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Ecological aspects of distribution of potential toxin-producing micromycetes on stored apple fruit

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The effect of ecological aspects of distribution of potential toxin-producing micromycetes on stored apple fruit was investigated at the Lithuanian Institute of Horticulture and Institute of Botany in 2004–2005. Fungi of twenty species belonging to eight genera were isolated from rotten and healthy apple fruits. *Penicillium expansum* and *Penicillium italicum* showed a high frequency of occurrence and were isolated from 50% and 17% healthy apples and from 83% and 67% of rotten fruits respectively. Seventeen species belonging to five genera were isolated from the storehouse air and dust. Eight species belonging to three genera were isolated from storehouse air. *Aspergillus amstelodami, A. clavatus, Cladosporium cladosporioides, C. herbarum, Penicillium clavigerum, P. corymbiferum, P. oxalicum, P. roqueforti* were identified as airborne fungi in the storehouse. Six species (*Acremonium roseum, Alternaria alternata, Aspergillus repens, Penicillium expansum, P. janthinellum, P. lanosum*) belonging to four genera were isolated from the storehouse dust. Two species, *Aspergillus ustus* and *Penicillium cyclopium*, were identified in the storehouse air and dust.

Key words: apples, micromycetes, microbial contamination

INTRODUCTION

During the past few decades, phytotoxins have been employed as tools contributing to fundamental discoveries in plant pathogenesis, host specificity, the mechanisms of resistance and susceptibility, secondary metabolism, fungal genome organization, plant cell and organelle functions and fungal ecology (Bondy, Pestka, 2000; Dunkle, 2005).

A good understanding of the ecological factors that favour infection and the production and growth of toxins is an essential condition for setting up effective strategies for reducing the level of mycotoxins in agricultural production. Mould and toxins develop under certain specific conditions of temperature, humidity and the level of gases in the air (Andersen, Frisvad, 2002). Contamination of fruit by fungi causes not only high post-harvest losses, but also could be a source of toxic substances harmful to humans (De Vries et al., 2002). More than 100000 species of fungi by 10% are plant pathogens and less than 100 species of fungi are responsible for the majority of post-harvest diseases (Eckert, Ratnayake, 1983; McCollum, 2002). The most important post-harvest diseases of apple are blue mould caused by *Penicillium expansum* Link, brown rot caused by *Monilinia fructigena* Honey, *Gloeosporium* rot caused by *G. album* Osterw and *G. fructigenum* Berk. (McCollum, 2002; Amiri, Bompeix, 2005). The leading position in toxic micromycetes belongs to the genera *Penicillium* Link., *Aspergillus* Link, *Fusarium* Link, *Alternaria* Nees (Lugauskas, Stakėnienė, 2001).

The aim of the work was to investigate the ecological aspects of contamination of stored apples and to elucidate the potential toxin-producing micromycetes.

METHODS

The effect of the ecological aspects of the distribution of potential toxin-producing micromycetes on stored apple fruit was investigated at the Lithuanian Institute of Horticulture and Institute of Botany in 2004–2005.

There were inspected 300 fruits (100 from each of three replicate trees) of the same maturity, size and free of physical damage and fungal infection in each control plot. The yield was kept in a storehouse with controlled storage conditions: the temperature was adjusted to 0–2 ˚C and relative air humidity to 90%. The orchard management activities and fungicide spray program were carried out according to recommendations accepted at the Lithuanian Institute of Horticulture (Uselis et al., 2005).

Microbial contamination was tested immediately after apple harvesting and after three months of storage in a storehouse. To isolate micromycetes, 1 cm^2 pieces of apples were plated onto Malt, Czapek Dox and Potatoes Dextrose agars. Plates were incubated at 26 ± 2 ˚C

for seven days in the dark. Grown fungi were isolated and ascribed to taxonomic groups following Ainsworth and Bisby's handbook (eighth edition, Hawksworth et al., 1995). The identification was performed according to particular manuals (Raper, Thom, 1949; Raper et. al., 1965; Gams, 1971; Ellis, 1971, 1976; Boerema, Dorenbosch, 1973; Милко, 1974; Domsch et al., 1980; Ramirez, 1982; Brandenburg, 1985; Samson et al., 2000). The distribution frequency (%) was calculated according to Мирчинк (1988).

RESULTS AND DISCUSSION

Fungi of twenty species belonging to eight genera were isolated from rotten and healthy apple fruits (Table 1). From healthy apples, eight species belonging to four genera were isolated. *Penicillium expansum* and *Penicillium italicum* showed a high frequency of occurrence and were isolated from 50% and 17% of healthy apples, and from 83% and 67% of rotten fruits respectively. From healthy apples, other species were isolated with insignificant frequency. Mycological analysis of rotten fruits yielded 19 fungal species belonging to seven genera. *Penicillium cyclopium* (28%), *P. roqueforti* (28%), *P. corylophilum* (25%), *Rhizopus oryzae* (33%), *Trichoderma viride* (38%) were isolated with moderate frequency (Table 1).

In a previous investigation, fruits and berries grown in Lithuania were infected most often by such fungi as *Sclerotinia sclerotiorum, Absidia butleri, Alternaria alternata, Drechslera biseptata, Sphaerotheca morsuvae, Aspergillus niger, Eurotium herbariorum, Geotrichum fermentans* and by numerous species of the genus *Penicillium* Link. The predominant species depended on the species of fruit or berries and on the surrounding ecological conditions (Lugauskas, Stakėnienė, 2001). Apples were mostly contaminated by *Penicillium expansum* and *P. italicum* fungi. The diversity of fungal species was higher on stored apples than on those freshly harvested (Levinskaitė et al., 2005). Similar results were recorded by H. Hasan (2000), R. Castoria et al. (2005). The genus *Penicillium* fungi can produce a variety of toxins: ochratoxin, patulin, citrinin, penicillic acid, roqoefortine, penitrem, etc. *Aspergillus* fungi can excrete aflatoxins, ochratoxin, sterygmocystins, tremorgens, versicolorins, malformins and cytochalasins, etc. *Fusarium* is known to produce trichotecenes, ipomenanols, moniliformin, zearalenone, and *Alternaria* excrete alternariol, altenuene, altenusin and altertoxins, etc. (De Vries et al., 2002; Frisvad, 2005). One of the most widespread fungi is *Penicillium expansum*, particularly distributed on apples; it is known as a patulin producer (Samson et al., 2000; Malmauret, 2002).

The widespread fungus *P. italicum* produces deoxibrevianamide E and toxins of the tremorgen group. *Aspergillus niger*, which was also found on the apples tested, is known to produce toxins of malformines and naphtopyrene groups. There were found fungi of the species *Penicillium claviforme, P. roqueforti,* etc., which can be producers of patulin (Andersen et al., 2004; Amiri, Bompeix, 2005; Frisvad, Thrane, 2005). It should be mentioned that species of the genus *Penicillium* do-

Table 1. **Distribution of fungi on healthy and rotten apple fruits**

Fungal species	Distribution frequency, %	
	Healthy fruits	Rotten fruits
Acremonium roseum Petch	4	0
Alternaria alternata (Fr.) Keissl.	0	22
Aspergillus niger Tiegh.	3	17
Cladosporium cladosporioides (Frensen.) G. A. de Vries	0	5
Cladosporium herbarum (Pers.) Link	2	10
Fusarium lateritium Nees	0	13
Fusarium oxysporum Schltdl.	0	10
Penicillium biforme Thom	4	20
Penicillium brevicompactum Dierckx	10	17
Penicillium cyclopium Westling	0	28
Penicillium corylophilum Dierckx	0	25
Penicillium clavigerum Demelius	0	8
Penicillium corymbiferum Westling	0	10
Penicillium expansum Link	50	83
Penicillium italicum Wehmer	17	67
Penicillium janthinellum Biourge	0	9
Penicillium lanosum Westling	0	5
Penicillium roqueforti Thom	13	28
Rhizopus oryzae Went et Prins.	0	33
Trichoderma viride Pers.	0	38
Total species	8	19

Table 2. **Potentially toxin-producing micromycete species isolated from storehouse environment**

Micromycetes	Air	Dust
Acremonium roseum Petch	-	+
Alternaria alternata (Fr.) Keissl.	-	+
Aspergillus amstelodami (L. Mangin) Thom et Church	+	-
Aspergillus clavatus Desm.	+	-
Aspergillus repens (Corda) Sacc.	-	+
Aspergillus ustus (Bainier) Thom et Church	+	+
Cladosporium cladosporioides (Fresen.) G. A. de Vries	+	-
Cladosporium herbarum (Pers.) Link	+	-
Penicillium cyclopium Westling	+	+
Penicillium corylophilum Dierckx	-	+
Penicillium clavigerum Demelius	+	-
Penicillium corymbiferum Westling	+	-
Penicillium expansum Link	-	+
Penicillium janthinellum Biourge	-	+
Penicillium lanosum Westling	-	+
Penicillium oxalicum Currie et Thom	+	-
Penicillium roqueforti Thom	+	-
Total species	10	9

minated among potential toxin producers (Druch, Ragab, 2003).

Seventeen species belonging to five genera were isolated from storehouse air and dust (Table 2). Eight species belonging to three genera were isolated from the storehouse air. *Aspergillus amstelodami, A. clavatus, Cladosporium cladosporioides, C. herbarum, Penicillium clavigerum, P. corymbiferum, P. oxalicum, P. roqueforti* were identified as airborne fungi in the storehouse.

Six species belonging to four genera (*Acremonium roseum, Alternaria alternata, Aspergillus repens, Penicillium expansum, P. janthinellum, P. lanosum*) were isolated from storehouse dust (Table 2). Two species, *Aspergillus ustus* and *Penicillium cyclopium*, were identified in the storehouse air and dust.

However, the micromycetes that produce mycotoxins also develop on organic products in storage and in storage environment. *Acremonium roseum, Cladosporium cladosporioides, Penicillium clavigerum, P. corymbiferum* were detected in the storehouse air and on rotten apple fruits. *Alternaria alternata, Penicillium cyclopium, P. corylophilum, P. janthinellum, P. lanosum* were found in storehouse dust and rotten fruits (Tables 1, 2). Three species, *Cladosporium herbarum*, *Penicillium expansum* and *P. roqueforti*, were found on healthy and rotten fruits, and only *Penicillium roqueforti* was recorded in the storehouse air and dust. *Cladosporium herbarum* was found in the storehouse air, and *Penicillium expansum* was detected in the storehouse dust (Tables 1, 2). It is possible to suppose that micromycetes found in the storehouse environment were able to evoke a mixed fungal contamination on fruits. However, in-depth epidemiological studies on this point are needed to elucidate the role these micromycetes of storehouse environment may be playing in the pathologies of organic products (Andersen et al., 2004).

Penicillium and *Aspergillus* species are common and important moulds in the human environment and are among the main agents of spoilage of human and animal food. Many species are known to produce a diversity of toxins (Druch, Ragab, 2003; Hasan, 2005). A number of common moulds on decaying leaves, fruits are able to colonize celluloses building materials. Some species of *Alternaria, Cladosporium* are known to produce toxins. Moderate levels of these fungi are common as a result of outdoor air exchange with the interior of the building (Bondy, Pestka, 2000). The majority of mycotoxins are chemically stable and resistant to changes in temperature, conditions of storage and processing procedures.

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POTENCIALIŲ TOKSINUS PRODUKUOJANČIŲ MIKROMICETŲ PLITIMO ANT LAIKOMŲ OBUOLIŲ EKOLOGINIAI ASPEKTAI

S a n t r a u k a

2004–2005 m. Lietuvos sodininkystės ir daržininkystės institute ir Botanikos institute tirti potencialių toksinus produkuojančių mikromicetų plitimo ant laikomų obuolių ekologiniai aspektai. Nuo sveikų ir puvinių pažeistų obuolių nustatyti 20 rūšių mikromicetai, priklausantys 8 gentims. Nuo sveikų obuolių išskirti aštuonių rūšių mikromicetai, priklausantys keturioms gentims. *Penicillium expansum* ir *P. italicum* buvo labiausiai išplitę ant sveikų ir puvinių pažeistų obuolių: 50 ir 17% ant sveikų obuolių bei 83 ir 67% ant puvinių pažeistų obuolių. Kitų rūšių mikromicetai ant sveikų obuolių pasireiškė nežymiai. Puvinių pažeistų obuolių mikologinė analizė parodė, kad potencialiai toksiškų mikromicetų rūšių buvo daugiau, nustatyta 19 rūšių mikromicetai, priklausantys 7 gentims.

Ištyrus sandėlio aplinkos orą ir dulkes, nustatyti 17 rūšių mikromicetai iš penkių genčių. *Aspergillus amstelodami, A. clavatus, Cladosporium cladosporioides, C. herbarum, Penicillium clavigerum, P. corymbiferum, P. oxalicum, P. roqueforti* rūšių mikromicetai išskirti iš sandėlio oro. *Acremonium roseum, Alternaria alternata, Aspergillus repens, Penicillium expansum, P. janthinellum, P. lanosum* rūšių mikromicetai išskirti iš sandėlio dulkių. Dviejų rūšių mikromicetai *Aspergillus ustus* ir *Penicillium cyclopium* išskirti iš sandėlio dulkių ir oro.

Raktažodžiai: obuoliai, mikromicetai, mikrobiologinė tarša