IMPACT OF *TRICHODERMA* ISOLATES ON THE MYCELIUM DEVELOPMENT OF WILD STRAINS OF *COPRINUS COMATUS* (MÜLL.) S.F. GRAY

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Abstract: The impact of different isolates of three species of the *Trichoderma* genus on the development of wild strains of *Coprinus comatus* mycelium was investigated. Nine strains of *C. comatus* obtained from natural sites and one cultivated strain were used in the trail. The following *Trichoderma* isolates were used: *T. aggressivum* f. *europaeum, T. longibrachiatum* and *T. atroviride*. It was found that isolates of *T. aggressivum* f. *europaeum* reduced the development of the *C. comatus* mycelium to a much greater degree than isolates of *T. longibrachiatum* and *T. atroviride*.

Key words: *Trichoderma, Coprinus comatus,* wild strains, mycelium, individual biotic effect

INTRODUCTION

Diseases described as "green moulds" constitute a serious problem in the cultivation of several mushroom species all over the world. The main cause of these diseases are fungi of the *Trichoderma* genus. Infections of cultivations with species of this genus appeared in Western Europe at the beginning of 1980s (Hayes 1978; Gandy 1985; Sharma *et al*., 1999; Samuels *et al*., 2002). In Poland in 2002, infections of epidemic proportions were observed in *Agaricus bisporus* cultivations (Maszkiewicz 2006). The following *Trichoderma* isolates were obtained from the substrate prepared for *A. bisporus* cultivation: *T. harzianum, T aggressivum*, *T. atroviride* and *T. longibrachiatum* (Popiel *et al*. 2010). However, the highest losses are caused by the isolates of *Trichoderma aggressivum* f. *europaeum* which are characterised by considerable aggressiveness and high pathogenicity in relation to *A. bisporus* (Samuels *et al*. 2002; Williams *et al*. 2003).

C. comatus are mushrooms which can be cultivated in similar conditions and on identical substrate as *A. bisporus* (Stamets 2000). This species is cultivated on a commercial scale in China and Taiwan (Huang 1997; Ho and Peng 2006). Fruit bodies of *C. comatus* contain substances of antimutagenic, anticarcinogenic and antidiabetic nature and improve overall resistance of human organisms (Liu and Zhang 2003; Yu *et al*. 2009). Cultivated and wild strains of this species differ in productivity. Cultivations which utilise wild strains of this species make it possible to obtain hybrids of increased yield-forming potential (Siwulski *et al*. 2001). Producers of *A. bisporus* and *Pleurotus* in Poland are interested in undertaking production of cultivated mushrooms for medical purposes. There is no detailed information in the available literature on losses in *C. comatus* cultivation caused by fungi of the *Trichoderma* genus.

The aim of the performed investigations was to determine mutual interactions between the selected isolates of the *Trichoderma* genus and cultivated and wild strains of *C. comatus*.

MATERIALS AND METHODS

Strains of *C. comatus* used in the experiment are shown in table 1. They are derived from the mushroom collection of the Department of Vegetable Crops of the University of Life Sciences in Poznań, Poland.

Four isolates of fungi of the *Trichoderma* genus were used in the discussed experiment (Table 2). Identification of the two isolates of the *Trichoderma* genus used in the trial was carried out at the Institute of Plant Genetics, Polish Academy of Sciences in Poznan. Identification was done with the assistance of morphological observations and growth analyses of fungal cultures as well as molecular data based on the analysis of ITS1 and ITS2 sequences of rRNA coding genes and the sequence analysis of the *tef* gene segment coding the 1-a transcription elongation factor. One of the remaining two *Trichoderma* isolates was derived from the

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Table 1. Strains and origin of *C. comatus* used in the experiment

Strain	Date of harvest (2008)	Place of harvest	Site
Cp.211/B	VIII	Regional Directorate of State Forests (RDLP) Piła, Czołpa Forest District	meadow
Cp.316/1	IX	RDLP Piła, Krucz Forest District	brush
Cp.24/E	VII	RDLP Piła, Złotów Forest District	meadow
Cp.132/12	X	RDLP Szczecin, Goleniów Forest District	unused farmland
Cp.71/A/5	VIII	RDLP Szczecin, Karwin Forest District	brush
Cp.12/1	VI	RDLP Poznań, Pniewy Forest District	meadow
Cp.132/7	VII	RDLP Poznań, Oborniki Forest District	brush
Cp.407/M	X	RDLP Szczecinek, Borne Sulinowo Forest District	unused farmland
Cp.241/A	IX	RDLP Szczecinek, Czaplinek Forest District	meadow
Cop.43	–	Cultivar developed in the Department of Vegetable Crops of the University of Life Sciences in Poznań	–

Table 2. *Trichoderma* isolates used in the experiment

Isolate	Species	Origin
CBS 115901	*T. aggressivum* f. *europaeum*	Fungal Biodiversity Centre CBS Holland
T361	*T. aggressivum* f. *europaeum*	Vienna University of Technology
KW/27/5R	*T. longibrachiatum*	Jarocin mushroom farm
KW 36/11/B	*T. atroviride*	Kościan mushroom farm

Table 3. Score scale for the determination of individual biotic effect IBE (Mańka 1974)

Type of interaction between colonies	Points
Both colonies are in contact along a straight line	0
Colony A remains in contact with colony B along a slightly curved line so that it surrounds less than 1/3 of colony A	+1
Colony A remains in contact with colony B along a curved line so that it surrounds at least 1/3, but less than 1/2 of colony A	+2
Colony A remains in contact with colony B along a curved line so that it surrounds at least 1/2, but less than 2/3 of colony A	+3
Colony A remains in contact with colony B along a curved line so that it surrounds at least 2/3 or more of colony A	+4
Each millimetre of the inhibition zone is occupied by colony A	+1
Colony B is at least 1/3 but less that 1/2 smaller than its control colony developed individually on a separate plate	+2
Colony B is at least 2/3 smaller than its control colony developed individually on a separate plate	+3
Colony B completely undeveloped	+4

Fungal Biodiversity Centre of Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. The other isolate was from the Vienna University of Technology.

The experiment was carried out at the Department of Vegetable Crops, the University of Life Sciences in Poznań. A wheat-agar medium was used in the experiment since both *Trichoderma* genus fungi and *C. comatus* mycelium show good growth on it. *C. comatus* mycelium and mycelium of the *Trichoderma* isolate were inoculated 4 cm from each other, on Petri dishes. The mycelia of both the tested mushroom and competitive fungi inoculated on the Petri dish were characterized by the same sized discs which were 5 mm in diameter. A plastic tube of 5 mm in diameter was used to cut these discs out from the PDA substrate which was overgrown with the mycelium of the examined mushroom and fungi. The applied experimental cultures of the *Trichoderma* were 7 days old, whereas those of the *C. comatus* were 14 days old. Petri dishes with the inoculated media were incubated without light access at a temperature of 25°C, and at relative air humidity ranging from 80–85%. Every 24 hours, growth measurements of the mycelium of the examined species were performed and interactions between the developing mycelia were analysed. The individual biotic effect (IBE) was determined on the basis of the scale of biotic relationships elaborated by Mańka (1974) (Table 3). The extent of the surrounding of one colony by the other, the width of the inhibition zone, and growth confinement of the infestation of one colony by the other were observed.

RESULTS

The highest IBE index ranging from +3 to +5 was determined in the plate test for the two following strains of *T. aggressivum* f. *europaeum*: CBS 115901 and T361 (Table 4). Strain CBS 11 5901 exhibited a high IBE index which for six strains of *C. comatus* amounted to +4, for two strains in the interaction with *C. comatus* it amounted to +5 and in

Table 4. IEB index for *T. aggressivum* f. *europaeum*, *T. longibrachiatum* and *T. atroviride* isolates

C. comatus strain	*T. aggressivum*		*T. longibrachiatum*	*T. atroviride*
	CBS 11 59 01	T361	KW 27/5R	KW 36/11/B
Cp.211/B	+4	+5	+4	+3
Cp.316/1	+5	+4	+3	+3
Cp.24/E	+4	+5	+4	+3
Cp.132/12	+5	+5	+4	+3
Cp.71/A/5	+3	+4	+3	+2
Cp.12/1	+4	+4	+3	+3
Cp.132/7	+4	+5	+4	+4
Cp.407/M	+4	+3	+3	+2
Cp.241/A	+3	+4	+3	+3
Cop.43	+4	+5	+4	+3

two cases was lower and reached +3. An even higher IBE index was exhibited by the T361 strain. Its IBE, in the interaction with the examined *C. comatus* strains, amounted to +5 for five strains, to +4, for four strains and to +3, for one strain.

In the case of the *T. longibrachiatum* isolate, IBE indices varied and ranged from +3 to +4. The value of the IBE index amounting to +4 was determined in the case of 5 *C. comatus* strains.

The *T. atroviride* KW 36/11/B isolate exhibited a considerably varied IBE index in interactions with the examined *C. comatus* strains. The lowest IBE index in the above-mentioned isolate for Cp 71/A/5 and Cp 407/M strains amounted to +2. The highest index for this isolate, amounting to +4 was determined in the interaction with the Cp 132/7 strain. In all remaining cases, the IBE value for the *T. atro*viride isolate – KW/11/B amounted to +3.

DISCUSSION

There is no precise information in the available literature regarding the impact of fungi of the *Trichoderma* genus on the development of the *C. comatus* mycelium. In the discussed experiment, nine wild strains of *C. comatus* obtained from natural sites, and one cultivated strain were used. It is evident from the analysis of the obtained research results that *T. aggressivum* f. *europaeum* isolates confined the development of the *C. comatus* mycelium to a considerably greater extent than *T. longibrachiatum* and *T. atroviride* isolates. Furthermore, the obtained results revealed that the *C. comatus* mycelium of the examined strains exhibited an antagonistic reaction in relation to CBS 11 59 01 and T361 strains of the *T. aggressivum* f. *europaeum* species. According to the literature on the subject, *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus eryngii* showed an antagonistic response to the above-mentioned isolates (Savoie *et al*. 2001). It should be emphasized that no similar response was observed in the case of *A. bisporus* cultivation (Mamoun *et al.*, 2000). The performed investigations confirmed that *C. comatus* exhibits a certain defensive response in relation to aggressive strains of *T. aggressivum* f. *europaeum* similar to the mushroom species mentioned above. The inhibition of the *C. comatus* mycelium growth by the *T. longibrachiatum* and *T. atroviride* species was considerably less in comparison with the inhibition caused by *T. aggressivum* f. *europaeum*. The IBE index in the above-mentioned case amounted, mostly to +3, and in six cases to +4, and in two cases to +2.

On the basis of the performed investigations it can be concluded, that *C. comatus* strains exhibited a significantly greater antagonistic response in relation to isolates of various fungal species of the *Trichoderma* genus than *A. bisporus* as well as some species from the *Pleurotus* sp. genus. Earlier experiments carried out by the authors (Siwulski *et al*. 2009; Sobieralski *et al*. 2009) demonstrated that the examined *A. bisporus* and *P. ostreatus* strains failed to exhibit any capability for a defensive response in relation to *T. aggressivum* f. *europaeum* isolates. It can be said, on the basis of the obtained research results, that the *C. comatus* strains obtained from natural sites exhibited a mixed antagonistic response in relation to the isolates of the *Trichoderma* genus. Some of the examined wild strains: Cp 71/A/5 and Cp 407/M showed a considerable antagonistic response towards isolates of the *Trichoderma* genus. This response was definitely stronger compared to the cultivated Cop43 strain which was treated as a control. *C. comatus* strains derived from natural sites, which demonstrated the above-mentioned capability, can provide valuable genetic material with potential application in *C. comatus* creative breeding.

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POLISH SUMMARY

WPŁYW RÓŻNYCH IZOLATÓW GRZYBÓW RODZAJU *TRICHODERMA* NA ROZWÓJ GRZYBNI DZIKICH RAS CZERNIDŁAKA KOŁPAKOWATEGO *COPRINUS COMATUS* (MÜLL.) S.F. GRAY

Zbadano wpływ różnych izolatów *Trichoderma* na rozwój grzybni czernidłaka kołpakowatego *Coprinus comatus.* Wykorzystano dziewięć ras *C. comatus* pozyskanych ze stanowisk naturalnych, oznaczonych jako Cp.211/B, Cp.316/1, Cp.24/E Cp.132/12, Cp. 71/A/5, Cp.12/1, Cp.132/7, Cp.407/M i Cp. 241/A oraz jedną odmianę uprawną Cop. 43. Użyto izolatów *T. aggressivum* f. *europaeum:* CBS 115901 i T361, a także izolatów *T. longibrachiatum:* KW/27/5R *oraz T. atroviride:* KW 36/11/B. Doświadczenie przeprowadzono na pożywce agarowej-pszennej. Ustalono wskaźniki indywidualnego efektu biotycznego (IEB) pomiędzy grzybniami *Trichoderma* i *C. comatus*. Stwierdzono, że szczepy *T. aggressivum* f. *europaeum* w znacznie większym stopniu ograniczały rozwój grzybni *C. comatus* niż szczepy *T. longibrachiatum* oraz *T. atroviride*. Najwyższy wskaźnik IEB wahający się od +3 do +5 zaobserwowano dla obu szczepów *T. aggressivum* f. *europaeum* : CBS 115901 oraz T361.