Effect of Antagonistic *Fusarium* spp. and of Different Commercial Biofungicide Formulations on Fusarium Wilt of Lettuce

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Five experimental trials were carried out to test different biological control agents against Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f.sp. *lactucae.* In the presence of a very high disease incidence, the best results in terms of disease control as well as increased growth response were shown by *Trichoderma harzianum* T 22 (RootShield), which, at 3 g l^{-1} of substrate, provided very consistent results. *F. oxysporum* IF 23 gave good disease control but in two out of five trials reduced the biomass produced. Less consistent, but still significant results were provided by *F. oxysporum* MSA 35, at 3 g l^{-1} of substrate, and by *Trichoderma viride* TV 1. The two *F. oxysporum* agents Fo 251/2 and Fo 47 and the mixture of *T. harzianum* + *T. viride* (Remedier) partially reduced disease incidence but were less effective than the above mentioned. Less interesting results were offered by *Streptomyces griseoviridis* (Mycostop). The results obtained show that biological control can play a role in the management of Fusarium wilt of lettuce.
KEY WORDS: Antagonistic *Fusarium oxysporum*; biocontrol agents; *Fusarium oxysporum* f.sp. *lactucae*; *Lactuca sativa*; *Trichoderma* spp.

INTRODUCTION

Fusarium wilt of lettuce, recently observed in Italy (5), has emerged as a major production problem in Lombardy (northwestern Italy), where every year repeated cropping of lettuce is carried out in the same soil. Symptoms were first observed on several varieties at thinning, when seedlings (30 days old) appeared wilted. Affected plants were stunted and developed yellowed leaves and brown or black streaks in the vascular system. The outbreaks of Fusarium wilt in northern Italy occurred on spring and summer leaf lettuce, particularly on varieties of the Batavia type, grown for processed lettuce (6).

A similar disease was described in Japan in 1967 (15), in the United States in 1993 (14) and in Taiwan in 1998 (13). The Italian isolates of *Fusarium oxysporum* f.sp. *lactucae (Fol)* have been identified by pathogenicity tests and molecular tools as belonging to race 1, similar to the USA isolates (6,16).

The causal agent of Fusarium wilt, *Fol,* is both seedborne and seed-transmitted and seeds may be important in disseminating the pathogen (7). The pathogen is spreading in several lettuce-producing areas in Italy. Disease management is complicated by the limited availability of registered fungicides and by the intensive cropping system adopted by local growers. Lettuce Fusarium wilt management commonly relies on the integration of various control measures, such as soil disinfestation, use of resistant cultivars, and seed

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dressing (10). However, the fact that *Fol* is seed transmitted (7) makes soil disinfestation only partially effective. Moreover, only some of the varieties of lettuce with resistance to Fusarium wilt are of commercial interest, while most of the varieties belonging to the Batavia type are susceptible to Fusarium wilt (6).

The limited efficacy of chemical control measures and the reduced availability of resistant cultivars of commercial interest have prompted the evaluation of biological control strategies for Fusarium wilt of lettuce. Non-chemical methods for wilt control are required particularly in the case of farms practicing organic agriculture, a sector steadily growing in Italy, due to increasing interest by consumers.

The objective of this work was to test the effectiveness of selected biocontrol agents chosen among strains of saprophytic *F. oxysporum* (previously shown to have potential as biocontrol agents for the control of Fusarium wilt diseases; 1,4,8), as well as of locally available commercial formulations of *F. oxysporum*, *Trichoderma* spp. and *Streptomyces griseoviridis* that indicate Fusarium wilts among target pathogens (3). The results obtained on lettuce under glasshouse conditions in five experimental trials carried out during the years 2006 and 2007 are reported.

MATERIALS AND METHODS

Fungal strains A highly virulent strain of *Fol*, isolated in 2002 from infected lettuce plants collected at Bergamo (northwestern Italy) and code-named FOL 10, was used throughout the experiments. Eight biocontrol agents were used throughout the experiments (Table 1): four biocontrol strains of *F. oxysporum,* three of them being commercial formulations (one – Microsan – sold as soil amendment) and one – Fo MSA 35 – grown in the laboratories of AGROINNOVA and applied as chlamydospores dispersed in talc. In addition, three *Trichoderma*-based formulations and one *S. griseoviridis*-based product were used (Table 1).

Delivery system Isolate FOL 10 of *Fol* for greenhouse bioassays was grown on liquid casein hydrolysate and maintained on a rotary shaker at 100 rpm for 14 days at 28°C. The biomass produced after centrifugation (8,000 rpm at 10°C) was prepared as dry talc powder (biomass : talc, 2:1 w/w). After 20 days at 25°C, the number of chlamydospores per gram of talc was evaluated on Komada selective medium. FOL 10 was added to the substrate as chlamydospores dispersed in talc powder at a final concentration of 5×10^4 CFU ml^{-1} of soil. Soil infestation with the pathogen was carried out at transplant. Non-inoculated substrate served as control.

The biocontrol agents were applied at the doses reported in Tables 3–7, 6 days before transplant. In the case of the commercial formulations, the doses applied corresponded to those recommended by their distributors, whereas for Fo MSA 35 doses already tested against other Fusarium wilts were applied (1). In the case of all commercial formulations, the CFU value was checked before use by serial plating on potato dextrose agar containing 25 mg l^{-1} of streptomycin sulphate.

Lettuce cultivars 'Lattuga verde', 'Foglia di quercia rossa' and 'Batavia gentilina' (Table 2), of known susceptibility to Fusarium wilt (9), were used in the various trials. Seeds were sown in a steamed (30 min at 70°C) soil mixture (peat: perlite: sand, 60:20:20 vol/vol) in plug trays and maintained at 25°C. Fifteen one-day-old seedlings were transplanted into a steamed substrate (peat: perlite: clay, 60:20:20 vol/vol) in 15-*l* containers. Each container was filled with 10 *l* of substrate, transplanted with ten lettuce

TABLE 1. Details of the biocontrol agents used in the trials

Product	Producer	Microorganism	CFU g^{-1} dry wt	Suggested dose	Applied dose (g l^{-1})
Fusarium oxysporum Biofox	Isagro Ricerca srl, Novara, Italy	*F. oxysporum* Fo 251/2	1×10^7	20 g m^{-2}	2–3
RootShield granules	Bio Works Inc., Fairport, NY, USA	*Trichoderma harzianum* strain T22	1×10^7	3-4 g l^{-1}	1–6
Mycostop	Verdera Oy, Espoo, Finland	*Streptomyces griseoviridis* strain K61	1×10^8	0.25-0.5 l m^{-2}	0.005–0.1
Microsan	Natural Plant Protection, Nogueres, France	*F. oxysporum* Fo 47	1×10^7	Soil amendment; no label	2–4
Remedier	Isagro Ricerca srl, Milano, Italy	*T. harzianum* (strain ICC012) + *Trichoderma viride* (strain ICC080)	5×10^7 + 5×10^7	0.25 g l^{-1}	0.25–2
Trichoderma TV1	Agribiotec srl, Cavezzo, Italy	*T. viride*	1×10^7	0.5-1 g l^{-1}	1–3
F. oxysporum MSA 35	AGROINNOVA, Univ. of Torino, Italy	*F. oxysporum* MSA 35	1×10^8		2–4
F. oxysporum IF 23	Agribiotec	*F. oxysporum*	1×10^8	0.25 g l^{-1}	1–4

TABLE 2. Details of the trials conducted (in four replicates)

Trial no.	Cultivar[z]	Inoculation with *Fusarium lactucae* (2006)	Treatment (2006)	Trial start, 2006 (transplanting)	Trial end	Duration (days)
1	Lattuga	21/06	16/06	21/06	21/07/06	30
2	Foglia	16/08	10/08	16/08	20/09/06	40
3	Foglia	16/08	10/08	16/08	20/09/06	40
4	Batavia	5/10	29/09	5/10	20/11/06	50
5	Batavia	1/12	25/11	1/12	23/01/07	54

[z]Lattuga = Lattuga verde (Franchi); Foglia = Foglia di quercia rossa (Nunhems); Batavia = Batavia gentilina (Four).

TABLE 3. Effectiveness of different biocontrol agents against Fusarium wilt of lettuce (Trial 1)

Treatment	Microorganism	Dose (g l^{-1})	Disease index[z] (0–100)	Biomass[z] (g)
Non-inoculated control	–	–	9.0 a	136.0 ab
Inoculated control	–	–	72.9 cd	55.5 abc
Fo 251/2	*Fusarium oxysporum* 251/2	2	35.4 abc	147.5 a
Fo 251/2	*F. oxysporum* 251/2	3	42.4 abc	153.5 a
RootShield	*Trichoderma harzianum*	3	24.3 ab	178.3 a
RootShield	*T. harzianum*	4	59.0 bcd	83.8 abc
RootShield	*T. harzianum*	6	67.4 cd	66.3 abc
Mycostop k61	*Streptomyces griseoviridis*	0.005	61.8 bcd	71.3 abc
Mycostop k61	*S. griseoviridis*	0.01	54.9 bcd	98.6 abc
Fo 47	*F. oxysporum* 47	2	46.5 abc	124.8 abc
Fo 47	*F. oxysporum* 47	3	63.2 bcd	84.3 abc
Fo MSA 35	*F. oxysporum* MSA 35	2	58.3 bcd	71.0 abc
Fo MSA 35	*F. oxysporum* MSA 35	3	59.0 bcd	77.0 abc
Fo IF 23	*F. oxysporum* 23	2	86.8 d	12.9 c
Fo IF 23	*F. oxysporum* 23	3	30.0 abc	106.2 abc

[z]Within columns, values followed by a common letter do not differ significantly at *P*=0.05, based on Tukey's test.

plants and considered as one replicate; there were four replicates of each of the five trials (for details, see Table 2). A completely randomized block design was used. Plants were maintained in a glasshouse at temperatures ranging from 25 to 32°C and watered daily. Relative humidity varied between 50% and 70%.

Data collection Typical symptoms of Fusarium wilt started to be visible 8–15 days after transplant. Plants were checked weekly for disease development, wilted plants were counted at regular intervals and completely wilted plants were removed. The final disease rating was done 30–54 days after inoculation. The disease index used throughout the experiments covered a range of 0 to 100 (0= healthy plant; 25 = initial symptoms of leaf chlorosis; 50 = severe leaf chlorosis and initial symptoms of wilting during the hottest hours of the day; 75 = severe wilting symptoms and initial symptoms of leaf chlorosis; 100 = plant totally wilted, leaves completely necrotic). Biomass, expressed as fresh weight per container of lettuce produced at the end of the trial, was also evaluated.

Statistical analysis The data from all the experiments were analyzed by Tukey's significant difference multiple comparison test (*P*=0.05; 18). Disease index data were transformed to the respective arcsin values prior to statistical analysis.

RESULTS

Disease incidence (DI) was very high in four out of five trials (Tables 3,4,5,7). Only in trial 4 (Table 6) did the inoculated control have a DI of only 19.1 (out of a maximum of 100). Non-inoculated control plants had a DI ranging from 0 (trials 4 and 5) to 21.3 (trial 3), confirming the possibility of introduction of the pathogen *via* infected seeds.

Although a certain degree of variability was observed among trials, most of the biological control agents tested resulted in a reduction in the severity of Fusarium wilt of lettuce. The most consistent results were offered by *T. harzianum* T 22 (RootShield), applied at 3 (or 4) g l^{-1} of substrate, followed by the *F. oxysporum* Fo IF 23, applied at

TABLE 4. Effectiveness of different biocontrol agents against Fusarium wilt of lettuce (Trial 2)

Treatment	Microorganism	Dose (g l^{-1})	Disease index[z] (0–100)	Biomass[z] (g)
Non-inoculated control	–	–	19.3 ab	169.7 abcd
Inoculated control	–	–	57.5 ab	92.4 cd
Fo 251/2	*Fusarium oxysporum* 251/2	2	41.9 ab	130.3 abcd
Fo 251/2	*F. oxysporum* 251/2	3	70.6 b	69.7 cd
RootShield	*Trichoderma harzianum*	1	63.8 ab	67.5 cd
RootShield	*T. harzianum*	2	53.8 ab	231.8 ab
RootShield	*T. harzianum*	3	29.4 ab	181.6 abc
RootShield	*T. harzianum*	4	28.1 ab	168.2 abcd
Mycostop k61	*Streptomyces griseoviridis*	0.005	65.0 ab	75.4 cd
Mycostop k61	*S. griseoviridis*	0.01	68.1 b	83.7 cd
Fo 47	*F. oxysporum* 47	2	63.8 ab	50.3 d
Fo 47	*F. oxysporum* 47	3	63.1 ab	82.1 cd
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.25	35.6 ab	171.1 abcd
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.5	35.6 ab	234.5 ab
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080080)	1	27.5 ab	176.3 abcd
TV 1	*T. viride*	1	59.4 ab	108.8 bcd
TV 1	*T. viride*	2	61.9 ab	68.5 cd
TV 1	*T. viride*	3	32.5 ab	240.4 a
Fo MSA 35	*F. oxysporum* MSA 35	2	48.1 ab	220.8 ab
Fo MSA 35	*F. oxysporum* MSA 35	3	49.4 ab	191.5 abc
Fo MSA 35	*F. oxysporum* MSA 35	4	47.5 ab	163.4 abcd
Fo IF 23	*F. oxysporum* 23	2	15.6 a	158.3 abcd
Fo IF 23	*F. oxysporum* 23	3	20.0 ab	164.7 abcd
Fo IF 23	*F. oxysporum* 23	4	50.0 ab	84.0 cd

[z]Within columns, values followed by a common letter do not differ significantly at *P*=0.05, based on Tukey's test.

2–3 g l^{-1} of substrate and by the mixture of *T. harzianum* + *T. viride* (Remedier), applied at 0.25–1 g l^{-1}. Less consistent, but still interesting results were provided by *F. oxysporum* MSA 35 at 3 g l^{-1} of substrate and by *Trichoderma viride* TV 1. The two *F. oxysporum* Fo 251/2 and Fo 47 did partially reduce DI, but were less consistent. *S. griseoviridis* (Mycostop) partially reduced Fusarium wilt at doses of 0.05–0.1 g l^{-1}.

The treatment with *T. harzianum* T 22 (RootShield) was also very effective in terms of biomass production (Tables 3–7), followed by Fo MSA 35. *F. oxysporum* IF 23 sometimes caused a reduction in the biomass (Tables 3 and 6).

Better activity of the most efficient biocontrol strains was generally observed in trials 4 and 5 (Tables 6 and 7), characterized by a lower presence of *Fusarium*-infected seeds in comparison with trials 1-3 (Tables 3-5), as indicated by the higher DI observed in the non-inoculated controls.

DISCUSSION

The results obtained show that biological control can be successfully used in the management of Fusarium wilt of lettuce. The lettuce crop, particularly in the case of varieties grown for ready-to-use processed salads, plays a large economic role in different areas, particularly in the Lombardy region, where highly specialized farms have developed for this type of production.

TABLE 5. Effectiveness of different biocontrol agents against Fusarium wilt of lettuce (Trial 3)

Treatment	Microorganism	Dose (g l^{-1})	Disease index[z] (0–100)	Biomass[z] (g)
Non-inoculated control	–	–	21.3 abcd	168.5 ab
Inoculated control	–	–	45.0 de	109.4 b
Fo 251/2	*Fusarium oxysporum* 251/2	2	38.8 bcde	173.7 ab
Fo 251/2	*F. oxysporum* 251/2	3	31.3 abcd	181.0 ab
RootShield	*Trichoderma harzianum*	1	46.3 de	141.1 ab
RootShield	*T. harzianum*	2	39.4 bcde	202.5 ab
RootShield	*T. harzianum*	3	15.7 abc	246.5 a
RootShield	*T. harzianum*	4	22.5 abcd	197.9 ab
Mycostop k61	*Streptomyces griseoviridis*	0.005	26.3 abcd	171.9 ab
Mycostop k61	*S. griseoviridis*	0.01	17.3 abc	181.4 ab
Fo 47	*F. oxysporum* 47	2	41.9 cde	147.3 ab
Fo 47	*F. oxysporum* 47	3	48.8 e	138.0 ab
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.25	7.5 a	208.3 ab
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.5	26.3 abcde	211.0 ab
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080080)	1	21.9 abcde	200.0 ab
TV 1	*T. viride*	1	24.4 abcde	175.3 ab
TV 1	*T. viride*	2	32.8 abcde	132.5 ab
TV 1	*T. viride*	3	16.9 abc	166.0 ab
Fo MSA 35	*F. oxysporum* MSA 35	2	23.8 abcde	225.8 ab
Fo MSA 35	*F. oxysporum* MSA 35	3	31.3 abcde	133.3 ab
Fo MSA 35	*F. oxysporum* MSA 35	4	30.0 abcde	161.3 ab
Fo IF 23	*F. oxysporum* 23	2	23.3 abcde	185.0 ab
Fo IF 23	*F. oxysporum* 23	3	12.5 ab	172.4 ab
Fo IF 23	*F. oxysporum* 23	4	16.3 abc	150.0 a,b

[z]Within columns, values followed by a common letter do not differ significantly at *P*=0.05, based on Tukey's test.

The limited availability of chemical control measures, the only partial control offered by seed dressing as well as by soil disinfestation, and the still limited availability of commercially interesting resistant cultivars of lettuce – make some of the tested biological control agents an adequate and reliable control measure, to be combined with other control methods. In particular, *T. harzianum* T 22 and some of the strains of *F. oxysporum* tested look promising. The findings with *T. harzianum* T 22 confirmed its already known good activity in terms of biological control as well as effect on plant productivity. Research data accumulated over the past few years have produced an understanding of the way by which this strain interacts not only with the target pathogens, but especially with plant and soil components (19). The capability of *T. harzianum* T 22 as a growth promoter is well documented for many crops (11). This strain induces increased root formation in treated plants, and improvements in root development frequently have been associated with increases in yield and biomass (12). Increased growth response was also shown by Fo MSA 35. A proper formulation of this strain could improve its performance. The reduced efficacy of IF 23 when applied at the highest dosage, as well as the biomass reduction observed in some trials, can be explained, at least partially, by its mechanisms of action, mostly based on induction of host resistance. It is well known that biocontrol agents can evince some side effects (2).

TABLE 6. Effectiveness of different biocontrol agents against Fusarium wilt of lettuce (Trial 4)

Treatment	Microorganism	Dose (g l^{-1})	Disease index[z] (0–100)	Biomass[z] (g)
Non-inoculated control	–	–	0 a	128.5 bcd
Inoculated control	–	–	19.1 e	35.0 de
Fo 251/2	*Fusarium oxysporum* 251/2	2	10.1 cd	173.0 bc
Fo 251/2	*F. oxysporum* 251/2	3	9.9 cd	80.9 cde
RootShield	*Trichoderma harzianum*	3	6.1 abcd	255.9 ab
RootShield	*T. harzianum*	4	7.3 abcd	153.1 bcd
Mycostop k61	*Streptomyces griseoviridis*	0.05	8.5 bcd	86.7 cde
Mycostop k61	*S. griseoviridis*	0.1	10.2 d	93.3 cde
Fo 47	*Fusarium oxysporum* 47	2	9.5 cd	102.5 cde
Fo 47	*F. oxysporum* 47	3	12.7 de	112.8 cde
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.25	8.0 abcd	319.3 a
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	0.5	10.9 de	174.3 bc
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080080)	1	6.9 abcd	118.3 bcd
TV 1	*T. viride*	1	7.5 abcd	82.8 cde
TV 1	*T. viride*	2	4.7 abcd	39.8 cde
TV 1	*T. viride*	3	0 a	9.6 e
Fo MSA 35	*F. oxysporum* MSA 35	2	7.1 abcd	80.3 cde
Fo MSA 35	*F. oxysporum* MSA 35	3	5.4 abcd	60.2 cde
Fo MSA 35	*F. oxysporum* MSA 35	4	5.9 abcd	56.5 cde
Fo IF 23	*F. oxysporum* 23	2	5.6 abcd	83.4 cde
Fo IF 23	*F. oxysporum* 23	3	1.9 abc	39.3 cde
Fo IF 23	*F. oxysporum* 23	4	0.3 ab	42.8 cde

[z]Within columns, values followed by a common letter do not differ significantly at *P*=0.05, based on Tukey's test.

The use of lettuce seeds contaminated by *F. lactucae* might affect the efficacy of biological control. In our trials, biocontrol was highest in the two trials (4 and 5) where disease incidence in non-inoculated controls was zero, thus indicating that the seeds used for the trials were not originally infected by *F. lactucae*. The efficacy of biocontrol was lower in the presence of seeds already infected by *F. lactucae*. This could have practical implications and more limited efficacy of biocontrol could be expected in the presence of heavily infected seeds.

The most effective biocontrol agents could indeed represent one more tool for growers where lettuce is very intensively grown, being easily combinable with agronomic practices (*e.g.* use of partially resistant cultivars) and other control measures (*e.g.* soil disinfestation). There are many possibilities of combination: the aim should be to obtain a synergistic, rather than additive effect. For this reason, it is very important to have a complete understanding of the mode of action and mechanism of biocontrol (17). Another important feature of biological control is the possible induction of increased growth response, an interesting phenomenon in the case of high value vegetable crops. Moreover, biological control is particularly interesting for those farms which practice organic farming production: in this case, the use of resistant cultivars and the application of biocontrol agents represent the main tools for disease management.

TABLE 7. Effectiveness of different biocontrol agents against Fusarium wilt of lettuce (Trial 5)

Treatment	Microorganism	Dose (g l^{-1})	Disease index[z] (0–100)	Biomass[z] (g)
Non-inoculated control	–	–	0 a	245.0 abcde
Inoculated control	–	–	60.6 e	241.9 abcde
Fo 251/2	*Fusarium oxysporum* 251/2	2	21.9 abcd	324.3 a
Fo 251/2	*F. oxysporum* 251/2	3	13.8 abcd	265.6 abcd
RootShield	*Trichoderma harzianum*	3	10.0 abcd	246.8 abcde
RootShield	*T. harzianum*	4	16.3 abcd	220.1 abcde
Mycostop k61	*Streptomyces griseoviridis*	0.05	35.0 bcde	232.4 abcde
Mycostop k61	*S. griseoviridis*	0.1	32.5 bcde	265.1 abcd
Fo 47	*Fusarium oxysporum* 47	3	11.3 abcd	293.8 abc
Fo 47	*F. oxysporum* 47	4	38.8 cde	263.7 abcd
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	1	39.4 cde	147.1 e
Remedier	*T. harzianum* (strain ICC012) + *T. viride* (strain ICC080)	2	40.0 de	190.5 cde
TV 1	*T. viride*	1	18.8 abcd	181.2 de
TV 1	*T. viride*	2	25.0 ab	208.8 bcde
TV 1	*T. viride*	3	55.0 e	148.0 e
Fo MSA 35	*F. oxysporum* MSA 35	2	8.8 abc	312.1 ab
Fo MSA 35	*F. oxysporum* MSA 35	3	17.5 abcd	306.6 ab
Fo MSA 35	*F. oxysporum* MSA 35	4	21.9 abcd	293.3 abc
Fo IF 23	*F. oxysporum* 23	2	15.0 abcd	272.2 abcd
Fo IF 23	*F. oxysporum* 23	3	18.1 abcd	189.3 cde
Fo IF 23	*F. oxysporum* 23	4	10.0 abcd	211.1 bcde

[z] Within columns, values followed by a common letter do not differ significantly at P=0.05, based on Tukey's test.

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