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ORIGINAL PAPER

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INCIDENCE OF POSTHARVEST FUNGAL DISEASES OF APPLES IN INTEGRATED FRUIT PRODUCTION

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ABSTRACT

In 2014–2017 an investigation was carried out into the occurrence of fungal storage diseases of five apple varieties (Red Jonaprince, Gala, Golden Delicious, Gloster and Ligol) in the Sandomierz orchard region. The fruit was stored at a CA cold storage room with ULO controlled atmosphere for six months. Occurrence of eight storage diseases was found. The most frequently occurring disease was bull's eye rot and the losses caused thereby were even 24% of the affected fruit. The cultivars most susceptible to this disease were the 'Golden Delicious' and 'Ligol' apples; the least susceptible were the 'Gloster' ones. The apples were significantly less affected by the fungi that cause brown rot, grey mould rot, blue mould rot and apple scab. Very seldom were the symptoms of calyx end rot, mouldy core and core rot, and anthracnose. Varying severity of infection of the varieties was noted in each season of observation.

Key words: apple, postharvest fungal diseases, CA/ULO long storage, identification, incidence, integrated fruit production, cultivars

INTRODUCTION

Poland is the largest producer of apples in the EU (almost 37% of the total harvest in 2018) and one of the largest exporters of this fruit in the world. The occurrence of a large number of storage diseases, which cause sizeable financial losses to growers, has been observed in Poland for many years. Apple pathogens comprise a large group of fungi. The types of diseases caused by them are highly varied among countries. Their incidence also depends on the variety, weather conditions during the growing period and cultivation operations. After the harvest, the occurrence of diseases is influenced by the storage conditions as well as proper care to avoid damaging the fruit (wounds) in the course of sorting and preparing it for market. The prevalence of various apple rots may be different for each variety. In our climatic conditions the most dangerous disease affecting the fruit during storage is bull's eye rot. Using PCR techniques, Michalecka et. al [2016a] found that the disease is caused by three fungi belonging to genus *Neofabraea – Neofabraea alba* (Guthrie) Verkley, *Neofabraea perennans* Kienholz, *Neofabraea kienholzii* (Seifert, Spotts & Lavesque). It was also demonstrated that the predominant species is *N. alba*. This disease is poses a comparably grave threat in other European countries and the USA [Cameldi et al. 2016, Pesicova et al. 2016, Vico et al. 2016, Aquilar 2017, Kingsnorth et al. 2017]. The impact of each of the apple pathogens can change radically over the period of 10–15 years. This might be affected by the process in which pathogens causing fruit rots become resistant to some of the active substances used as fungicides [Blazek et al. 2006, Weber and Palm 2010, Grantina-Ievina 2015]. The global warming contributes to the constant ap-

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pearance of new species of pathogenic fungi which have thus far been unknown in many regions where apples are grown. During the last 50 years, there has been observed a considerable variation in terms of the fungal species and their respective effect on fruit infections in different parts of the world. The increase in the average annual temperature has caused the appearance of such apple pathogens as *Glomerella acutata* Guerber & Correll, *Diplodia seriata* De Not., *Phacidiopycnis washingtonensis* Xiao & Rogers, *Colletotrichum* spp., *Phacidium lacerum* Fr. and *Shpaeropsis pyriputrescens* Xiao & Rogers [Xiao et al. 2004, Weber 2011, Mari et al. 2012, Ivic et al. 2013, Baroncelli et al. 2014]. A major problem with recognising the symptoms and identifying the pathogens that cause apple rots is the hidden (i.e. latent) character of the course of the disease. The infection occurs before the harvest in the orchard and the signs of the disease commonly appear first after several months of controlled atmosphere (CA) storage. Most fungi infect the fruit before the harvest and the gates of infection are lenticels. Some rot-causing fungi infect apples through wounds (mechanical damage to the skin), also inside cold storage warehouses; they are the so-called wound pathogens. This concerns in particular *Penicillium expansum* Link. and *Botrytis cinerea* Pers. They colonise a lot of non-disinfected box pallets and cold rooms, and having a without large infection potential (spores) they infect the fruit. The prevalence of storage rots is effectively reduced by fungicide spraying applied 1–3 weeks prior to the harvest. However, the use of such preparations immediately before harvesting involves the risk of too high levels of pesticide residues in apples [Poulsen et al. 2009, Weber and Palm 2010, Szymczak et al. 2016]. An alternative for the chemical method might be the use of biological preparations or treating apples with hot water after the harvest.

The aim of the investigation was to provide a comprehensive assessment of the incidence of storage diseases on apples of the five most popular cultivars grown in orchards managed using integrated production.

MATERIALS AND METHODS

The investigation was carried out during three storage seasons in 2014–2017. Apples came from orchards located in the north-west part of the Sandomierz orchard region (Zdanów). The trees were planted on M9 rootstock in 2004. The investigation comprised five apple cultivars: 'Red Jonaprince', 'Gala', 'Golden Delicious', 'Gloster' and 'Ligol'. The orchards were managed in accordance with the Integrated Production System. Standard disease prevention measures (mainly with regard to apple scab) were applied in them. Fruit of each cultivar was protected chemically against storage diseases 1–3 weeks prior to the harvest. The harvest date for the individual cultivars was determined each year based on the starch test and a measurement of firmness. After being harvested and preliminarily cooled, the apples were transported to a CA/ULO-storage room. Storage parameters were +1°C; O_2 – 1.5% and CO_2 – 2.5%. The fruit was stored in plastic box pallets. After 6 months 2500 fruits from each variety were chosen for research. After being taken out of the cold room the box pallets were left in rooms with a temperature of 15–17°C. After 7 days the assessment was commenced in order to determine the extent of the infection by pathogenic fungi. Each disease was identified on a preliminary basis based on the aetiological signs and characteristic lesions (such as rotting or discolouration). Where there were substantial difficulties in identifying individual lesions (especially in the initial phase of their development), laboratory methods were used. The affected parts of the fruit were rinsed and disinfected, and then put in Petri dishes containing PDA medium. Following a number of cleavages pure fungal cultures were obtained and these were subsequently identified microscopically using mycological keys.

RESULTS

The cultivars included in the investigation were characterised by varied susceptibility to fungal storage diseases (Tab. 1). Over the 3 seasons of the study the highest infection rates were found for the 'Golden Delicious' cultivar. In the 2014/2015 season, 28.5% of the apples were infected, followed by 12.3% in 2015/2016 and 22.2% in 2016/2017. Equally high susceptibility to fungal storage diseases was found for the 'Ligol' cultivar. In the 2014/2015 season infection was found on 26.5% of the apples. In subsequent years storage diseases affected 12.8% (2015/2016) and 7.8% (2016/2017) of the apples. Lower suscepti-

Table 1. The occurrence of postharvest fungal diseases of apple in integrated production

Cultivar season storage	Apple fruits with symptoms of fungal diseases after 6 months storage (%)							
	BE*	BR	BM	GM	CR	AA	AS	ZG
Red Jonaprince								
2014/2015	1.5ab**	1.5i	1.0f	1.2f	0.1c	0.2c	1.0i	0a
2015/2016	4.0c	3.0l	0.6e	2.0h	0a	0a	0.2c	0.1b
2016/2017	2.5bc	1.0f	0.2b	0.8e	0.1b	0a	0a	0.2c
Mean	2.7B	1.8D	0.6D	1.3D	0.006B	0.006B	0.4C	0.1B
Gala								
2014/2015	10.4e	0.8e	0.2b	0.3c	0.1b	0a	0.3d	0a
2015/2016	4.8d	1.5i	0.4d	0.1a	0a	0a	0.6g	0a
2016/2017	6.6d	2.0k	0.1a	0.8e	0a	0a	0.1b	0a
Mean	7.2C	1.3C	0.2A	0.4B	0.003A	0A	0.3B	0A
G. Delicious								
2014/2015	21.1h	3.5m	1.2g	1.4g	0.7f	0.1b	0.4e	0.1b
2015/2016	9.8e	1.2g	0.1a	0.8e	0.2d	0a	0.1b	0.1b
2016/2017	15.0g	4.0n	0.1a	2.2i	0.1b	0.2c	0.5f	0.1b
Mean	15.3E	2.9E	0.4C	1.4E	0.3C	0.1C	0.3B	0.1B
Gloster								
2014/2015	0.8a	1.8j	1.0f	1.2f	1.2g	0a	0.8h	0.1b
2015/2016	1.0 ab	0.1a	0.2b	0.3c	0.3g	0.1b	0.1b	0a
2016/2017	0.2a	0.4d	0.1a	0.1a	0.5e	0.1b	0.6g	0.2c
Mean	0.7A	0.8B	0.4B	0.5C	0.7D	0.06B	0.5D	0.1B
Ligol								
2014/2015	24.1i	1.3h	0.1a	0.6d	0.1b	0a	0.2c	0.1b
2015/2016	12.0f	0.3c	0.2b	0.1a	0a	0a	0.1b	0.1b
2016/2017	6.7d	0.2b	0.3c	0.2b	0a	0a	0.3d	0.1b
Mean	14.2D	0.6A	0.2A	0.3A	0.03A	0A	0.2A	0.1B

* Symbols: BE – bull's eye rot (*Neofabraea alba*, *Neofabraea perenans*); BR – brown rot (*Monilinia fructigena*); BM – blue mould rot (*Penicillium expansum*); GM – grey mould rot (*Botritis cinerea*); CR – calyx end rot (*Sclerotinia sclerotiorum*, *Neonectria galligena*, *Alternaria alternata*, *Fusarium lateritium*, *Fusarium avenaceum*, *Phoma* spp.); AA – anthracnose (*Colletotrichum acutatum*); AS – apple scab (*Venturia inaequalis*); ZG – core rot and mouldy core (*Alternaria alternata*, *Fusarium lateritium*, *Fusarium avenaceum*).
** Means having same letter were not significantly different by Fishers comparison at $p < 0,05$ level.
Lowercase letters indicate the means for each year, capital letters main factors

bility was found for the 'Gala' cultivar where 12.1% of all the apples showed symptoms of disease in the 2014/2015 season. In subsequent storage seasons the infection rates were 7.4% and 9.6%. A significantly smaller number of infected fruit was observed for the 'Red Jonaprince' cultivar. 'Gloster' was the cultivar with the lowest number of fruit with symptoms of disease. In the first year the rate was 6.9% while in subsequent years it was only 2.1% (2015/2016) and 2.2% (2016/2017). A very interesting issue concerns the percentage of each storage disease in the cultivars included in the study. In 2014/2015, a total of 28.5% of apples of the 'Golden Delicious' cultivar showed symptoms of disease. However, as many as 21.1% within this group of fruit were apples with symptoms of bull's eye rot. An even higher percentage of bull's eye rot was observed for the 'Gala' cultivar (10.4%) with 12.1% of infected fruit. For the 'Ligol' cultivar, 26.5% of the apples were infected out of which as many as 24.1% were fruit with symptoms of bull's eye rot. The other diseases only accounted for very small percentage percentages, i.e. brown rot (1.3%); grey mould (0.6%); storage scab (0.2%); blue mould, calyx end rot and mouldy core and core rot (0.1% each).

Completely different ratios were observed for the 'Gloster' cultivar (6.9% of infected fruit) where bull's eye rot only accounts for 0.8%; brown rot 1.8%; blue mould 1.0%; grey mould 1.2%; calyx end rot 1.2%; storage scab 0.8% and mouldy core and core rot 0.1%.

In the 2015/2016 season a lower incidence of storage diseases was observed as compared with the 2014/2015 season with the exception of the 'Red Jonaprince' cultivar (9.9% of infected fruit). The highest infection rate was found for the 'Ligol' cultivar (12.8%) where bull's eye rot accounted for as many as 12.0% of the cases. The other diseases only accounted for very small percentage percentages, i.e. brown rot (0.3%); blue mould (0.2%); grey mould, storage scab and calyx end rot (0.1% each). Also in 2015/2016 'Gloster' was the least infected cultivar (2.1% of apples). Here, however, the share of bull's eye rot increases to 1.0% with a lower proportion of the fungi causing brown rot, blue mould, grey mould, calyx end rot and storage scab. On the other hand, there appear symptoms of apple anthracnose caused by *Colletotrichum acutatum* Simmonds (0.1%).

In the 2016/2017 season a lower infection rate was found compared with the previous years. 'Golden Delicious' was the most infected cultivar (22.2%) and the share of bull's eye rot was 15.0%. A higher rate of infection by fungi of genus *Monilinia* was observed (4.0%) as well as the highest percentage of apples infested by *B. cinerea* (2.2%) causing grey mould in the three seasons. Another important disease in that season was storage scab (0.5%). The share of the other diseases was small, i.e. in the range of 0.1–0.2%. The lowest number of storage diseases was noted for the 'Gloster' cultivar (2.2%). However, the largest shares were accounted for by apples with symptoms of apple scab (0.6%), calyx end rot (0.5%) and brown mould (0.4%). Bull's eye rot and mouldy core and core rot only accounted for 0.2% each. Due to the specific structure of the calyx, this cultivar is characterised by a large shares, as compared with other cultivars, of calyx end rot (0.5%) and mouldy core and core rot (0.2%). A small percentage (0.1%) of apple anthracnose was also found here. In summarising the results of the 3-year observation, it should be stated that bull's eye rot was the most commonly occurring storage disease. Its share among all the diseases was high at 70.2% on average in the three seasons. The prevalence of the other diseases was significantly lower (Tab. 1).

DISCUSSION

Fungal diseases pose a particular threat to apples during their growing period as well as after the harvest during the storage. In the moderate climate and the absence of appropriate crop protection, the most substantial loss of crops may be caused by apple scab (*V. inaequalis*). On the other hand, bull's eye rot is currently the most prevalent fungal disease in Poland and many countries around the world [Bryk and Kruczyńska 2005, Giraud and Bompeix 2012, Soto-Alvear et al. 2013, Grantina-Ievina 2015]. In some years it causes storage losses reaching even over 50%. The causative agents are fungi of genus *Neofabraea* (*N. alba*, *N. perennans*, *N. malicorticis* and *N. kienholzii*). The occurrence of the three above species, except *N. malicorticis*, has been confirmed in Poland and the Czech Republic [Michalecka et al. 2016b, Pesicova et al. 2016]. In our own studies, only the occurrence of

N. alba (90%) and *N. perennans* (10%) was confirmed in the course of identifying isolates. The species that is definitely prevalent in Europe is *N. alba*. In Serbia *N. alba* is the only species considered to be the causative agent of the disease [Vico et al. 2016]. Research has confirmed the high vulnerability of the 'Golden Delicious', 'Ligol' and 'Gala' cultivars to bull's eye rot. The susceptibility of each cultivar to this disease may depend on the number of lenticels as the gates of infection on the fruit skin. The extent of infestation also depends on the harvest maturity of the apples. Differences with regard to infestation of each cultivar in each season may be caused by the weather conditions in the pre-harvest period; this is especially true of moisture content. The most important factors determining fruit infestation are the sanitary conditions in the orchard (presence of sources of infection) and the crop protection programmes. At present, the most effective method is the application of appropriate fungicides in the pre-harvest period [Blazek et al. 2006, Jonsson et al. 2009, Vico et al. 2016]. The second most common disease was brown rot caused by *M. fructigena* (13.2%). Symptoms of the disease were most frequently found on fruits of the 'Golden Delicious' cultivar where between 1.2% and 4% of the apples were infected. The least affected cultivar was 'Ligol' (0.2–1.3%). Blazek et al. [2006], Ivic et al. [2012] and Novotny et al. [2019] found in their studies that infestation of apples by *Monilinia fructigena* Aderh. et Ruhl. was at a similar level. The greater extent of infestation was due to the large infection potential of the pathogen in the form of mummified fruit and infected shoots in the crowns of trees. They were a source of primary infections like in the case of *Neofabraea* spp. The next disease in terms of the amount of infected fruit is grey mould. In many studies this disease is considered as the second most prevalent after bull's eye rot. In the course of our own study varied extent of infestation of the cultivars was observed in each year. The largest amounts of apples infected by *B. cinerea* were found for the 'Golden Delicious' (1.4%) and 'Red Jonaprince' (1.3%) cultivars. Konstantinou et. al. [2011] in Greece demonstrated that *B. cinerea* is one of the most common (23.6%) pathogens that cause rotting in stored apples. Ivic et al. [2012] and Novotny et al. [2019] report that *B. cinerea* infects apples in the ranges of 0.2–0.53% and 0.15–0.35%, respectively. This pathogen belongs to the group of fungi that infect fruit through skin defects (wounds). Infections may occur after harvesting during storage and marketing. A high level of infestation by *B. cinerea* and *P. expansum* as well as *M. fructigena* may indicate bad sanitary conditions inside the storeroom. Blue mould, the causative agent of which was *P. expansum*, occurred less frequently and its share among all the diseases was 3.4%. This disease occurs in different orchard regions and affects between 0.1% and 6.6% of fruit in integrated cultivation [Ivic et al. 2013, Juhnevica-Radenkova et al. 2016, Novotny et al. 2019]. Apple scab *Venturia inaequalis* (Cooke) Winter is an example of a disease where the infection and growth of the pathogen takes place in the orchard during the growing period. Symptoms of late infections are hardly noticeable and become apparent first during storage in the form of the so-called storage scab. The investigation revealed that scab symptoms were most common among the 'Gloster' (0.5%) and 'Red Jonaprince' (0.4%) cultivars. It also occurred to a small extent in each year of observation during the investigation reported in this paper. The occurrence of signs of disease at the storeroom indicates inappropriate crop protection during the growing season. Florian et. al [2018] reports that apples with symptoms of storage scab may account for between 17.7% and 25% of the affected fruit. Calyx end rot and mouldy core and core rot has so far been less prominent in Poland. In addition to *Sclerotinia sclerotiorum* (Lib.) De Bary, calyx end rot may be caused by a number of other pathogenic fungi. In the course of the tests and observations for this paper, several other fungi were identified to species. The most frequently isolated ones were *B. cinerea*, *Neonectria galligena* (Bres.) Rossman & Samuels, *Alternaria alternata* (Fr.) Keissl., *Fusarium lateritium* Ness and *Phoma* spp. Similar results were obtained by Weber and Dralle [2013] who described blossom end rot. Mouldy core and core rot was mainly caused by *A. alternata*, *F. lateritium* and *Fusarium avenaceum* (Fr.) Sacc. An interesting fact is the occurrence of apple rot caused by *C. acutatum* which is termed in Poland as apple and pear anthracnose. This species occurred in regions with a considerably more moderate climate and posed a threat to apple orchards. It has occurred in small amounts for several years but its prevalence is increasing. In the course of the inves-

tigation, its incidence was confirmed on fruit of the 'Red Jonaprince', 'Golden Delicious' and 'Gloster' cultivars. There are suggestions that the prevalence of the disease is actually greater but due to the similarity with the early stages of development it was classified as bitter rot.

CONCLUSION

1. The biggest threat to orchards in integrated production are fungal diseases, which have a high infectious potential in mummified fruit and infected shoots. This applies particularly to bull's eye rot and brown rot.

2. Fungi that cause blue mold and gray mold rot mainly infect apples through wounds after harvest.

3. Global warming has contributed to the emergence of new pathogens. An example is apple rot caused by *C. acutatum*, whose incidence is increasing.

4. A variation was found in the extent of infestation between the cultivars in each year of observation. This might have been caused by varied weather conditions contributing to infection, varied harvest maturity of the apples as well as the crop protection programmes that were used in the pre-harvest period.

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