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FUSARIUM ROT OF ONION AND POSSIBLE USE OF BIOPRODUCT*

ABSTRACT: Several species of *Fusarium* are causal agents of onion rot in field and storage. Most prevalent are *F. oxysporum* f. sp. *cepae* and *F. solani*, and recently *F. proliferatum*, a toxigenic species. Most frequently isolated fungi in our field experiments were *F. solani* and *F. proliferatum* with different pathogenicity. Certain differences in antagonistic activity of *Trichoderma asperellum* on different isolates of *F. proliferatum* and *F. solani* have been found in *in vitro* study in dual culture, expressed as a slower inhibition of growth of the former, and faster of the latter pathogen. Antagonistic abilities of species from genus *Trichoderma* (*T. asperellum*) are important, and have already been exploited in formulated biocontrol products in organic and conventional production, in order to prevent soil borne pathogens inducing fusarium wilt and rot. The importance of preventing onion infection by *Fusarium* spp., possible mycotoxin producers, has been underlined.

KEY WORDS: *Allium cepa* (onion), antagonism, bioproduct, *Fusarium* rot, *Trichoderma asperellum*

INTRODUCTION

Several species of *Fusarium* are associated with rot and deterioration of onion in field and storage. Most often, *F. oxysporum* Schlecht. f. sp. *cepae* (H. N. Hans.) W. C. Snyder & H. N. Hansen causes the rot of a basal plate, *F. solani* (Mart.) Appel & Wollenw. in early growth stage, but recently, the attention has been paid to *F. proliferatum* (Matsushima) Nirenberg, as a toxigenic species producing important mycotoxins. Only few reports on *Allium* disease caused by *Fusarium* species, in section Liseola, were published. *F. proliferatum* was reported on onion in Italy (Manneruccі et al., 1987), and in Germany as mycotoxin producing fungus in garlic (Seefelder et al., 2002).

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As a Causal agent of garlic bulb rots, it is also capable of rotting actively growing plants, but also curing garlic bulbs, as it was reported in North America. The same year, du Toit and Inglis (2003) reported this pathogen on 70% of the harvested bulbs of white varieties, but not on yellow and red onion in Columbia Basin of Central Washington and Dugan et al. (2003) reported it as a pathogen of serious impact on garlic and onion in the Pacific North West. Because of documented mycotoxin production, increasing disease pressure and currently unknown etiology, the documentation of instances of *F. proliferatum* attacking bulbs is strongly warranted.

In Serbia, the presence of *F. proliferatum* on garlic and white onion varieties was observed in 2000 (Stanković et al., 2005), and fumonisin B_1, beauvericin and fusaproliferin producing strains were confirmed (Stanković et al., 2007).

Chemical measures in management of soil borne pathogens, especially those infecting onion in late vegetative phase, are of limited or no value. Therefore the presence of root colonizing antagonist seems acceptable for many reasons, including self propagation, longer persistence in soil environment, and ecological and toxicological benefit for workers and consumers.

Many *Trichoderma* strains have been identified as having potential applications in biological control, and eight of commercial products (Bio-Fungus, Belgium; Root Pro and Trichoderma 2000, Israel; Trochoject, Trichopel, Trichodowels and Trichoseal, New Zealand; TUSAL, Spain; Trieco, India; Trichodex, Hungary) are available against pathogenic *Fusarium* species (Monte, 2001).

The objectives of this study were: (i) determination of main *Fusarium* species causing onion rot; (ii) evaluation of their pathogenicity on onion under ambient laboratory conditions; (iii) study of potential antagonistic effects of *Trichoderma asperellum* Samuels, Lieckfeldt, Nirenberg (teleomorph *Hypocrea asperella* Starbäck) on *Fusarium* species.

MATERIAL AND METHODS

Isolation of pathogens

Onion bulbs were inspected for possible presence of *Fusarium* species. The samples from diseased tissue were disinfected in 0.1% sublimate solution, rinsed in sterile water and planted onto potato dextrose agar (PDA) amended with streptomycin sulphate (50 mg L^{-1}). The isolates were purified and underwent preparation for determination.

The fungi were single-spored and sub-cultured on PDA under permanent darkness at 25°C, on carnation leaf agar (CLA), and on nutrient synthetic agar (SNA) under an alternating temperature of 25°C during day and 20°C at night, with 12 h photoperiod. On the basis of morphological characteristics, *Fusarium* isolates were identified according to Gerlach and Nirenberg (1982), Nelson et al. (1983) and Burgess et al. (1994).

Pathogenicity test

Artificial infection of onion sets (disinfection, wounding, immersion in spore suspension) (16 sets/repetition) was done according to du Toit and Inglis (2003), incubated in moist chamber at 20°C and examined daily for symptoms during two weeks.

After two weeks, onion sets were rinsed in water, surface sterilized for 10 min in 0.5% sodium hypochlorite, and cross-sectioned to visualize punctures. After incubation 2-day mycelia was transferred to solid PDA medium amended with streptomycin sulphate.

The used isolates and re-isolates were inoculated on PDA in the same Petri dish and compared.

Antagonistic experiments in vitro

T. asperellum, active moiety of biological product Trifender (BioVed), ability to antagonize onion pathogens *F. solani* and *F. proliferatum* (obtained from field experiment) was tested in laboratory experiment under controlled conditions.

T. asperellum antagonism was investigated in dual culture plates against tested phytopathogenic *Fusarium* species. The experiments were conducted in Petri dishes on solid PDA in darkness at 25°C the temperature which enables growth of both pathogens and *T. asperellum.* Agar plates (10 ml per Petri dish) were inoculated with agar disks (5 mm in diameter), cut from the edges of *Fusarium* colonies growing on solid PDA medium. Two days after the first inoculation, *Trichoderma* was inoculated in the same way 6 cm apart from the position of the first inoculum. The plates were incubated at 25°C in darkness, for two to three weeks, and examined daily for growth rates and morphological characteristics, such as colony appearance, colony diameters, sporulation and pigment formation.

RESULTS AND DISCUSSION

Determination of Fusarium species

After undergoing the mycological procedure, *F. proliferatum* and *F. solani* were found to be the most frequently isolated species. Three isolates of each — *F. proliferatum* (Figures 1, 3, 4) and *F. solani* (Figures 2, 4, 6) were selected for pathogenicity test and study of antagonistic effect of *T. asperellum* (Figures 7, 8).

Pathogenicity of F. proliferatum and F. solani on onion bulbs

Re-isolation of the fungal strains confirmed that the symptoms on the infected sets were caused by corresponding isolates of *F. proliferatum* or *F. solani* used for inoculation.

In Tab. 1, the percent of sprouted onion sets, the percent of sets with long sprouts, and of *Fusarium* positive are given. The results from dual culture growth rate of fungi both confronted and non-confronted, as well as days spent to meet colony edges or to overlap, are given in Table 1 and in Figured 9—14.

Tab. 1 — Effects of *F. solani* and *F. proliferatum* on onion sets after artificial infection and antagonistic effect of *Trichoderma asperellum* in dual culture experiment

Fusarium spp.	Isolate No	Percentage of onion sets			overgrown by *T. asperellum* in	
		sprouted	long sprout	diseased	days	cm
F. solani	F6IIB	81,3	25	43.8	7—11	0.2—2.2
F. solani	FKIIIA	50	12,3	87.5	7—11	1.1—2.2
F. solani	F 5/IV bel	50	25	100	5—40	0.5—2.5
F. proliferatum	FKIII dan	18.8	6.3	100	7—40	0,5—1
F. proliferatum	F 8/I	37.5	18,8	81.3	6—8	0.7—1
F. proliferatum	F 3/IIIB	43.8	18.8	100	10	1.0
Noninfected		81.3	56.3	12.5		

Our research confirmed the pathogenicity of *F. proliferatum* on yellow cultivars opposite to du Toit and Inglis results (2000) who isolated pathogens from white cultivars. The differences were found between the isolates of both pathogens: in the percent of the diseased and sprouting sets (Tab. 1, Fig. 15—20). The highest number of well-developed sprouts appeared in non-infected control sets, that underwent the same procedure, but with sterile water immersion. Very aggressive isolates infected 100% of onion sets. When compared to the percentage of positive infected sets, *F. proliferatum* isolates with low overgrowth in dual culture test, appeared highly pathogenic and inhibited sprouting from 18.8 to 50.0% (Table 1). The high percent of set infection coincided with lowest percent of sprouting and sprout elongation, and in dual culture test there was minimum inhibition of *F. proliferatum* isolate FK III "dance", and no inhibition in case of isolate F 3IIIB by *T. asperellum*.

Fusarium species inhibit the sprouting and sprout elongation. *Trichoderma* species are known as being capable of deactivating some enzymes formed by pathogens. These traits should be taken into account when judging the influence on sets sprouting.

Antagonistic activity of Trichoderma asperellum in vitro

Results of comparing the antagonistic activity of *T. asperellum* and different isolates of *F. proliferatum* and *F. solani* indicated different degrees of antagonism. Mycelia growth was affected to different degrees, depending on specific interactive microbial couplet.

More days were needed for *Trichoderma* species to approach *F. proliferatum* than *F. solani* colonies. *F. solani* colonies were overgrown by myce-

lium of *T. asperellum* to a higher extent, in comparison to *F. proliferatum* isolates, of which some were not completely overgrown even after 5 weeks.

The investigated strain of *T. asperellum* was able to overgrow the isolates of *F. solani* mycelia and produce conidia on their surface, indicating mycoparasitic action (Figures 21—23).

In case of *F. proliferatum* isolates the difference in *T. asperellum* mycelia growth was found (Figures 24—26). The isolate F K-III dance of *F. proliferatum*, during the antagonistic interaction, produced distinct colour at the confronting colony edge (Figure 26). In some cases, a clear inhibition zone was visible and radial growth pathogen was limited to small extent (Figure 24), whereas for isolate F 8/I of *F. proliferatum*, two concentric rings of sporulation were formed in the zone of confrontation (Fig. 25).

T. asperellum decreased the rate of mycelia growth of *F. solani* and *F proliferatum*. However, its ability to completely overgrow the colony was demonstrated only with *F. solani* isolates. It took five days to grow to the edge of the colony, and eleven days to overgrow the same. However, after short period of growth retardation, *T. asperellum* grew over the colony of *F. proliferatum* to a certain limit, but never completely until after 40 days (Table 1).

One could speculate that *T. asperellum* is capable of mycoparasitism of *F. solani* but not of *F. proliferatum.* The consequence could be a less powerful protection against *F. proliferatum* infection. Speculations on other employed suppression mechanisms ought to be confirmed in future experiments, to explain the good effect of *T. asperellum* bioproduct on onion protection in field.

The experiments were conducted at 26°C in darkness, at temperature optimal for growth of both pathogens and *T. asperellum*, as it was known from the literature data that *T. asperellum* prefer swarm soil conditions. Its optimum growth *in vitro* is at 30°C. *Fusarium* species are also growing well on this temperature, and infection of bulbs occurs late in the season, often aided either by some mechanical or insect injury, or by water or heat stress in soil. Therefore we speculated that, once present in soil, *T. asperellum* would develop and overgrow the bulb roots, preventing infection of *Fusarium* species. Once it establishes in the bulb microenvironment, it would significantly protect root and bulb from pathogens.

The differences in behavior in dual culture between the isolates determined as *F. proliferatum* are of interest for clarifying their physiological profile. In future experiments, the isolates should be tested to detect the nature of specific reaction.

Not only one mechanism is involved in *Trichoderma — Fusarium*, or other pathogens interaction — from competition for root exudates to fungi super parasitism. The nature of this particular relation, in case of onion pathogen, remains to be further clarified.

T. asperellum has been recently shown to induce systemic resistance in plants through a mechanism that employs jasmonic acid and ethylene signal--transduction pathways. *Trichoderma* activates plant defence mechanisms, which results in infection suppression the leaf pathogen *Pseudomonas syringae* pv. *lacrymans* (Smith & Bryan) Young et al (S h o r e s h et al., 2006).

Suppressive effect of *T. viride* (Pers ex Gray) Gorenz strain B35 on onion pathogen *Pyrenochaeta terrestris* (Hansen) was explained by production of extra cellular hydrolytic enzymes (protease, cell wall degrading enzymes), (Pietr, et al., 2004). Applied as a bulb treatment before planting, it increased significantly the marketable yield, during three following seasons. The observed stimulating effect on the growth suggested the induction of systemic resistance, resulting in soil-borne pathogen suppression.

Managing the health of onion was the focus of many investigations, due to poor chemical protection from soil pathogens, such as *Fusarium* and *Sclerotium* species. Products accepted even in organic vegetable production are based on several biocontrol organisms, such as *Trichoderma* species and *Coniothirium minitans*. *T. harzianum*, Rifai T-22HC and T22-Planter Box (Bio Works), formulated for broadcast seed treatment, in-furrow spray and transplant starter. They extand root protection beyond chemical seed treatment: protects roots against diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium* species and create stronger root systems.

Biocontrol agent use is justified by its persistence and self propagation in soil, different modes of action to pathogen, activation of resistance, low toxicity for mammals, consumers and field workers, beneficial effect on root and plant development, permission to use in organic production, etc. Disadvantages are short shelf life, in most cases sensitivity to environment effects, and necessity for know how education of farmers.

Fusarium rot are hard to control being seed and soil-borne, long persistant (over 4 years), capable of infection and spread in field and storage. So far, the best solution is the use of resistant short-day and intermediate onion varieties. Therefore integrated measures: cultural, breeding and biological or chemical, are the only justified approach to onion protection nowadays.

Fusarium species infecting onion, affect the health safety of agricultural workers, especially those associated with processing and store houses, as well as the consumers. Fumonisin B_1, beauvericin and fusaproliferin producing strains were confirmed as products of *F. proliferatum* on garlic and white onion variety in Serbia (Stanković et al., 2005, 2007). However, the other *Fusarium* species are of interest as well. Onychomycosis is usually caused by *F. solani* and *F. oxysporum*, but Hattori et al. (2005) reported *F. proliferatum* as a causal agent of onychomycosis for the first time. It was reported to cause suppurative thrombophlebitis in an immunocompromised patient (Murray et al., 2003), endophalmitis after cataract surgery (Ferrer et al., 2005), and disseminated infection in a child with lymphoblastic leukemia (Summerbell et al., 1988). Use of bioproducts would greatly contribute to an improvement of health safety of both producers and consumers.

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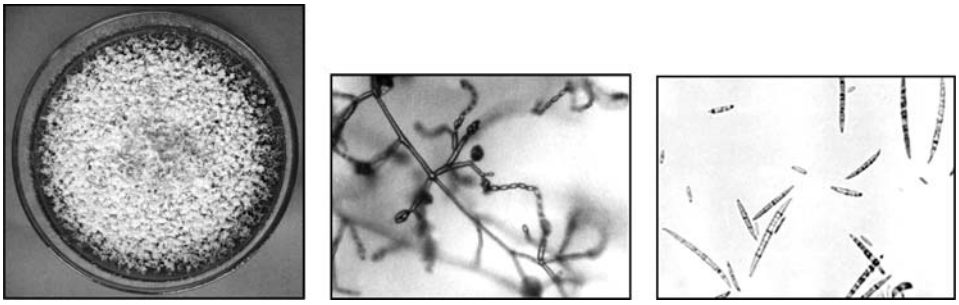

Fig. 1, 3, 4 — Colony of *F. proliferatum* on PDA and morphological characteristics

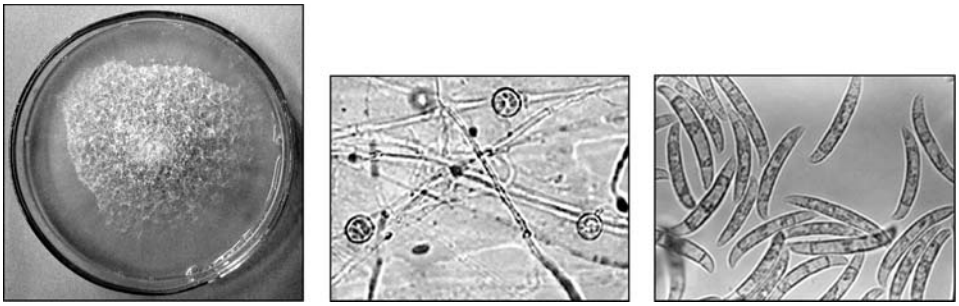

Fig. 2, 5, 6 — Colony of *F. solani* on PDA and morphological characteristics

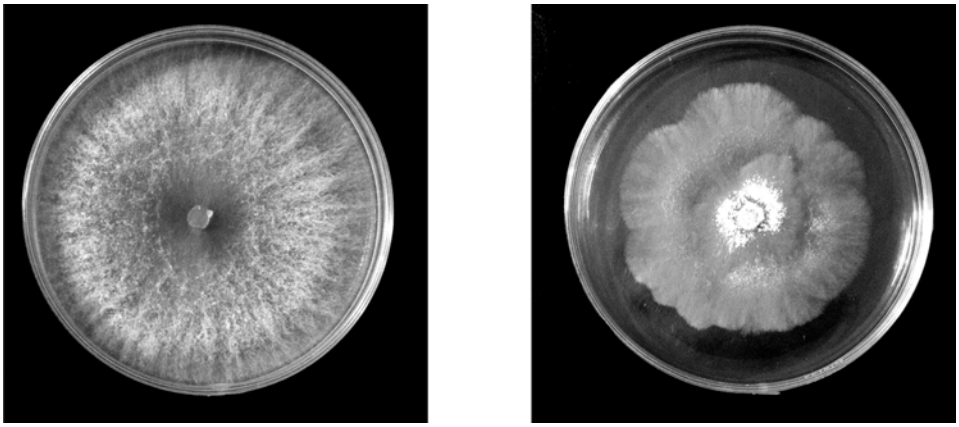

Fig. 7. and 8 — *Trichoderma* colony on PDA not amended (7) and amended (8) with Streptomycin sulphate at 50 ppm

Fig. 9. *Fusarium solani* isolate F 6-IIB

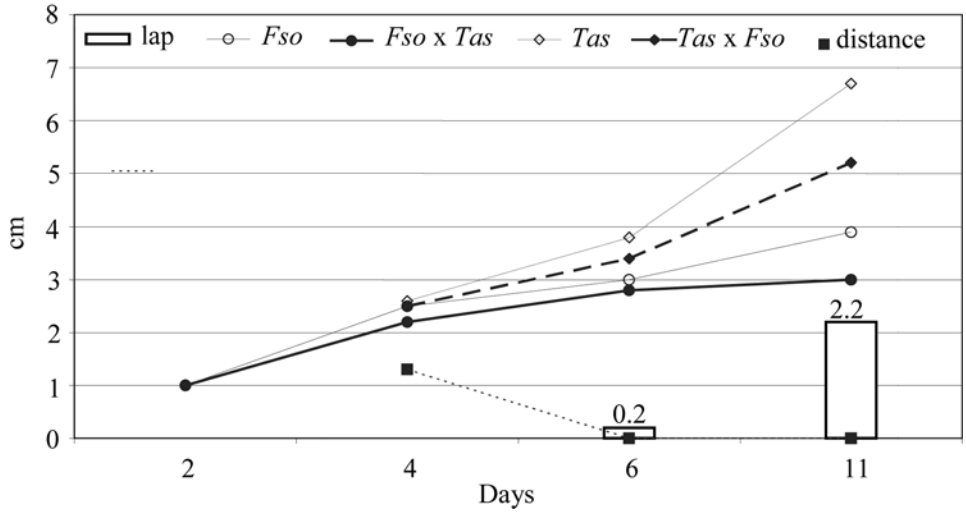

Fig. 10. *Fusarium solani* isolate FKIIIA

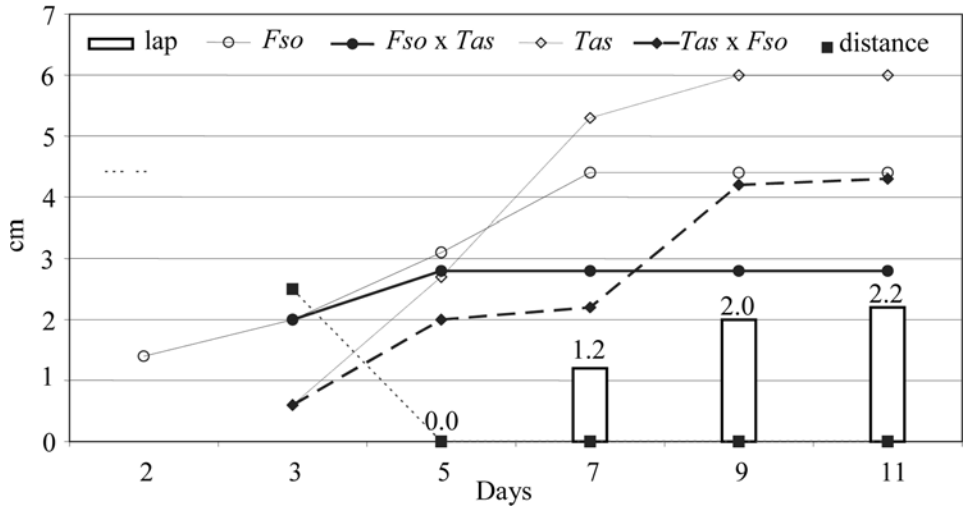

Fig. 11. *F. solani* isolate – F 5/IV belo

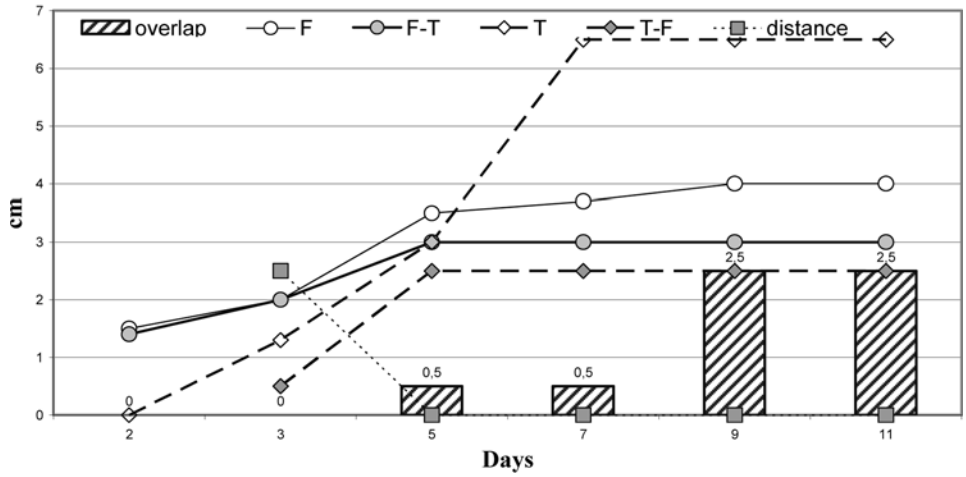

Fig. 9, 10, 11 — Mutual effects of *Trichoderma asperellum* (Tas) and *Fusarium solani* (Fso) isolates in dual culture; presented as radial mycelia growth (cm); growth of *F. solani* confronted with *T. asperellum* (Fso x Tas); growth of *T. asperellum* confronted with *F. solani* (Tas x Fso); black squares present distances between colony edges; columns present colonies overlap in cm.

Fig. 12. *Fusarium proliferatum* isolate F 8/I

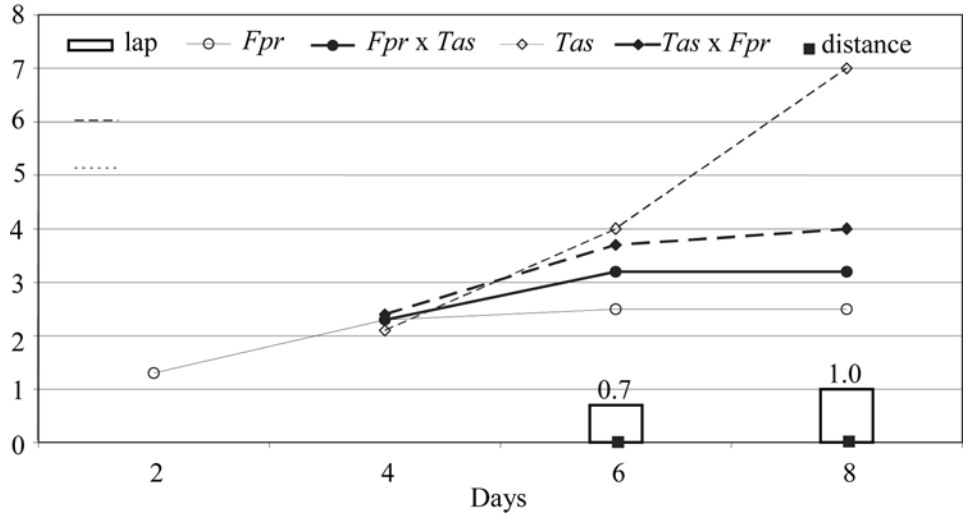

Fig. 13. *Fusarium proliferatum* isolate F K-III dance

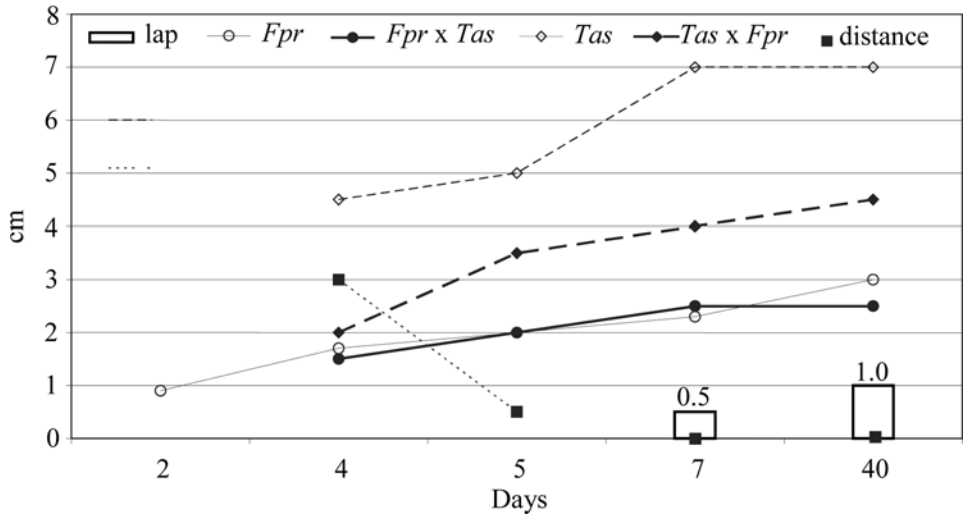

Fig. 14. *Fusarium proliferatum* isolate F 3-III B

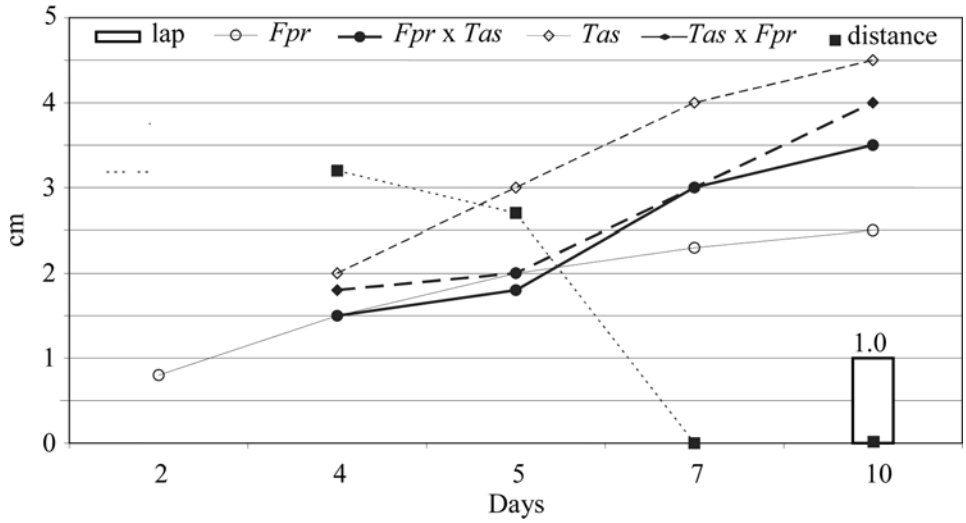

Fig. 12, 13, 14 — Mutual effects of *Trichoderma asperellum* (Tas) and *Fusarium proliferatum* (Fpr) isolates in dual culture; presented as radial mycelia growth (cm); growth of *F. proliferatum* confronted with *T. asperellum* (Fpr x Tas); growth of *T. asperellum* confronted with *F proliferatum* (Tas x Fpr).; black squares present distances between colony edges; columns present colonies overlap in cm.

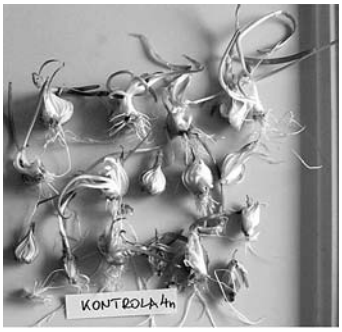

Fig. 15 — Control — non infected onion sets in pathogenicity test

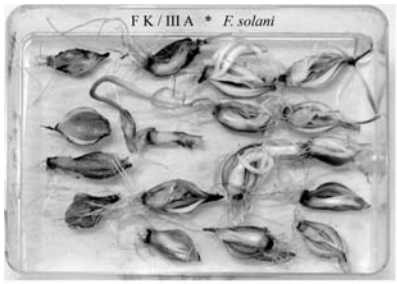

Fig. 16,

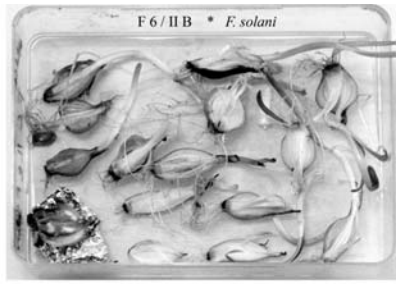

Fig. 17.

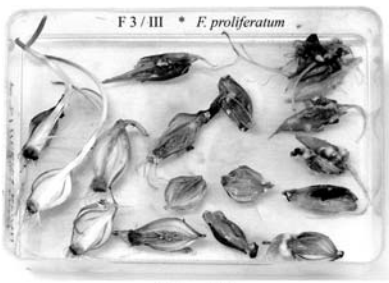

Fig. 18.

Fig. 19.

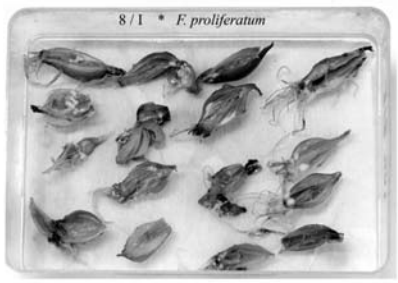

Fig. 20.

Fig. 16—20 — Onion sets artificially infected with different isolates of *F. solani* (16, 17) and *F. proliferatum* (18, 19, 20)

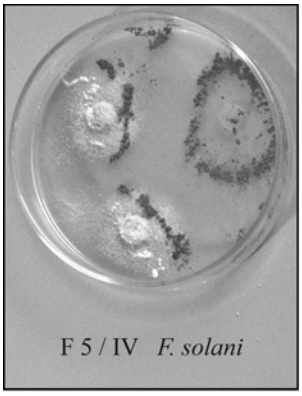

Fig. 21.

Fig. 22.

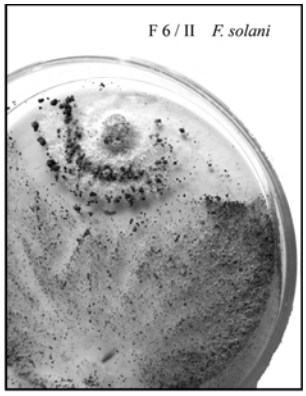

Fig. 23.

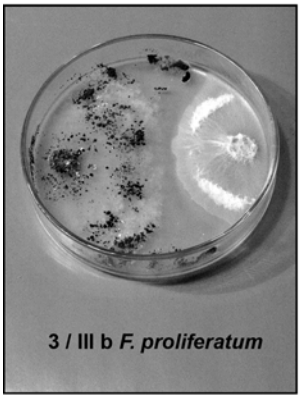

Fig. 24.

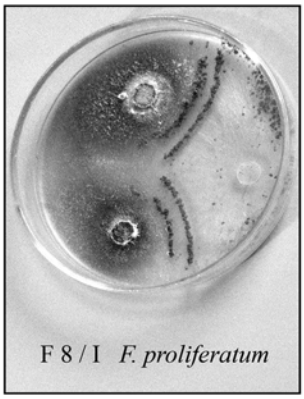

Fig. 25.

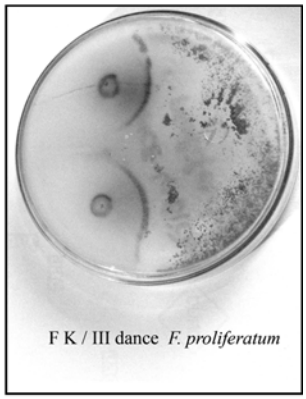

Fig. 26.

Fig. 21—26: Antagonism testing on solid media PDA: *F. solani* challenged by *T: asperellum* (21, 22, 23); *F. proliferatum* challenged by *T. asperellum* (24, 25, 26)

ФУЗАРИОЗНА ТРУЛЕЖ ЛУКА И МОГУЋНОСТ ПРИМЕНЕ БИОПРЕПАРАТА

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Резиме

Фузариозна трулеж лука најчешће је проузрокована врстама *Fusarium oxysporum* f. sp. *cepae* и *F. solani*, а и одскора и токсикогени *F. proliferatum* у нашим условима гајења и чувања лука у складиштима. Утврђена је највећа учесталост *F. proliferatum* и *F. solani* на луковицама из поља. Разлике изолата у патогености испољиле су се у различитом утицају на клијање лучица, издуживање клице и интензитет пропадања. Утврђена је различита антагонистичка активност *Trichodrema asperellum* на изолате *F. proliferatum* и *F. solani*, спорија инхибиција у случају првог и изражена у случају другог патогена у *in vitro* огледима. Антагонистичка својства врста из рода *Trichoderma* се искоришћавају за формулацију биолошких препарата примењивих у органској и конвенционалној производњи, у превенцији обољења лука која проузрокују земљишни паразити, пре свега фузариозног увенућа и трулежи. Истакнут је значај биолошких препарата у заштити здравствене безбедности произвођача и потрошача, с обзиром да су ови патогени потенцијални продуценти микотоксина.