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# IMPACT OF *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM* TH2 ON THE YIELDING OF *AGARICUS BISPORUS*

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## Abstract

The effect of infection with *Trichoderma aggressivum* f. *europaeum* isolates on yielding of white and brown strains of *Agaricus bisporus* mushrooms was investigated. Two strains of white and two of brown mushrooms were used in the experiments. Substrates were inoculated with two different isolates of *T. aggressivum* f. *europaeum* Th2. The substrate inoculation with the two isolates resulted in very serious mushroom yield decreases. The examined brown mushroom strains showed significantly smaller yield decreases in comparison with the white strains.

**Key words:** *Agaricus bisporus*, brown strains, white strains, *Trichoderma*, yielding

## Introduction

One of the most serious hazards for cultivation of mushroom and oyster mushroom in Poland is infection by green moulds of the *Trichoderma* genus. They contribute to huge losses in mushroom-growing houses and oyster mushroom cultivations as well as in spawn companies. Despite investigations conducted in many research centers all over the world, so far no effective methods for control of the pathogenic fungi in production of cultivated mushrooms have been developed. Non-aggressive isolates occurring in mushroom-growing houses include Th1 and Th3 biotypes (Fletcher 1994, Fletcher et al. 1989, Seaby 1987). Aggressive biotypes of *T. 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 *T. 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 Th4 biotype is found in North America while Th2 in Europe. Aggressive biotypes Th2 and Th4 differ from mor-

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phologically similar *T. harzianum* and *T. atroviride* strains by their growth rate (Samuels et al. 2002).

The objective of the performed investigations was to determine the impact of infection by aggressive isolates of biotype *T. aggressivum* f. *europaeum* Th2 on yielding of white and brown strains of *Agaricus bisporus*.

## Material and methods

The following strains of mushroom were used in the experiment: Amycel Alpha producing large fruiting bodies and Amycel 2600 forming small fruiting bodies from the Amycel Company and brown strains FB30 and FB31 deriving from the Italspawn Company. The tested isolates of *T. aggressivum* f. *europaeum* Th2 derived from two mushroom cultivations in Wielkopolska area (Central-West Poland).

Experimental substrates were inoculated independently with each of the two different isolates T12/3 and T17/4 belonging to *T. aggressivum* f. *europaeum* (Th2) biotype. The discussed experiment was established in an air-conditioned chamber and the mushrooms were cultivated in plastic boxes (38 × 30 × 18 cm). The substrate used in these experiments was prepared from rye straw and chicken droppings deriving from a compost farm. Each box was filled with 6 kg of the substrate. The substrate was inoculated with granular mycelium prepared according to the recipe recommended by Lemke (1971). Maternal mycelium of the examined strains was prepared in the laboratory of the Department of Vegetable Crops of Poznań University of Life Sciences and next it was multiplicated in a spawn company in Łobez. 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 *T. aggressivum* f. *europaeum* isolates was also prepared on wheat grains. The substrate was infected with the mycelium of 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 the 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. Sphagnum peat deacidified with chalk to pH 7.5 and disinfected thermally was applied as a cover.

Experiments were established in fully randomized design, in four replications and two cultivation cycles. *Agaricus bisporus* carpophores were harvested for six 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 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 two cultivation cycles.

## Results

In all the examined cases, inoculation of the substrate with *T. aggressivum* f. *europaeum* isolates T12/3 and T17/4 resulted in very significant decrease in mushroom yields. Yields obtained from control plots ranged from 15.2 to 20.2 kg per 1 $m^2$. In general, brown strains yielded worse than the white ones. In the first experiment with *T. aggressivum* f. *europaeum* T12/3 isolate (Fig. 1), the mushroom FB30 strain yielded 16.3 kg/$m^2$ and the FB31 strain – 17.4 kg/$m^2$, whereas the experimental white strains Amycel Alpha and A 2600 yielded better, 18.5 and 19.4 kg/$m^2$, respectively. Similarly, in the second experiment with T17/4 isolate the brown strains gave significantly lower yields in comparison with the white ones and the trend was very similar. When analyzing yields of the mushroom strains infected with the *T. aggressivum* f. *europaeum* T12/3 biotype, it seems that the decrease in yields of brown strains was considerably smaller in comparison with the white strains. The FB30 strain infected with the T12/3 strain yielded 7.2 kg/$m^2$, while the FB31 strain infected with the same strain yielded 6.1 kg/$m^2$. On the other hand, the experimental white strains yielded 4.2 kg/$m^2$ in the case of the Amycel Alpha strain infected with the T12/3 strain and 3.6 kg/$m^2$ in the case of the Amycel 2600 strain infected with the same isolate. A similar tendency was observed in the second experiment (Fig. 2), when the FB30 strain was infected with the *T. aggressivum* f. *europaeum* T17/4 isolate. In this case, the yield reached 9.4 kg/$m^2$, while in the case of the FB31strain infected with the same isolate – 7.3 kg/$m^2$. The applied white strains yielded distinctly lower harvests: the Amycel Alpha strain infected with the T17/4 isolate yielded 4.8 kg/$m^2$, whereas the Amycel 2600 strain infected with the same isolate yielded only 2.2 kg/$m^2$.

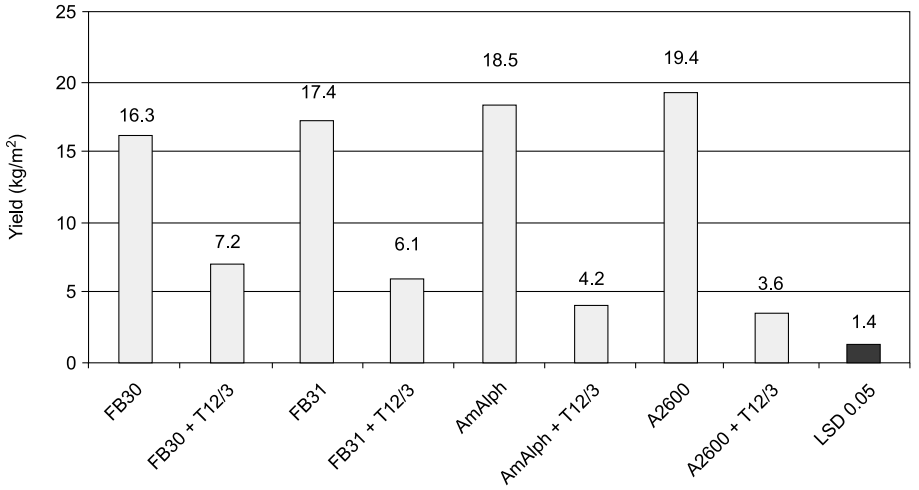


Fig. 1. Yielding of white and brown strains of *Agaricus bisporus* infected with *Trichoderma aggressivum* f. *europaeum* T12/3 isolate

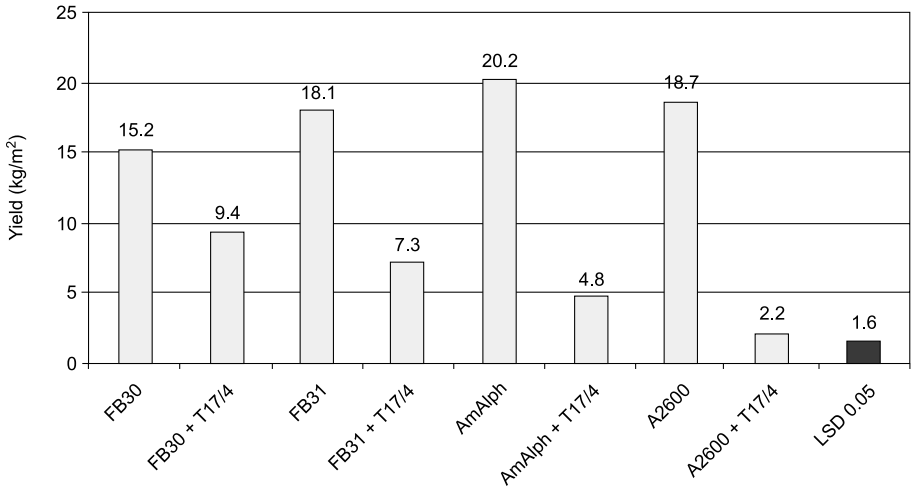


Fig. 2. Yielding of white and brown strains of *Agaricus bisporus* infected with *Trichoderma aggressivum f. europaeum* T17/4 isolate

## Discussion

In continental Europe, the highest losses in mushroom cultivation are caused by the aggressive biotype *Trichoderma aggressivum* f. *europaeum* Th2 (Ospina-Giraldo et al. 1998). According to Williams et al. (2003) *T. aggressivum* f. *europaeum* displays high aggressiveness. This claim was fully corroborated by the results obtained by the authors of this article. The experiment was carried out in an air-conditioned chamber and, hence, the question arises to what extent the obtained results can be reliable for production conditions. However, a number of earlier experiments carried out by the authors in plastic boxes using relatively small amounts of substrate were fully reliable (Kiśluk and Siwulski 1996, Sobieralski and Siwulski 1998, 2002 a, 2002 b, Sobieralski et al. 2007). Generally speaking, yields obtained in an air-conditioned chamber in boxes containing small quantities of substrate are significantly lower than those obtained in production conditions (Sobieralski and Siwulski 2006).

The performed experiments proved that the infection of the experimental substrate with aggressive isolates of *T. aggressivum* f. *europaeum* resulted in very significant yield losses. What deserves special attention is the recorded different response of the experimental mushroom strains to the infection. In general, it can be concluded that brown strains exhibited a significantly lower yield decrease in comparison with white strains. On the other hand, healthy brown strains gave considerably lower yields than the white ones. It should also be emphasized that there is no specific information in the available world literature on the subject about susceptibility or tolerance of individual strains of mushroom to aggressive forms of

the *Trichoderma* genus isolates. In addition, it appears that experimentally confirmed lower sensitivity to infections by *T. aggressivum* f. *europaeum* isolates may have practical and breeding significance. However, further experiments are necessary to elaborate practical recommendations.

## Streszczenie

WPŁYW *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM* TH2
NA PLONOWANIE PIECZARKI DWUZARODNIKOWEJ
(*AGARICUS BISPORUS*)

Celem badań było określenie wpływu infekcji wywołanej przez grzyby rodzaju *Trichoderma* na plonowanie białych i brązowych odmian pieczarki *Agaricus bisporus*. W doświadczeniu użyto odmian białych pieczarki: Amycel Alpha i Amycel 2600 oraz odmian brązowych: FB30 i FB31. Podłoża inokulowano dwoma różnymi izolatami: T12/3 oraz T17/4, należącymi do biotypu *T. aggressivum* f. *europaeum* (Th2). Doświadczenie prowadzono w komorze klimatyzowanej w skrzynkach plastikowych zawierających 6 kg podłoża uprawowego. Inokulacja podłoża agresywnymi izolatami *T. aggressivum* f. *europaeum* prowadziła do bardzo znacznego zmniejszenia plonów pieczarki. Reakcja badanych odmian na porażenie badanymi izolatami była zróżnicowana. Odmiany brązowe reagowały znacznie mniejszym spadkiem plonu niż odmiany białe.

### Literature

Fletcher J.T., 1994: Preliminary investigations of the pathogen and weed mould problems of mushrooms in Australia. Report of project Mu/303 Horticultural Research and Development Corporation, NSW, Gordon. 2072.

Fletcher J.T., White P.F., Gaze R.H., 1989: Mushrooms: pest and disease control. Intercept Limited, Andover, England.

Kiśluk A., Siwulski M., 1996: Porównanie plonowania kilku odmian pieczarki dwuzarodnikowej *Agaricus bisporus* (Lange) Sing. In: Materiały II Ogólnopolskiego Sympozjum „Nowe rośliny i technologie w ogrodnictwie". Ed. M. Siwulski. HaKa, Poznań: 173–176.

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

Ospina-Giraldo M.D., Royse D.M., Thon M.R., Chen X., Romaine C.P., 1998: Phylogenetic relationships of *Trichoderma harzianum* causing mushroom green mold 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 mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94, 1: 146–170.

Seaby D.A., 1987: Infection of mushroom compost by *Trichoderma* species. Mushroom J. 179: 355–361.

Seaby D.A., 1996: Differentiation of *Trichoderma* taxa associated with mushroom production. Plant Pathol. 45: 905–912.

Sobieralski K., Siwulski M., 1998: Porównanie plonowania 4 polskich odmian pieczarki dwuzarodnikowej w warunkach uprawy towarowej. In: Materiały I Ogólnopolskiej Konferencji „Zasoby genowe roślin użytkowych – gromadzenie, ocena i wykorzystanie", Puławy. IHAR, Radzików: 48.

Sobieralski K., Siwulski M., 2002 a: Porównanie metod oceny plonowania wybranych odmian pieczarki. In: Materiały Ogólnopolskiej Konferencji Naukowej „Jakość warzyw i ziół na tle uwarunkowań uprawowych i pozbiorczych". Wyd. SGGW, Warszawa: 115.

Sobieralski K., Siwulski M., 2002 b: Porównanie plonowania wybranych kultur krzyżówkowych pieczarki białej i brązowej. In: Materiały Ogólnopolskiej Konferencji Naukowej „Jakość warzyw i ziół na tle uwarunkowań uprawowych i pozbiorczych". Wyd. SGGW, Warszawa: 114.

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

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. In: Spontaneous and induced variation for the genetic improvement of horticultural crops. Ed. P. Nowaczyk. University of Technology and Life Science in Bydgoszcz University Press, Bydgoszcz: 341–345.

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. Environ. Microbiol. 69, 7: 4192–4199.

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