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IMPACT OF INFECTIONS WITH TWO *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM* ISOLATES ON THE YIELDING OF SOME WILD STRAINS OF *AGARICUS BISPORUS* (LANGE) IMBACH

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Abstract: The effect of infections with *Trichoderma aggressivum* f. *europaeum* on the yielding of seven wild and one commercial strain of *Agaricus bisporus* (Lange) Imbach. was investigated. Wild strains of the mushroom were derived from natural habitats of Poland. The cultivation substrate was inoculated with two different isolates of *T. aggressivum* f. *europaeum* Th2. It was found that infections of the substrate with these isolates resulted in a very serious decrease in mushroom yield. The examined mushroom strains showed different reactions to the infections with *Trichoderma* isolates.

Key words: mushroom, wild strains, green mould, yielding

INTRODUCTION

For at last two decades, green moulds of the *Trichoderma* genus are one of the most serious hazards for the mushroom production in Western Europe and Poland. The moulds contribute to huge crop losses in mushroom cultivation (Seaby 1998; Sharma *et al*. 1999; Maszkiewicz 2006). Despite investigations conducted in many research centers all over the world, so far no effective methods of control of the pathogenic fungi in production of cultivated mushrooms have been developed. Aggressive biotypes of *Trichoderma harzianum* Th2 and Th4 were classified by Samuels *et al*. (2002). In the literature on the subject, the aggressive biotype Th2 is referred to as *Trichoderma aggressivum* f. *europaeum* and the Th4 biotype as *T. aggressivum* f. *aggressivum*. Williams *et al*. (2003) indicated that both above-mentioned biotypes were more aggressive in comparison with other isolates of *T. harzianum*. The first reports about the appearance of green mould in mushroom cultivations in Poland date back to 2002 (Szczech *et al*. 2008). The authors of this study showed that the most frequently isolated species of the *Trichoderma* genus identified in mushroom producing farms included: *T. harzianum*, *T. atroviride*, *T. aggressivum* and *T. longibrachiatum*. In addition, using PCR, multiplex PCR, RAPD techniques and DNA sequence analysis, they also found that the examined 24 isolates, derived from Polish mushroom-growing houses, belonged to the *T. aggressivum* Th2 biotype. Studies conducted by Sobieralski *et al*. (2009) proved the different susceptibility of white and brown strains of *Agaricus bisporus* (Lange) Imbach. for infections with *Trichoderma* isolates. Wild strains of *A. bisporus* which show high resistance to infections could be used in breeding.

The objective of the performed investigations was to determine the impact of infection with isolates of biotype *T. aggressivum* f. *europaeum* Th2 on yielding of wild strains of *Agaricus bisporus*, derived from natural habitats in Poland.

MATERIALS AND METHODS

Strains of *A. bisporus* and isolates of *T. aggressivum* used in our experiments came from the collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops of Poznań University of Life Sciences. The following seven wild strains of mushroom came from sites in Poland situated in the area of the Notecka Primeval Forest (Central-West Poland): Kr1/B, Bi2/7, Kw3/S, An4/A, St5/8, Ha6/F, Mi7/E as well as a commercial strain F62 of the Italspawn Company were used in the experiments. Table 1 gives sites from which mushroom strains used in the experiments were obtained. The tested isolates of *T. aggressivum* f. *europaeum* derived from two mushroom-growing plants in the Wielkopolska area (Central-West Poland). Isolate TB/7/12 was obtained from the substrate from a mushroom-growing house in Kłoda near Rydzyny, and isolate TKR/3 – from a compost farm in Rakoniewice. Using the techniques of PCR, multiplex

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PCR and RAPD, it was determined that the above-mentioned isolates belonged to the *T. aggressivum* f. *europaeum* (Th2) biotope. Furthermore molecular species identification was based on sequence analysis of the internal transcribed spacers (ITS1 and ITS2) of the rRNA gene cluster. DNA sequencing and sequence similarity analysis was performed according to the methods described by White *et al*. (1990) and Błaszczyk *et al*. (2005).

Table 1. List of collection data, site and designation of the mushroom *A. bisporus* used in the investigations

No.	Collection date	Location of collecting site	Designation
1	VIII	Kruteczek	Kr1/B
2	VIII	Biała	Bi2/7
3	IX	Kwiejce	Kw3/S
4	VIII/IX	Antoniewo	An4/A
5	IX	Stajkowo	St5/8
6	VIII	Hamrzysko	Ha6/F
7	IX	Miały	Mi7/E

In the experiments, cultivation substrates were inoculated with two different isolates TB/7/12 and TKR/3 belonging to the *T. aggressivum* f. *europaeum* (Th2) biotype. The discussed experiments were established in an air-conditioned chamber and the cultivation was conducted in plastic boxes measuring 38x30x18 cm. The substrate used in these experiments was prepared from rye straw and chicken droppings obtained from a compost farm in Rakoniewice. Each box was filled with 6 kg of the substrate. The material used for inoculation was granular mycelium prepared according to the recipe recommended by Lemke (1971). Maternal and granular mycelium of the examined strains was prepared in the biological laboratory of the Department of Vegetable Crops of Poznań University of Life Sciences. The infection, using *T. aggressivum* f. *europaeum* isolates, was carried out 10 days after the inoculation of the substrate with the *A. bisporus* mycelium. Granular mycelium of isolates was also prepared on wheat grains. The substrate was infected by the mycelium of the tested *T. aggressivum* f. *europaeum* isolates in the amount of 100 cm^3 per box. The substrate was thoroughly mixed with the mycelium and subjected to further incubation. The incubation took place at a temperature of 25°C and relative air humidity of 85–90%. Once the substrate was overgrown by mushroom mycelium, it was covered with a 5 cm thick cover. High peat deacidified by chalk to pH = 7.5 and disinfected thermally was applied as a cover.

Two separate experiments for TB/7/12 and TKR/3 isolates in the same design were conducted. Experiments were established in fully randomized design, in 4 replications and 2 cultivation cycles. Harvest of *A. bisporus* carpophores was carried out for a period of 6 weeks.

Yields of carpophores were determined on the basis of harvested fruiting bodies calculated per square meter of cultivation. Cultivations carried out in boxes which were not infected by *T. aggressivum* f. *europaeum* isolates were treated as the control.

When comparing experimental results, the analysis of variance for factorial experiments was applied using Duncan's test at the level of significance $\alpha = 0.05$. The results of experiments were discussed on the basis of mean values from 2 cultivation cycles.

RESULTS AND DISCUSSION

The highest losses in the cultivation of mushrooms in Europe are caused by the aggressive strain of *T. aggressivum* f. *europaeum* Th2 (Ospina-Giraldo *et al*. 1998). Many research centres all over the world are currently involved in research aiming at developing effective methods to control *Trichoderma* genus fungi in cultivations of mushroom and oyster mushroom. Most frequently, scientists in their studies focus on issues associated with the development, identification and classification as well as physiology of the above-mentioned fungi at biological, phytopathological or molecular levels. For these purposes, scientists use appropriate analytical and computer techniques (Chen *et al*. 1999). Nevertheless, specific conditions occurring in a cultivation hall, *i.e.* abundance of organic matter as well as high temperature and humidity create optimal conditions and favour the development of the above-mentioned fungi. A number of different fungal species of the *Trichoderma* genus can be found in substrates used for mushroom cultivation but they do not exert a significant influence on mushroom yields. Species characterised by relatively low pathogenicity comprise: *T. viride*, *T. atroviride*, *T. pseudokoningi*, *T. hamatum* (Fletcher *et al*. 1989). According to Williams *et al*. (2003), *T. aggressivum* f. *europaeum*, exhibits considerable aggressiveness in relation to mushroom mycelium. Our performed experiments support the above opinion. All the examined mushroom strains suffered significant yield losses following substrate infection with *T. aggressivum* f. *europaeum* TB/7/12 and TKR/3 strains (Figs. 1, 2). The trial was carried out in an air-conditions chamber, in plastic boxes of small capacity (6 kg) which questions the reliability of the obtained results in comparison with conditions prevailing in commercial cultivation. However, earlier investigations of the authors proved that experiments carried out in such boxes containing small quantities of substrate are fully reliable (Sobieralski and Siwulski 2002; Sobieralski *et al*. 2007). It should be emphasised, that yields obtained in boxes of relatively small substrate volume are lower in comparison with yields obtained in production conditions (Sobieralski and Siwulski 2006).

Yields from the control plots, uninfected with *Trichoderma* ranged from 4.8 to 14.6 kg/m^2, with F62 strain giving the highest yields of 14.6 and 13.9 kg/m^2, respectively. From among strains obtained from natural sites, the highest yields in both experiments were recorded by the Kr1/B strain (13.1 and 11.4 kg/m^2). The remaining experimental strains were characterized by lower yields in comparison with the above-mentioned strain. The lowest yields (5.4 and 4.8 kg/m^2) were recorded for the Ha6/F strain. The performed yielding analysis of strains infected with *T. aggressivum* f. *europaeum* isolates revealed that the TKR/3 isolate reduced yields of the examined mushroom strains far more significantly than the TB/7/12 isolate. The Kr1/B

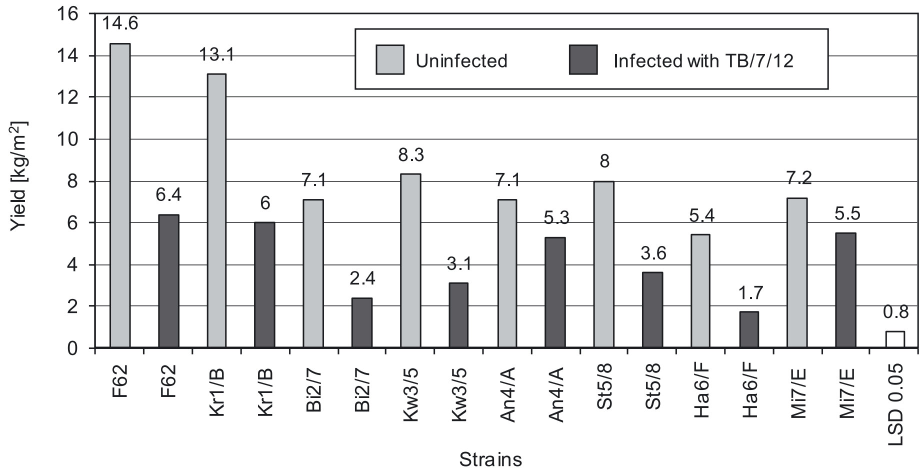

Fig. 1. Impact of substrate infection with the TB/7/12 isolate of *T. aggressivum* f. *europaeum* on yields of seven wild and one commercial strain of *A. bisporus*

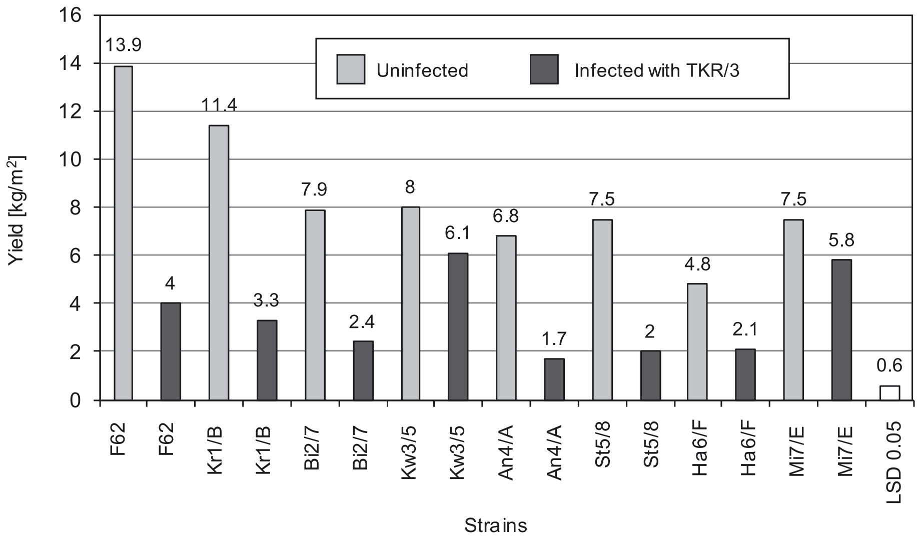

Fig. 2. Impact of substrate infection with the TRK/3 isolate of *T. aggressivum* f. *europaeum* on yields of seven wild and one commercial strain of *A. bisporus*

mushroom strain infected with the TKR/3 strain gave yields of 3.3 kg/m^2, while when infected with the TB/7/12 isolate, it yielded 6.0 kg/m^2. The same occurred in the case of the commercial strain F62; a greater yield loss was observed following substrate infection with the TKR/3 isolate (4.0 kg/m^2) than with the TB/7/12 isolate (6.4 kg/m^2). On the other hand, in the case of the Kw3/5 strain, a stronger effect of yield loss was found in the case of infection with the TB/7/12 isolate (3.1 kg/m^2) in comparison with the infection with the TKR/3 isolate (6.1 kg/m^2). A similar response of mushroom to infections with aggressive isolates of fungi of *Trichoderma* genus was obtained by the authors in their earlier studies (Sobieralski *et al*. 2009).

Percentage yield loss gives a much better illustration of the above-presented interrelationships (Table 2). Yield losses in the case of infection with the TB/7/12 isolate ranged from 25.3 to 68.5%, while in the case of the TKR/3 isolate – from 22.7 to 75.0%. The highest yield losses in all the examined mushroom strains were caused by the infection with the TKR/3 isolate. The An4/7 strain turned

Table 2. Yield losses in mushroom strains infected with *T. aggressivum* f. *europaeum* isolates in comparison with yields from uninfected cultivations

Isolate of *Trichoderma*	Strain	Yield loss [%]
TB/7/12	F62	56.2
	Kr1/B	54.2
	Bi2/7	66.2
	Kw3/5	62.6
	An4/7	25.3
	St5/8	68.5
	Ha6/F	55.0
	Mi7/E	23.6
TKR/3	F62	71.2
	Kr1/B	71.1
	Bi2/7	69.6
	Kw3/5	23.7
	An4/7	75.0
	St5/8	73.3
	Ha6/F	56.2
	Mi7/E	22.7

out to be the least resistant to infections as yield losses reached 75%. The infection of the cultivation substrate with both *T. aggressivum* f. *europaeum* isolates caused very high yield losses in the mushroom strain designated as St5/8 (68.5 and 73.3%). The lowest yield loss was recorded in the case of strain Mi7/E (22.7%).

The results of the performed investigations confirm, fairly unequivocally, the significant impact of substrate infection with aggressive isolates of the *Trichoderma* genus on yield losses both of a commercial strain as well as wild strains. The performed experiments failed to demonstrate higher resistance of wild strains of mushroom in comparison with the commercial strain to substrate infections with *T. aggressivum* f. *europaeum*. It should be emphasized that there is no unequivocal and detailed data in world literature on the subject concerning susceptibility of individual mushroom strains to aggressive isolates of the *Trichoderma* genus. Investigations demonstrating lower susceptibility for infections with aggressive fungi of the *Trichoderma* genus may have both practical and breeding implications.

REFERENCES

Błaszczyk L., Tyrka M., Chełkowski J. 2005. PstIAFLP based markers for leaf rust resistance genes in common wheat. J. Appl. Genet. 46 (4): 357–364.

Chen X., Romaine C.P., Tan Q., Schlagnhauter B., Ospina-Giraldo M.D., Royse D.J., Huff D.R. 1999. PCR-based genotyping of epidemic and preepidemic *Trichoderma* isolates associated with green mould of *Agaricus bisporus*. Appl. Environ. Microbiol. 65 (6): 2674–2678.

Fletcher J.T., White P.F., Gaze R.H. 1989. Mushrooms Pest and Disease Control. 2nd. ed., Intercept Limited, Andover, England, 174 pp.

Lemke G. 1971. Mycelenzucht und Fruchtkorperprokuktion des Kulturchampignons *Agaricus bisporus* (Lange) Sing. Gartenbauwissenschaft 36 (18): 19–27.

Maszkiewicz J. 2006. Choroby grzybowe. p. 61–79. In: „Ochrona Pieczarek" (E. Dmowska, M. Ignatowicz, M. Lewandowski, J. Maszkiewicz, J. Szymański, Z. Uliński, eds.). Hortpress, Warszawa.

Ospina-Giraldo M.D., Royse D.M., Thon J.R., Chen X., Romaine C.P. 1998. Phylogenetic relationships of *Trichoderma harzianum* causing mushroom green mould in Europe and North America to other species of *Trichoderma* from world-wide sources. Mycologia 90 (1): 76-81.

Samuels G.J., Dodd S.L., Gams W., Castlebury L.A., Petrini O. 2002. *Trichoderma* species associated with the green mould epidemic of commercial grown *Agaricus bisporus*. Mycologia 94 (1): 146–170.

Seaby D.A. 1998. *Trichoderma* as a weed mould or pathogen in mushroom cultivation. p. 267–287. In: "*Trichoderma* and *Gliocladium*. Vol. 2. Enzymes, Biological Control and Commercial Application" (G.E. Harman, C.P. Kubicek, eds.). Taylor & Francis, London, 394 pp.

Sharma H.S.S., Kilpatrick M., Ward F., Lyons G., Burns L. 1999. Colonization of phase II compost by biotypes of *T. harzianum* and their effect on mushroom yield and quality. Appl. Microbiol. Biotechnol. 51 (5): 572–578.

Sobieralski K., Siwulski M. 2002. Porównanie metod oceny plonowania wybranych odmian pieczarki. In: Mat. Ogólnopol. Konf. Nauk. „Jakość warzyw i ziół na tle uwarunkowań uprawowych i pozbiorczych". Warszawa, 27–28 czerwca 2002, 115 pp.

Sobieralski K., Siwulski M. 2006. Wpływ podłoża na plonowanie kultur jednozarodnikowych i krzyżówkowych pieczarki dwuzarodnikowej *Agaricus bisporus* (Lange) Sing. In: III Ogólnopol. Konf. „Zasoby genowe roślin w ochronie różnorodności biologicznej". Lublin, 27–29 czerwca 2006, 118 pp.

Sobieralski K., Siwulski M., Frużyńska-Jóźwiak D., Górski R. 2009. Impact of *Trichoderma aggressivum* f. *europaeum* Th2 on the yelding of *Agaricus bisporus*. Phytopathologia 53: 5–10.

Sobieralski K., Siwulski M., Grzebielucha I., Nowak M. 2007. Yielding and morphological characteristics of carpophores of single-spore cultures, crossbred cultures and brown strains of mushroom *Agaricus bisporus* (Lange) Imbach. Spontaneous and induced variation for the genetic improvement of horticultural crops. Univ. Technol. Life Sci., University Press: 341–345.

Szczech M., Staniaszek M., Habdas H., Uliński Z., Szymański J. 2008. *Trichoderma* spp. – the cause of green mold on polish mushroom farms. Veg. Crops Res. Bull. 69: 105–114.

White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. In: "PCR Protocols: A Guide to Methods and Applications" (M.A. Innis, D.H. Gelfand, J.J. Shinsky, T.J. White, eds.). Academic Press, San Diego.

Williams J., Clarkson J.M., Mils P.R., Cooper R.M. 2003. Saprotrophic and mycoparasitic components of aggressiveness of *Trichoderma harzianum* groups toward the commercial mushroom *Agaricus bisporus*. Appl. Enivron. Microbiol. 69 (7): 4192–4199.

POLISH SUMMARY

WPŁYW INFEKCJI IZOLATAMI *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM* NA PLONOWANIE KILKU DZIKICH RAS PIECZARKI DWUZARODNIKOWEJ *AGARICUS BISPORUS* (LANGE) IMBACH

Celem przeprowadzonych badań było określenie wpływu infekcji grzybów z rodzaju *Trichoderma* na plonowanie kilku ras pieczarki dwuzarodnikowej *Agaricus bisporus* (Lange) Imbach pochodzących ze stanowisk naturalnych w Puszczy Noteckiej. W doświadczeniu użyto szczepów pieczarki oznaczonych jako: Kr1/B, Bi2/7, Kw3/S, An4/A, St5/8, Hab6/F, Mi7/E oraz odmiany produkcyjnej F62 firmy Italspawn. Podłoże uprawowe infekowane było dwoma różnymi izolatami *T. aggressivum* f. *europaeum*, tj. TB/7/12 oraz TKR/3. Uprawę prowadzono w plastikowych skrzynkach, w komorze klimatyzowanej. Po zainfekowaniu podłoży badanymi izolatami *Trichoderma* stwierdzono znaczną obniżkę plonu. Reakcja badanych ras pieczarki na porażenie izolatami *T. aggressivum* f. *europaeum* była zróżnicowana. Największą obniżkę plonu stwierdzono przy infekcji podłoża uprawowego izolatem TKR/3, tj. od 22,7 do 75,0%.