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The effects of biological and chemical controls on fungal communities colonising tomato (*Lycopersicon esculentum* Mill.) plants and soil

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ABSTRACT

Tomato plants (Rumba Ożarowska cultivar) grown in the greenhouse of the University of Warmia and Mazury in Olsztyn were treated with the biological control agent Polyversum WP, the growth promoter Biochikol 020 PC, the growth regulator Asahi SL, a mycorrhizal inoculum, and the fungicide Bravo 500 SC. Untreated plants served as the control. After fruit harvest, soil, stem and root samples were collected, and fungi were isolated in the laboratory. The applied biological and chemical control agents effectively reduced the abundance of fungi, including pathogenic species, colonising tomato plants and soil. The fungicide Bravo 500 SC showed the highest efficacy. Amongst the biological control agents, Biochikol 020 PC and the mycorrhizal inoculum were most effective in controlling stem colonisation by pathogens, while Polyversum WP offered the best protection of tomato roots and soil.

Key words: tomato stems and roots, soil, pathogenic fungi, saprotrophic fungi

INTRODUCTION

Tomatoes grown in the soil and hydroponically, in the greenhouse and in the open field, can be infested by bacterial pathogens (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato, Erwinia carotovora* subsp. *carotovora*), fungus-like organisms (*Phytophthora* spp.) and fungi (*Alternaria* spp., *Botrytis cinerea, Cladosporium fulvum, Colletotrichum coccodes, F. oxysporum, F. oxysporum* f. sp. *radicis-lycopersici, Rhizoctonia solani, Sclerotinia* spp., *Verticillium* spp.) (Gullino and Garibaldi 2001, Tomescu and Negru 2003). Preventive measures include crop rotation, sustainable fertilisation, the maintenance of adequate soil organic matter levels, and seed dressing in ground cultivation (Kokalis-Burelle 2002) as well as soil solarisation, temperature control and adequate ventilation in greenhouse growing (Veloukas et al. 2006). Developing new varieties with improved resistance is also an important consideration (Panthee and Chen 2010). Bacterial and fungal bio-control agents and biotechnological control agents have become increasingly popular recently. In the greenhouse, powdery mildew on tomato plants has been effectively treated with Trichodex, Biosept 33 SL, Biochikol 020 PC and Tytanit (Cerkauskas et

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al. 2000, Borkowski and Nowosielski 2001), while Bion 50 WP and Biochikol 020 PC lowered the rates of infection caused by *Clavibacter michiganensis* subsp. *michiganensis* (Macias 2002). Fungi of the genus *Trichoderma* offers protection against grey mould in tomatoes and peppers (Salas-Brenes and Sauchez-Garita 2006). *Gliocladium catenulatum* strain J1446, isolated from the soil, showed good control efficacy of diseases caused by the following pathogens: *Pythium, Phytophthora, Rhizoctonia, Alternaria, Didymella* and *Botrytis* (Niemi and Lahdenpera 2000). The species *T. harzianum* reduced the symptoms of *R. solani* infection in greenhouse tomatoes (Montealegre et al. 2007). Various fungicides (azoxystrobin, cymoxanil, maneb, fenamidone, chlorothalonil) provided effective control of *P. infestans* infection, thus affecting tomato fruit yields (Tofoli et al. 2003). Trifloxystrobin was reported to offer better control of *C. fulvum* infection when applied preventively, not curatively (Veloukas et al. 2006). In a study by Mironova and Marin (2008), tomato plants were sprayed with a mixture of *P. fluorescens* and the fungicide Ridomil Gold MZ 67,8 WG to protect them against *P. infestans* infection, which improved the yield and quality of fruit. Monaco et al. (2009) demonstrated that the use of non-pathogenic isolates of *Fusarium, Epicoccum nigrum* and *T. harzianum* might be a viable alternative to fungicides in the control of *B. cinerea* on tomatoes.

The objective of this study was to determine the effects of biological and chemical control agents on the composition of fungal communities colonising tomato plants and soil.

MATERIAL AND METHODS

Tomatoes of the Rumba Ożarowska cultivar were grown in the greenhouse of the University of Warmia and Mazury in Olsztyn in 2006–2007. Four-week-old tomato seedlings were planted in pots filled with peat substrate and compost soil at a ratio of 1:3. The experiment involved the following treatments: tomato plants sprayed three times with 0.2% Asahi SL (sodium para-nitrophenolones – 0.3%, sodium ortho-nitrophenoles – 0.2%, sodium 5-nitroguaiacolan – 0.1%; 20 ml per plant), plants sprayed three times with 2% Biochikol 020 PC (20% chitosan; 40 ml per plant), plants sprayed three times with 0.1% Polyversum WP (oospores of *Pythium oligandrum*; 40 ml per plant), mycorrhizal inoculum (*Glomus* spp. - Mycoflor®) applied to the roots of seedlings prior to planting, tomato plants sprayed twice with Bravo 500 SC (50% chlorothalonil; according to the recommendations of the Institute of Horticulture in Skierniewice). Every treatment comprised six plants per pot. Pots with unprotected plants served as the control. After fruit harvest, soil, stem and root samples were collected. Following disinfection with 50% ethanol and 1% sodium hypochlorite, 0.5 cm pieces of stems and roots were placed on a PDA medium (five replications). After seven days of incubation, fungal colonies were inoculated onto agar slants for later microscopic identification according to standard keys and monographs. Soil samples collected at a depth of up to 5 cm under each plant were placed in dishes and mixed with a rotary motion. 1 g of the resultant fraction was mixed thoroughly with 149 g fine sand in a flask with a rotary motion for 10 minutes. 300 mm^3 of the mixture was poured with a Martin medium (50°C). Fungal colonies were inoculated onto agar slants for later microscopic identification (Ellis 1971, Skirgiełło et al. 1979, Nelson et al. 1983).

RESULTS AND DISCUSSION

The most abundant fungal community was isolated from soil samples. A higher number of fungal species were identified in soil samples (36) than in stem and root samples (21 and 18, respectively – Tab. 1). The applied chemical and biological control agents effectively reduced all fungi counts, including pathogenic species, in all analysed environments (tomato stems, roots and soil). The fungicide Bravo 500 SC showed the highest efficacy, but no pathogens were found in soil samples collected in the Polyversum WP treatment, either. The growth regulator Asahi SL contributed to the lowest reduction in the abundance of the causal agents of tomato diseases, as compared with the control treatment. According to reference data (Picard et al. 2000), the development of fungal pathogens and fungus-like organisms can be inhibited by Polyversum WP due to its strong mycoparasitic and competitive abilities. In a study by Patkowska (2006), Polyversum WP and Biosept 33 SL protected the seeds and seedlings of different vegetable species against *A. alternata, B. cinerea, F. culmorum, F. oxysporum, F. solani, S. sclerotiorum* and *R. solani*. In the present experiment, the predominant pathogens on tomato stems (from 12.5% in the fungicide treatment to 41% in the control treatment) were *Fusarium* species, which colonised tomato plants in all treatments (Fig. 1a). Differences in the abundance of pathogenic fungi, antagonistic, *Mucorales* and *Penicillium*

Table 1. Number of fungus colonising tomato organs and soil

Fungi	stems						roots						soil					
	C	MI	Po	Bi	A	Br	C	MI	Po	Bi	A	Br	C	MI	Po	Bi	A	Br
Acremonium strictum W. Gams			2	2														
Alternaria alternata (Fr.) Keissler*	4	2	6	2			2		1		6	2	16					
Aureobasidium pullulans (de Bary) Arnaud													1			4	2	1
Aspergillus sp.																	1	
Botrytis cinerea Pers*	2		2		4								4			5		
Chaetomium globosum Hughes										3				2	4	1		
Cladosporium cladosporioides (Fres.) de Vries	2		2			4	2								2			
Colletotrichum coccodes (Wallr.) Hughes*			2	2			25	19	18	24	19	12	1	1		1		
Epicoccum spp.											3							
Fusarium avenaceum (Fr.) Sacc.*	2				2													
Fusarium culmorum (W.G.Sm.) Sacc*	2		2				2		1	2	1		17			1		
Fusarium concolor Corda *	14	2	4	6	4		3	2	2	1	1							
Fusarium nivale (Fries) Cesati *																1	1	
Fusarium oxysporum Schlecht *	2	6	4		4	4	2	1	2	2			2	1		1		
Fusarium poae (Peck.) Wollenweber *				2									1					
Fusarium sulphureum Schlechtendahl *														1				1
Geotrichum candidum Link																		1
Gilmaniella humicola Barron													1					
Gliocladium catenulatum Gilman Abbott **														1		1		
Gliocladium penicillioides Corda**				6	2													
Gliomastix murorum (Corda) Hughes													1	26		8		2
Humicola fuscoatra Traaen																1	3	
Humicola grisea Traaen														2				1
Humicola nigrescens Omvik													2				3	
Mortierella alpina Peyronel	6			2		4	12	5	2	3	7			3	17		1	1
Mortierella isabelina Quademans			6	4			2		4	5	4	11		3				
Mucor circinelloides von Tieghem						3		2	5	2								
Mucor hiemalis Wehmer	6		8	4	6	6	2	2	2	11	8	3						
Paecilomyces niveus Stolk et Samson**																		14
Paecilomyces lilacinus (Thom) Samson**		4		4	2								9		9	11	7	
Paecilomyces variotti Bainier**														22	8		7	13
Papulaspora irregularis Hotson										1								
Penicillium spp.	2		6	4	8	5	2	5	1			2	19	39	34	25	15	13

cont. **Table 1**

Periconia macrospinosa Lefebvre, Johnson																1		1
Phialophora sp.																1	3	
Phoma eupyrena Sacc.															3		1	
Rhizoctonia solani Kühn*														3		2	6	
Rhizopus nigricans Ehrenberg	20	24	4	2		6	9	12	17	4	7	10	2	2	2	4	2	1
Sporormia spp.													3			1		1
Sporotrichum olivaceum Fries**													27	4			15	13
Trichoderma hamatum (Bon) Bain**		2		4										1	1		1	1
Trichoderma harzianum Rifai**			2				1											1
Trichoderma polysporum (Link, Pers.)**									3								1	4
Trichoderma viride Persoon ex S.F.Gray**			2					2		1			1	1	8		1	2
Zygorhynchus spp.							1		2									
Yeast-fungi		6			2				4	4		1	167	129	111	127	95	79
Non sporulating fungi							2						11	1	2	2		
Total	62	46	52	44	40	32	67	50	64	63	56	41	285	242	201	198	165	150

C – control, MI – mycorrhizal inoculum, Po – Polyversum, Bi – Biochikol 020 PC, A – Asahi, Br – Bravo 50 SC, * – pathogenic fungi, ** – antagonistic fungi

spp. between particular control treatments were significant (Tab. 2). The remaining pathogenic species, *A. alternata, B. cinerea* and *C. coccodes,* were less abundant on tomato stems. *C. coccodes* attacks tomato leaves, stems, roots and fruits, and disease severity is determined by weather conditions and the age of plant tissue (Byrne et al. 1997).

Pandey (2006) and Minuto et al. (2006) reported that tomato plants could also be infected by *C. capsici*, the causal agent of root rot. Tomato plants sprayed with Bravo 500 SC were least frequently colonised by pathogens. Azoxystrobin applied with Fixade or alone significantly reduced the symptoms of early blight and late blight on tomato plants (Oliveira et al. 2003). As demonstrated by Shashi-Kamal et al. (2007), spraying *Lycopersicon esculentum* plants with the following fungicides: carbendazim, copper oxychlorite, metalaxyl and mancozeb + tiophanate - methyl, applied alone or in combination, and seed dressing with *T. harzianum* and *P. fluorescens,* lowered the rates of leaf infections caused by *Alternaria, Cercospora* and *Oidium*, and fruit infections caused by *Colletotrichum* and *Alternaria.* Fungi showing antagonistic activity against pathogens were isolated only from the stems of tomato plants treated with bio-control agents. Species of the order *Mucorales* had a high share of saprotrophic fungi (15% - 60%).

The most abundant pathogen colonies were found on tomato roots, in particular in unprotected plants. Differences in the abundance of these fungi between particular control treatments were not significant (Tab. 2). The predominant species was *C. coccodes,* the causal agent of anthracnose, whose share ranged from 30% to 40% depending on the treatment (Fig. 1b). Fungi of the genus *Fusarium* were isolated less frequently, and they did not colonise the roots of fungicide-treated plants. Vatchev and Hadjidimitrov (2006) reported that fungicides applied in combination (Ridomil Gold MC 68 WP + Benomyl 50 WP, Previcur 607 SL + Benomyl 50 WP) provided better protection against the causal agents of tomato root rot (*F. oxysporum* f. sp. *radicis-lycopersici, C. coccodes, P. nicotianae, P. lycopersici, R. solani*). In the present study, *A. alternata* did not colonise the roots of tomato plants treated with mycorrhizal inoculum and plants sprayed with Biochikol 020 PC. According to Ait-Barka et al. (2004), Biochikol induces morphological and structural changes in fungal cells, thus inhibiting the growth of pathogens. Pięta et al. (2006) and Kurzawińska (2007) both highlighted the significant role played by Biochikol

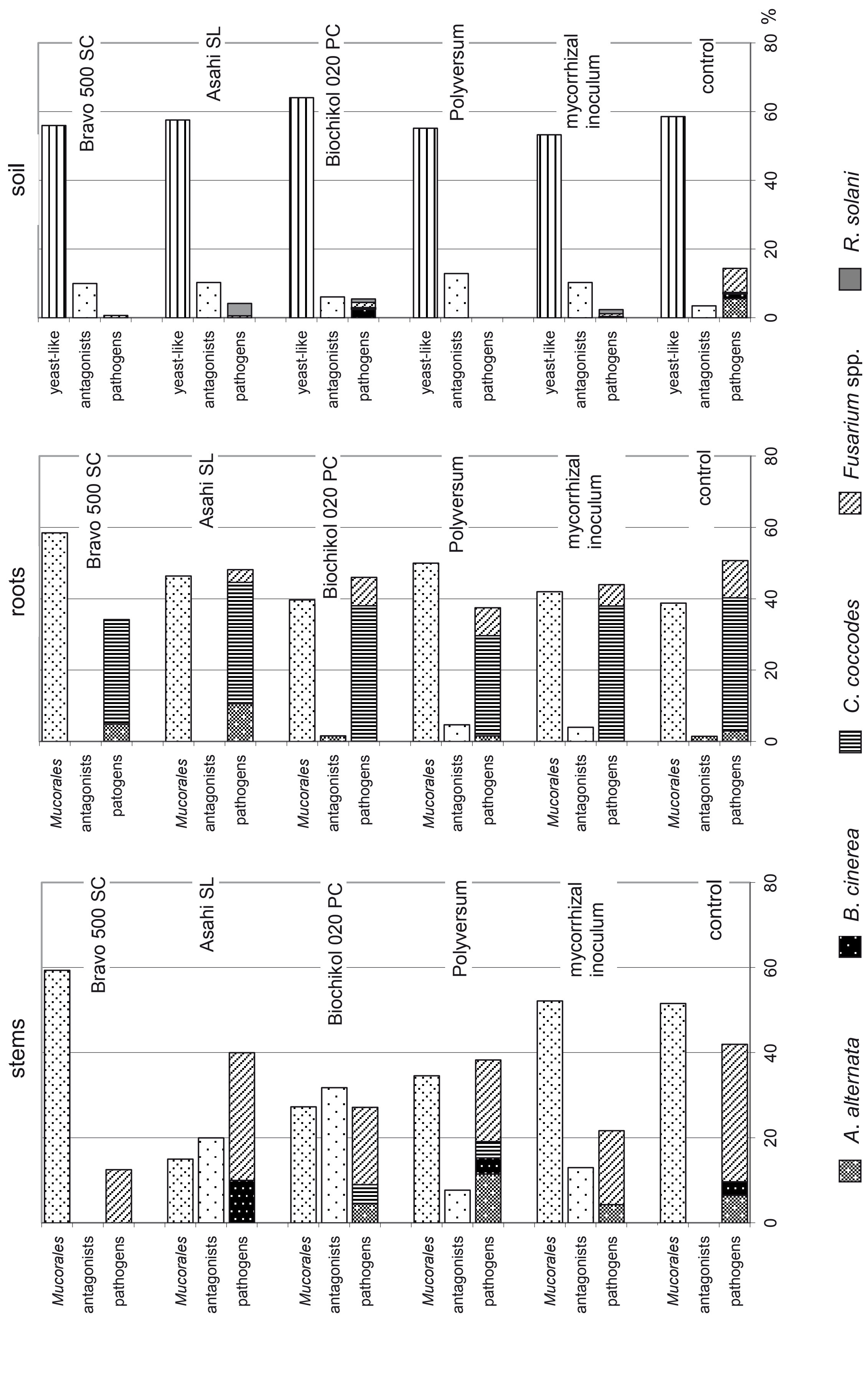

Figure 1. The influence of biological and chemical protection on the percentage of fungi isolated from tomato stems, roots, and soil

Table 2. Most frequently isolated fungi from tomato organs and soil (mean numbers of isolates for years)

Treatments	Stems				Roots				Soil			
	Path	Antag	Muc	Pen	Path	Antag	Muc	Pen	Path	Antag	Muc	Pen
Control	13.0 a*	0.0 c	16.0 a	1.0 bc	17.0 a	0.5 a	13.0 a	1.0 a	20.5 a	5.0 b	1.0 a	9.5 a
MI	5.0 c	3.0 b	12.0 ab	0.0 c	11.0 a	1.0 a	10.5 a	2.5 a	3.0 b	12.5 ab	4.0 a	19.5 a
Polyversum	10.0 ab	2.0 bc	9.0 b	3.0 ab	12.0 a	1.5 a	16.0 a	0.5 a	0.0 b	13.0 ab	9.5 a	17.0 a
Biochikol 020 PC	6.0 abc	7.0 a	6.0 bc	2.0 abc	14.5 a	0.5 a	12.5 a	0.0 a	5.5 b	6.0 ab	2.0 a	12.5 a
Asahi SL	7.0 abc	2.0 bc	3.0 c	4.0 a	13.5 a	0.0 a	13.0 a	0.0 a	3.5 b	8.5 ab	1.5 a	7.5 a
Bravo 500 SC	2.0 c	0.0 c	9.5 b	2.5 abc	7.0 a	0.0 a	12.0 a	1.0 a	0.5 b	17.5 a	8.0 a	6.5 a

Path - pathogens, Antag - antagonists, Muc - *Mucorales*, Pen - *Penicillium* spp., MI – mycorrhizal inoculum
*Means with the same letter do not differ significantly (Duncan test, p = 0.01)

020 PC in disease control in vegetables. In this experiment, species of the order *Mucorales* were isolated most frequently. Antagonistic *Trichoderma* species were much less abundant, as they did not colonise the roots of tomato plants sprayed with the growth regulator Asahi SL and the fungicide Bravo 500 SC.

The soil fungal community was dominated by yeast-like fungi, which accounted for 53.3% and 64% of all isolates in the treatments with mycorrhizal inoculum and Biochikol 020 PC, respectively (Fig. 1c). The desirable antagonistic species of the genera *Paecilomyces* and *Trichoderma* were isolated in greatest abundance from soil samples collected in the Polyversum treatment, where no pathogens were found. Those beneficial fungal species were present at very low numbers in the control treatment. The abundance of pathogenic fungi and *R. solani* was substantially lower in experimental treatments than in the control. Polyversum provided an effective control of the infection caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou et al. 1997). The findings of other researchers (Catxarrera et al. 2002, Barakat and Al-Masri 2009) also show that an increase in the population size of *Trichoderma asperellum* due to the application of organic fertilisers inhibited *F. oxysporum* infection in tomato plants. Mycorrhizal inoculum (*Glomus fasciculata*) decreased the severity of *P. aphidermatum* infection and increased fruit yield (Reddy et al. 2006).

CONCLUSIONS

1. The following fungal pathogens were isolated from tomato stems, roots and soil: *A. alternata*, *B. cinerea*, *C. coccodes*, *Fusarium* species and *R. solani*.
2. The applied biological and chemical control agents effectively reduced the abundance of fungi, including pathogenic species, colonising tomato plants and soil.
3. Unprotected tomato plants were abundantly colonised by pathogens. The fungicide Bravo 500 SC showed the highest efficacy in reducing the population size of pathogenic fungi.
4. The soil communities of yeast-like fungi were most abundant in all treatments. Pathogens were not found in soil samples collected in the Polyversum WP treatment, where antagonistic fungi were predominant.

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WPŁYW OCHRONY BIOLOGICZNEJ I CHEMICZNEJ NA ZBIOROWISKO GRZYBÓW KOLONIZUJĄCYCH ORGANY POMIDORA (*LYCOPERSICON ESCULENTUM* MILL.) I GLEBĘ

S t r e s z c z e n i e : Pomidor odmiany Rumba Ożarowska uprawiano w szklarni Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. W ochronie pomidora stosowano: preparat biologiczny Polyversum WP,

biotechniczny Biochikol 020 PC, regulator wzrostu Asahi SL, szczepionkę mikoryzową, fungicyd Bravo 500 SC (kontrola – rośliny niechronione). Po zbiorze owoców pobierano próby gleby, łodygi oraz korzenie w celu izolacji grzybów w laboratorium.

Zastosowana ochrona biologiczna i chemiczna ograniczały liczebność grzybów, w tym patogenów, zasiedlających organy pomidora i glebę spod uprawy. Największą skuteczność wykazał fungicyd Bravo 500 SC. Spośród elementów biologicznej ochrony Biochikol 020 PC i szczepionka mikoryzowa najbardziej ograniczały liczebność patogenów zasiedlających łodygi, a Polyversum WP – korzenie i glebę.

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