 30 to 40 cm and seed clove density of 27 cloves m⁻².

Monitoring of seed clove treatment efficacy throughout the garlic production process

Garlic plants (locally developed variety Ottolini) were sampled over the 2 years of this study, both from the field and postharvest, as shown in Table 2. Three crop growth stages were selected to monitor disease severity and fungal incidence in bulb basal plates and tissues: BBCH 15 (5th leaf visible; mid-April), BBCH 45 (half bulb final diameter; end of May), and BBCH 49 (harvest; end of June) (Lopez-Bellido *et al.* 2016; Mondani *et al.*, 2021b). Harvested bulbs were sun-dried for 1 month until the beginning of August in an open field (with coverage in case of rain) and were then stored in

cold chambers at –4°C for 9 months, according to the locally used management practices. Inspections of symptomatic bulbs postharvest were carried out four times: after 3 months (end October), 6 months (early February of the following year), and 9 months (mid-May of the following year) of cold chamber storage, and then 15 d after the bulbs were transferred from the cold chamber to room temperature (≈ 25°C), simulating the period after delivery to consumers. After 9 months of storage and 15 d at room temperature, symptomatic cloves and bulbs were counted, and fungi were isolated from bulb basal plates and cloves. Basal plates and bulbs were separately analyzed as previously described (Mondani *et al.*, 2021a). Bulb water activity (a_w) was measured at four critical steps of the production process: at BBCH49, at the end of natural drying, after 9 months of cold storage, and after 15 d at room temperature.

Inspections and fungus isolations from bulb basal plates

Four replicates of five plants per seed clove treatment (total of 20 plants) were sampled at three field growth stages (BBCH15, BBCH45, and BBCH49). Based on visual inspections, symptom severity on each bulb was scored using five categories (Mondani *et al.*, 2021b): 0%, asymptomatic; 10%, small brown spots near the basal plate (base of radicles); 35%, brown spots on one-half of the basal plate; 65%, brown spots on the entire perimeter of the basal plate, with or without white mycelia on the inner cloves and violet pigmentation on the radicles; or 90%, brown spots on the basal plate and bulb, necrotic radicles, and visible white mycelia. Basal plate severity index (BPSI) was calculated for each sampling time point by multiplying the number of plants in

Table 2. Experimental scheme of garlic plant/bulb sampling time points in the production chain, from growth in the field through postharvest cold storage and delivery^a.

Data collection time point	Field				Cold storage			Room temperature
	BBCH15	BBCH45	BBCH49	Drying	3 months	6 months	9 months	15 d
	April	May	June	July	October	February	May	May
<i>Basal plates</i>								
Inspection of bulb basal plates (BPSI)	20 plants	20 plants	20 plants					
Fungus isolations from bulb basal plates	20 plants	20 plants	20 plants				24 bulbs	24 bulbs
<i>Bulbs</i>								
Water activity ^b			five bulbs	five bulbs			five bulbs	five bulbs
Visual inspection of symptomatic bulbs (BSI)					120 bulbs	120 bulbs	120 bulbs	120 bulbs
Count of symptomatic cloves/bulb							24 bulbs	24 bulbs
Fungus isolations from bulbs							24 bulbs	24 bulbs

^aThe number of plants/bulbs reported at each sampling time point is the total number sampled for each seed clove treatment.

^bWater activity was measured at key steps in the chain.

Abbreviations: BBCH15, 5th leaf visible (mid-April); BBCH45, half of bulb final diameter (31 May); BBCH 49 (ripening; 30 June); BPSI, basal plate severity index; BSI, bulb severity index.

each disease severity category with the corresponding disease severity value and dividing by the number of plants collected per sampling time point (20).

Direct fungus isolation was performed by sampling a portion of basal plates from each bulb (whether it was symptomatic or asymptomatic), during field growth stages and postharvest. The samples were washed with tap water for 20 min, surface-disinfected with 1% NaO-Cl for 1 min, and plated on WA as previously described (Mondani *et al.*, 2021b). Resulting colonies were transferred to PDA and identified at the genus level (Schwartz and Mohan, 2016), and *Fusarium* colonies were identified at the species level based on morphological features (Leslie and Summerell, 2006). One isolate per morphological group was confirmed by molecular identification (Mondani *et al.*, 2021b) using established protocols (Nicolaisen *et al.*, 2009; Mbofung and Pryor, 2010).

Inspection and fungus isolation from bulbs

To simulate product selection by garlic producers, bulbs were inspected at four time points during storage (Table 2). Bulb disease severity was determined by touching the cloves and estimating the number that were emptied (0–100% emptiness per bulb), without removing the or disrupting the bulb sheath. As the mean number of cloves per bulb was 10, each empty clove represented a 10% increment in severity. Bulb severity index (BSI) was calculated with the following formula:

$$BSI = ([10 \times n_1] + [20 \times n_2] + [...] + [100 \times n_{10}]) / \text{no. of plants sampled},$$

where numbers from 10 to 100 represent the percentages of cloves emptied by the fungus, and n_1 to n_{10} are the number of bulbs in each severity category.

At the 9-month and 15-d time points, six bulbs were randomly selected from the four trial replicates based on selling quality category (bulb diam. = 35–46 mm, or >46 mm), for a total of 24 bulbs per seed bulb treatment. To verify disease severity, all sampled bulbs were peeled and the percentage of symptomatic cloves in each bulb was calculated with the following formula:

$$\% \text{ symptomatic cloves} = (\text{no. of cloves with brown spots} / \text{total no. of cloves in bulb}) \times 100.$$

Direct fungus isolation was carried out from cloves at 9 months and 15 d. Asymptomatic and symptomatic cloves (20 each) were randomly selected for each quality category and seed clove treatment (20 cloves \times seven seed clove treatments \times two categories \times two sampling time points \times 2 years = total of 1120 asymptomatic and 1120 symptomatic cloves). Clove portions were washed with tap water for 20 min and plated, and resulting fungus colonies were identified as reported above.

Determination of bulb water activities (a_w)

At BBCH 49, after 1 month of natural drying, at 9 months of cold storage, and after 15 d of room tem-

perature storage, a_w was measured using an Aqualab Pre instrument (Meter Group). Five bulbs were sampled from each seed clove treatment. Central cloves of each bulb were removed (two replicates) and used for analysis. Each measurement was repeated 3 times.

Statistical analyses

BPSI, BSI, % symptomatic cloves/bulb, and incidence of fungus species (*Fusarium* spp.) were arcsine-transformed to homogenize means (Clewer and Scarisbrick, 2001). Transformed data and a_w values were subjected to analyses of variance. Tukey's test was used to compare means ($P < 0.01$). Statistical analyses were carried out using PASW Statistics v25 (SPSS Inc.).

RESULTS

Basal plate inspections and fungus isolations

Basal plates were divided into severity categories, and BPSI was calculated at three growth stages (BBCH15, BBCH45, and BBCH49) during crop development, and at two postharvest time points (9 months and 15 d). During the postharvest period, all bulb basal plates were of severity category 3 (brown spots visible on the entire basal plate perimeter, with growing mycelia occasionally visible). This category corresponded to a severity value of 65%, and these data were excluded from the statistical analyses.

Statistically significant differences were detected between treatments ($P < 0.01$) based on the ANOVA. Untreated control plants had the greatest mean disease severity (43.7%), along with those treated with the commercial BCA product including *Trichoderma* and *B. subtilis* as active ingredients. Greatest disease reductions resulted from the chemicals tebuconazole (mean severity = 32.1%) and prochloraz + propiconazole (35.0%). Chemicals (mean BPSI = 33.6%) gave greater bulb rot reductions than the BCAs (mean BPSI = 37.1%), but *B. subtilis* and *S. griseoviridis* as single active ingredients gave similar results (mean BPSI = 35.6%) to the chemical treatments. Statistically significant differences were observed between the 2 years of the study, with a 12.9% greater mean disease severity in year 2 compared to year 1. Disease severity on bulb basal plates increased during the cropping seasons, from 4.1% at BBCH15 to 55.9% at BBCH49, and to 65% postharvest (Table 3).

The main fungus genus isolated from basal plates was *Fusarium*. Other genera were sporadically isolated, including *Rhizopus* (mean of 3.2% for all treatments and

years) analyzed), *Trichoderma* (1.0%), *Alternaria* (0.82%), and *Penicillium* (0.42%). Data for these other genera were excluded from the statistical analyses.

Two species of *Fusarium* were identified, namely, *F. oxysporum* and *F. proliferatum*. Active ingredients applied as seed clove treatments were not effective in controlling these two fungi on bulb basal plates (Table 3). Both *F. oxysporum* and *F. proliferatum* were detected at incidences comparable to those in the untreated control samples. Mean incidence of *F. oxysporum* was greater in year 1 (28.5%) than in year 2 (21.3%) ($P < 0.01$) (Table 3). The rates of isolation of the two species also varied according to the time point of sampling. Greatest incidence of *F. oxysporum* was at BBCH15 (mean = 38.2%) and BBCH49 (29.6%) ($P < 0.01$). *Fusarium proliferatum* showed a similar trend, with least incidence at BBCH45 (mean = 50.7%) and greatest at the time of harvest (BBCH49, 63.2%) ($P < 0.01$) (Table 3). After the cold storage period, when bulbs were stored at $\approx 25^\circ\text{C}$ for 15 ds, the two species showed opposite trends: *F. oxysporum* mean incidence on bulb basal plates decreased by 7.5% from 26.5% at 9 months, and to 19.0% at 15 d, whereas that of *F. proliferatum* increased by 12.5% from 52.1% to 64.6% ($P < 0.01$) (Table 3).

Bulb inspections and fungus isolations

Bulbs were first inspected by touch to estimate the percentage of cloves emptied by fungus pathogens, which is the method commonly used by garlic producers. Among the treatments, tebuconazole and bacterium BCAs gave the least bulb disease severity (mean BSI = 10.6%), followed by prochloraz + propiconazole (13.5%) and the fungus BCA (mean = 14.7%). All the treatments resulted in significant differences in disease severity ($P < 0.01$) relative to the untreated controls (16.6%) (Table 4).

After 9 months of cold storage, bulbs were peeled and the number of symptomatic cloves per bulb was counted. Tebuconazole was the most effective treatment for reducing disease symptoms (mean = 30.5% symptomatic cloves/bulb), and this treatment differed ($P < 0.01$) the untreated control (45.8% symptomatic cloves/bulb). All the other treatments showed comparable performance, with *B. subtilis* (35.4%) and *Trichoderma* + *B. subtilis* (36.8%) giving less disease than the untreated control. Differences in BSI were observed between the years of study (6.0% in year 1 and 20.1% in year 2; $P < 0.01$) (Table 4). BSI increased during bulb storage, but the number of symptomatic cloves in the bulbs was the same at 9 months and after 15 d at room temperature. However, bulbs with large diameters (>46 mm) had greater mean proportion (41.2%) of symptomatic

Table 3. Mean garlic bulb basal plate severity indices (BPSI), and mean incidence of fungi isolated from basal plates, for different field trial treatments, two growing seasons (Year 1, 2017–2018, and Year 2, 2018–2019), and field crop and postharvest sampling time points^a.

Parameter	BPSI (%)	Incidence (%)	
		<i>F. oxysporum</i>	<i>F. proliferatum</i>
<i>A Treatment</i> ^b	**	n.s.	n.s.
Control	43.7 d ^c	22.6	55.8
<i>Trichoderma</i> + <i>Bacillus subtilis</i>	39.8 cd	24.9	55.8
<i>T. harzianum</i> + <i>T. gamsii</i>	37.9 bc	25.9	55.1
<i>S. griseoviridis</i>	34.9 abc	25.9	57.0
<i>B. subtilis</i>	35.8 abc	24.0	59.0
Prochloraz + propiconazole	35.0 ab	23.4	60.6
Tebuconazole	32.1 a	27.5	55.0
<i>B Year</i>	**	**	n.s.
Year 1	34.5 a	28.5 b	56.2
Year 2	39.6 b	21.3 a	57.6
<i>C Sampling time point</i>	**	**	**
BBCH15	4.1 a	38.2 d	53.9 abc
BBCH45	51.0 b	11.1 a	50.7 a
BBCH49	55.9 c	29.6 cd	63.2 bc
9 months	§	26.5 bc	52.1 ab
15 days	§	19.0 ab	64.6 c
<i>Interactions</i>			
A × B	n.s.	n.s.	n.s.
A × C	n.s.	n.s.	n.s.
B × C	*	**	n.s.
A × B × C	n.s.	n.s.	n.s.

^aGarlic seed cloves were subjected to seven treatments. Experimental factor statistical significance (*, $P < 0.05$; **, $P < 0.01$; n.s., not significant $P > 0.05$) is indicated.

^bTreatment (A), year (B), and sampling time point (C) were factors in the analysis of variance of data.

^cMeans accompanied by the same letters are not different (Tukey's test, $P < 0.01$).

§ Indicates that data were not included in statistical analysis (see text).

cloves than small (36–46 mm) bulbs (34.0%; $P < 0.01$) (Table 4).

Fusarium was the main genus isolated from cloves (mean incidence = 31.3%), followed by *Penicillium*. (7.2%). Other genera, including *Rhizopus*, *Aspergillus*, and *Alternaria*, were isolated sporadically (mean incidence <2% across treatments and years), and were excluded from statistical analyses. Tebuconazole reduced *F. proliferatum* incidence on bulbs mean = 16.6%) relative to the untreated control (31.7%) ($P < 0.05$), whereas occurrence of *F. oxysporum* was unaffected by the

applied treatments (Table 4). Incidence of *Fusarium* spp. on bulbs was comparable between the 2 years of this study. However, only *F. proliferatum* showed significant differences between sampling time points, with incidence almost doubled after bulbs were removed from the cold chambers and stored at room temperature for 15 d (19.7% after 9 months and 31.3% after the subsequent 15 days; $P < 0.01$). Large bulbs had greater incidence of *F. oxysporum* (7.2%) than smaller bulbs (3.8%; $P < 0.05$) (Table 4). Mean incidence of *Penicillium* spp. was also reduced by tebuconazole (mean = 1.9%) compared to the untreated control (7.9%), and these fungi were isolated at a greater rate in year 2 (7.6%) than in year 1 (0.6%; $P < 0.05$).

Water potential of garlic cloves

No statistically significant differences ($P > 0.05$) in a_w values were detected during the four time points after harvest during storage, nor were there differences in these measurements between the applied treatments. However, differences were observed between the 2 years of this study, with greater overall a_w in year 1 (mean = 0.927) than in year 2 (0.947). Similarly, there were differences in this parameter between the sampling times, with the greatest mean a_w at harvest (0.976), which decreased after the natural drying process (to 0.874), before increasing during cold storage (to 0.940 at 9 months), and then in storage for 15 d at room temperature (to 0.959) (Table 5).

DISCUSSION

Garlic is propagated vegetatively, and the selection of healthy planting material is critical to ensure high quantity, quality, and consumer safety in bulb production. *Fusarium proliferatum*, the main causative agent of dry rot (Palmero *et al.*, 2012; Tonti *et al.*, 2012; Mondani *et al.*, 2021a; Mondani *et al.*, 2021b), is detected in significant proportions of apparently healthy garlic cloves (Dugan *et al.*, 2019), so selection of pathogen-free seed cloves on an industrial scale is difficult and usually not achievable.

In the present study, spray seed clove treatments with fungicides were assessed as a measure for reducing bulb dry rot severity and latent infections in symptomless bulbs. Efficacy of chemicals and BCAs was assessed for control of *F. proliferatum* and garlic dry rot, from early crop stages and through prolonged storage periods.

The tested active ingredients were selected according to published *in vitro* efficacy data and preliminary

Table 4. Mean garlic disease severity indices (BSI), percentages of symptomatic cloves/bulbs and mean incidence of fungi isolated garlic cloves, for different field trial treatments, two growing seasons (Year 1, 2017–2018, and Year 2, 2018–2019), and field crop and postharvest sampling time points^a. †.

Parameter	BSI (%)	% Symptomatic cloves/ bulb ^d	Incidence, %	
			<i>F. oxysporum</i>	<i>F. proliferatum</i>
<i>A Treatment^b</i>	**	**	n.s.	*
Control	16.6 c ^c	45.8 c	7.0	31.7 b
<i>Trichoderma + B. subtilis</i>	15.3 bc	36.8 ab	7.7	24.0 ab
<i>T. harzianum + T. gamsii</i>	14.1 bc	37.2 abc	5.3	28.4 ab
<i>S. griseoviridis</i>	11.1 a	40.2 bc	6.3	29.7 b
<i>B. subtilis</i>	11.4 a	35.4 ab	2.8	20.9 ab
Prochloraz + Propiconazole	13.5 b	37.5 abc	4.7	27.2 ab
Tebuconazole	9.3 a	30.5 a	4.7	16.6 a
<i>B Year</i>	**	n.s.	n.s.	n.s.
Year 1	6.0 a	38.8	6.7	27.1
Year 2	20.1 b	36.4	4.3	23.8
<i>C Sampling time point</i>	**	n.s.	n.s.	**
3 months	10.1 a			
6 months	11.8 b			
9 months	13.3 c	36.3	5.9	19.7 a
15 days	17.0 d	38.9	5.1	31.3 b
<i>D Category</i>		**	*	n.s.
Bulb diameter 36–46 mm		34.0 a	3.8 a	23.9
Bulb diameter ≥46 mm		41.2 b	7.2 b	27.1
Interactions				
A×B	**	*	**	*
A×C	n.s.	n.s.	*	*
A×D	n.s.	n.s.	n.s.	n.s.
B×C	**	n.s.	*	*
B×D	n.s.	**	**	n.s.
C×D	n.s.	n.s.	n.s.	n.s.
A×B×C	n.s.	n.s.	n.s.	n.s.
A×B×D	n.s.	n.s.	n.s.	n.s.
A×C×D	n.s.	n.s.	n.s.	n.s.
B×C×D	n.s.	n.s.	n.s.	n.s.
A×B×C×D	n.s.	n.s.	n.s.	n.s.

^aGarlic seed cloves were subjected to seven treatments. Experimental factor statistical significance (*, $P < 0.05$; **, $P < 0.01$; n.s., not significant $P > 0.05$) is indicated.

^bTreatment (A), year (B), sampling time point (C), and category (D) were factors in the analysis of variance of data.

^d % of symptomatic cloves/bulb = (No. of cloves showing brown spots / total No. of cloves in bulb) × 100.

^cDifferent lowercase letters indicate significant differences with Tukey's test ($P < 0.01$).

experiments conducted *in vitro* and in pots (Mondani *et al.*, 2021c). Triazoles are fungicides that block the demethylation step of sterol biosynthesis in cell membranes (Osborne and Scott, 2018), and these compounds inhibit *Fusarium* growth, with 50% lethal doses (LD₅₀s) ranging from 0.24 to 6.5 mg L⁻¹ and LD₉₀ of 10 mg L⁻¹/l (Müllernborn *et al.*, 2008; Marín *et al.*, 2013). BCAs were selected based on their modes of action, which likely reflect dif-

ferent mechanisms of interaction with *Fusarium* under field conditions. Bacterial BCAs produce compounds that inhibit plant pathogen growth and stimulate systemic immune responses in crops (Stein, 2005). *Bacillus subtilis* was shown to suppress *F. oxysporum* and *F. graminearum* mycelium growth and sporulation (Kim and Knudsen, 2013; Zhao *et al.*, 2014). *Streptomyces griseoviridis* can produce antibiotics and hydrolytic

Table 5. Mean water activity (a_w) in garlic cloves, for different field trial treatments, two growing seasons (Year 1, 2017–2018, and Year 2, 2018–2019), and field crop and postharvest sampling time points^a.

Parameter	a_w
A Treatment ^b	n.s.
Control	0.939
<i>Trichoderma</i> + <i>B. subtilis</i>	0.941
<i>T. harzianum</i> + <i>T. gamsii</i>	0.923
<i>S. griseoviridis</i>	0.942
<i>B. subtilis</i>	0.936
Prochloraz + propiconazole	0.934
Tebuconazole	0.945
B Year	**
Year 1	0.927 a ^c
Year 2	0.947 b
C Sampling time	**
BBCH49	0.976 d
Natural drying	0.874 a
9 months	0.940 b
15 days	0.959 c
Interactions	
A×B	n.s.
A×C	*
B×C	**
A×B×C	n.s.

^aGarlic seed cloves were subjected to seven treatments. Experimental factor statistical significance (*, $P < 0.05$; **, $P < 0.01$; n.s., not significant $P > 0.05$) is indicated.

^bTreatment (A), year (B), and sampling time point (C) were factors in the analysis of variance of data.

^cDifferent lowercase letters indicate significant differences with Tukey's test ($P < 0.01$).

enzymes that affect cell membranes in *F. oxysporum* and *F. proliferatum* isolated from cucurbit plants (Zhao *et al.*, 2013). In contrast, fungus BCAs such as *Trichoderma* spp. compete with pathogenic microbes for space and nutrients (Kubicek *et al.*, 2001; Kavitha and Nelson, 2013). Some *Trichoderma* spp. isolates were reported to rapidly colonize substrates and parasitize *F. oxysporum* mycelia within 48 h after inoculation (Sharma, 2011).

The present study assessed the efficacy of fungicides based on visible symptoms and incidence of *Fusarium* on garlic plant basal plates and bulbs. The effects of seed clove treatments on disease severity in basal bulb plates and plant roots (as measured by BPSI) confirmed previous results from trials conducted with plants grown in pots under conditions (sowing period and environment) comparable to open field cultivation (Mondani *et al.*, 2021c). Tebuconazole was the most effective active ingre-

redient (mean BPSI = 32.1%) and gave similar disease levels as prochloraz + propiconazole (35.0%) and bacterial BCAs (35.4%).

The treatments did not affect incidence of *F. oxysporum* or *F. proliferatum* on bulb basal plates, despite reducing disease severity. Additional analysis is required to determine the sites of *Fusarium* spp. colonization of the cloves, given that *F. proliferatum* has been detected in 25 to 50% of apparently healthy garlic bulbs (Dugan *et al.*, 2019). In preliminary studies in our laboratory, *F. proliferatum* was isolated from garlic cloves, which may explain the low efficacy of the seed clove treatments.

Differences in BPSI were observed between the 2 years of this study, with mean disease severity increasing from 34.5% in year 1 (2017–2018) to 39.6% in year 2 (2018–2019). Weather conditions differed over the 2 years (Mondani, *et al.*, 2021b): more rainfall and lower temperatures were recorded in the spring (mid-April until end of May) of year 2 than in year 1, when bulbs were between BBCH15 and BBCH45 growth stages. In year 1, spring rainfall was 258.3 mm and mean temperature was 14.6°C, while in year 2 spring rainfall was 82.8 mm and mean temperature was 18.7°C). This difference may have increased the impact of *Fusarium* on garlic bulbs in year 2. However, the active ingredient × year interaction effect was not significant, so irrespective of disease severity, the seed clove treatment reduced BPSI, but only by 9% to 27% relative to the untreated controls.

Tebuconazole and the BCAs *B. subtilis* and *S. griseoviridis* controlled dry rot in the garlic bulbs. A maximum of one or two cloves per bulb were emptied by the fungus (14% less BSI compared to the controls). After bulb peeling, tebuconazole was confirmed as the most effective active ingredient, with fewer visible symptoms on the cloves (35% lower) relative to the controls. *Bacillus subtilis* as single active ingredient, *Trichoderma* + *B. subtilis*, and *T. harzianum* + *T. gamsii*, so as the chemicals prochloraz + propiconazole, were less effective for reducing visible symptoms, although these treatments did not differ significantly from tebuconazole. The treatments had no effects on the incidence of *F. oxysporum* and very limited effects on *F. proliferatum* in cloves, with only tebuconazole (mean BSI = 16.6%) giving a statistically significant reduction in the abundance of these fungi compared to the controls (BSI = 31.7%). Other fungal genera (*Rhizopus*, *Aspergillus*, and *Alternaria*) were isolated sporadically and their occurrence was unaffected by the treatments applied to seed cloves.

The seed clove treatments did not affect water activity (a_w) in garlic cloves. Values above 0.90 (the threshold for *Fusarium* growth and metabolic activity; Marín *et al.*, 1996) were recorded during cold storage, and

these increased after the bulbs were removed from cold chambers (mean $a_w = 0.959$), and were then stored at a temperature ($\approx 25^\circ\text{C}$) that is favorable for *F. proliferatum* activity, as found in markets or after sale consumer homes. The increase in a_w is consistent with previous results (Mondani *et al.*, 2021a) and was expected because of the high relative humidity ($\approx 90\%$) set inside cold chambers to prevent garlic dehydration.

Application of fungicides or BCAs as sprayed seed treatments generally yielded positive results for controlling postharvest rot in garlic bulbs, and reduced visible symptoms, from the field stage through prolonged storage. This is unlike fungicides sprayed on garlic leaves, which was shown to be ineffectual (Gálvez Patón *et al.*, 2017). Tebuconazole was the most effective seed clove treatment, giving a 44% reduction in BSI, 33% reduction in visible rot symptoms, and 48% reduction of incidence of *F. proliferatum*. BCAs, and particularly *B. subtilis*, also performed well, giving 31% reduction of BSI, 23% reduction of visible symptoms, and 34% reduction of incidence of *F. proliferatum*. Therefore, promising BCAs are available for use in organic farming.

The impacts of these treatments on bulb dry rot cannot be considered as satisfactory, because 30-35% of bulbs were symptomatic, with incidence of *F. proliferatum* 16% recorded from the most effective treatment. The treatments had limited impacts on the incidence of both *F. proliferatum* and *F. oxysporum*, especially once the bulbs were transferred to room temperature. The $a_w > 0.94$ during cold storage, which increased in the last step of the garlic production chain (room temperature storage), probably promoted growth of the pathogenic fungi. One option to enhance garlic clove protection during germination and seedling development is to apply treatments as film coatings instead of sprays (Rocha *et al.*, 2019; Afzal *et al.*, 2020). On the other hand, the isolation of *F. proliferatum* from cloves is a further concern. Future studies should also investigate whether treatment of cloves can minimize the presence of fungi while preserving clove germinability. Persistence of treatments should also be determined, especially in the case of BCAs that can potentially survive cold storage and be reactivated once treated material is moved to room temperature.

This study contributes to the management of garlic dry rot as it has demonstrated positive effects achieved by seed clove treatments, although they can probably be improved. The study has also identified weak points in the garlic production chain as topics for future research. Further effort is required to provide stakeholders with safe and highly effective solutions for preventing and managing garlic dry rot.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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