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THE OCCURRENCE OF SOFT ROT (*JANTHINOBACTERIUM AGARICIDAMNOSUM*) IN MUSHROOM (*AGARICUS BISPORUS*) CROPS

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Abstract: This study evaluated the development of soft rot disease (*Janthinobacterium agaricidamnosum*) in *Agaricus bisporus* crops. Symptoms of the disease appeared at the end of the first break of the mushroom's harvest and mostly occurred in the later stages of cultivation (in the second break and between the 1st and 2nd crop). Distribution of lesions in growing rooms indicated that employees served as a vector of disease infection, which was spread when harvesting the mushrooms. An evaluation of the post-harvest mushrooms showed that fruiting bodies, especially those stored in closed containers, showed disease symptoms when infected at harvest.

Key words: *Agaricus bisporus, Janthinobacterium agaricidamnosum*, mushrooms, mushroom diseases, soft rot

INTRODUCTION

Bacteria in mushroom crops are common. They are present in the growing medium, casing soil, and production environment. Bacteria play an important role in mushroom production, especially in the formation of fruit bodies. However, some of the species occurring in mushroom crops are pathogens (Szumigaj and Szymanski 2009). Most of pathogenic bacteria in cultures of *Agaricus bisporus* belong to the genus *Pseudomonas* (Largeteau and Savoie 2010). In recent years, there has been more information about the incidence of different mushroom diseases. These diseases are displayed through the rapid decay of individual fruiting bodies. Apart from a few rare earlier cases of soft rot, this disease was not actually known until about decade ago (Desrumaux *et al.* 2004). On the other hand, a similar disease occurring in the UK on *A. bitorquis*, had already been described in the 1990s, but *A. bitorquis* was grown at a much higher temperature and humidity than button mushrooms (Lincoln *et al.* 1991).

Two bacterial species causing mushroom rotting were identified: *Burkholderia gladioli* pv. *agaricicola* and *Janthinobacterium agaricidamnosum* sp. nov. *Burkholderia gladioli* develops mainly in tropical and sub-tropical conditions, which explains its presence in *A. bitorquis* crops (Atkey *et al.* 1991; Lincoln *et al.* 1991). On the other hand, the incidences of *Janthinobacterium* are closely linked to the production of *A. bisporus* (Lincoln *et al.* 1999; Fermor and Lincoln 2000).

Bacterial soft rot in mushrooms does not usually cause significant yield losses but can have a very devastating impact on the profitability of fungi producing companies. Good quality mushrooms, collected in the growing room and delivered to the recipient, melt into shapeless, wet stuff after several hours of rapid rotting. Soft rot can have significant and long-term consequences for producers because it discourages consumers from buying mushrooms.

There is very little information in the literature about the development of soft rot during mushroom growth, and no reports about the development of the disease during storage of the crop.

In this study, the development of the disease under industrial mushroom production practices as well as the development of the disease during mushroom storage were analyzed.

MATERIALS AND METHODS

Monitoring of disease symptoms in infested mushrooms

Samples of mushrooms with clear symptoms of the disease were collected in the summer of 2010 from a Polish, industrial-scale, mushroom producing farm. Infested fruiting bodies were collected and transported in containers at 4°C to the laboratory for analysis. Observation of symptoms and photographic documentation were done.

Isolation of biological material

Bacteria (*J. agaricidamnosum*) were isolated by cutting off pieces of rotting mushrooms with a sterile scalpel or by taking swabs of the mushroom surface using sterile swab sticks. The collected material was transferred to a test tube and suspended in 50 mM of phosphate buffer. The cell suspension was transferred to a Petri dish which

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had King B medium (Pseudomonas F Agar, Difco). Single colonies of bacteria were obtained by serial dilution and re-transferred into the medium for identification.

Identification of *J. agaricidamnosum*

Individual colonies were identified on the basis of physiological tests and the use of nutrients, as previously described (Lincoln *et al.* 1999). Pathogenicity of *J. agaricidamnosum* strains was confirmed by applying 100 µl of suspension containing 10^6 cells on mushrooms fragments and incubating them at 20°C in a closed container. Lignin dipped in water was placed inside the container to maintain 85–95% humidity. As the control, 100 µl of water was applied to sections of mushroom fruit bodies, which were incubated under identical conditions.

Cultures from the air and air conditioners

Air from indoor rooms and the air conditioning systems (about 500 l) was analyzed for cultures using the portable tester M Air T air (Millipore).

Induced infection of the crop

Randomly selected healthy mushrooms from the 1st break were infected. One hundred µl of inoculum, prepared beforehand, was applied to the surface of the fruit bodies. Infected mushrooms were marked for further observations. As for the control, mushrooms grown under the same conditions and which were not infected, had 100 µl of water applied to their surface. The sample consisted of 15 repetitions.

Infection during harvest

A total of six experimental variants were prepared. Healthy, good-looking fruit bodies were selected on the second day of the 1st break after harvest. After being infected with 100 µl of bacterial inoculum which had been prepared beforehand, the mushrooms were placed in suitable containers along with other healthy mushrooms. Variants of the experiment using 3 kg open plastic crates and 250 g boxes tightly wrapped in plastic film are shown in table 1.

Statistical analyzes

Observations were performed in triplicate. The results were evaluated statistically using the analysis of variance by Statistica v.10 (Statsoft) software. The significance of differences was assessed with the Fischer-Snedecor test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Bacterial strains of *J. agaricidamnosum* – causal agent of mushroom soft rot (Lincoln *et al*. 1999) – were isolated from the infested, rotting mushrooms found in the growing rooms of a Polish mushroom farm. Strains of *Klebsiella planticola* and *Enterobacter cloacae* were also isolated from the fruiting bodies when the mushrooms were at the stage of complete decomposition.

Infection in the production environment

In the initial phase of infection, some infested fruiting bodies were observed in the cultivation halls. Their number did not exceed 1 pcs./100 m^2. Despite the successive removal of symptomatic mushrooms from the facility, the infection remained at a comparable level in near-by rooms. After the first observed disease symptoms, the fungi rapidly increased and showed serious symptoms of soft rot infestation. Single rotting fungi were already observed at the end of the 1st break. Then, a drastic increase in the number of infected mushrooms was observed between the 1st and 2nd break. A large number of infected fruit bodies was also present during the second break, despite the removal of infected mushrooms from the growing room (Fig. 1).

It is assumed that the optimum temperature for *J. agaricidamnosum* growth is between 16 and 18°C, and the maximum temperature is about 30°C (Fermor and Lincoln 2000). In addition to temperature, relative humidity (RH) is also very important during mushroom cultivation. Studies with brown mushroom varieties and hybrid varieties of type U3 indicated that RH clearly affects the development of the disease in brown mushroom varieties. There were significantly less symptoms of the disease, when mushrooms were grown in a RH of 85% compared to 95%. Studies based on artificial infection demonstrated that there were no signs of the disease at a relative humidity of less than 71.5%, some discoloration was observed on the fruiting bodies at 80% humidity, clear signs of decay were present at 85% RH, and obvious widespread mushroom rotting symptoms were recorded at 95% humidity (Lincoln *et al.* 1999).

The studies presented in this paper do not show a clear correlation between the intensity of observed disease symptoms and the climatic conditions in the mushroom growing room (Fig. 2). The highest incidence of soft rot symptoms was observed between the 1st and 2nd mushroom break and second break when the RH was relatively low; approximately 85%, while no signs of disease

Table 1. Packaging and temperature variants during storage of mushrooms

Designation of the experimental variant	Type of container	Storage temperature
Sk4	open plastic crate	4°C
Sk20	open plastic crate	20°C
Sk4-20	open plastic crate	4°C for 5 days, and then 20°C
Pu4	wrapped box	4°C
Pu20	wrapped box	20°C
Pu4-20	wrapped box	4°C for 5 days, and then 20°C

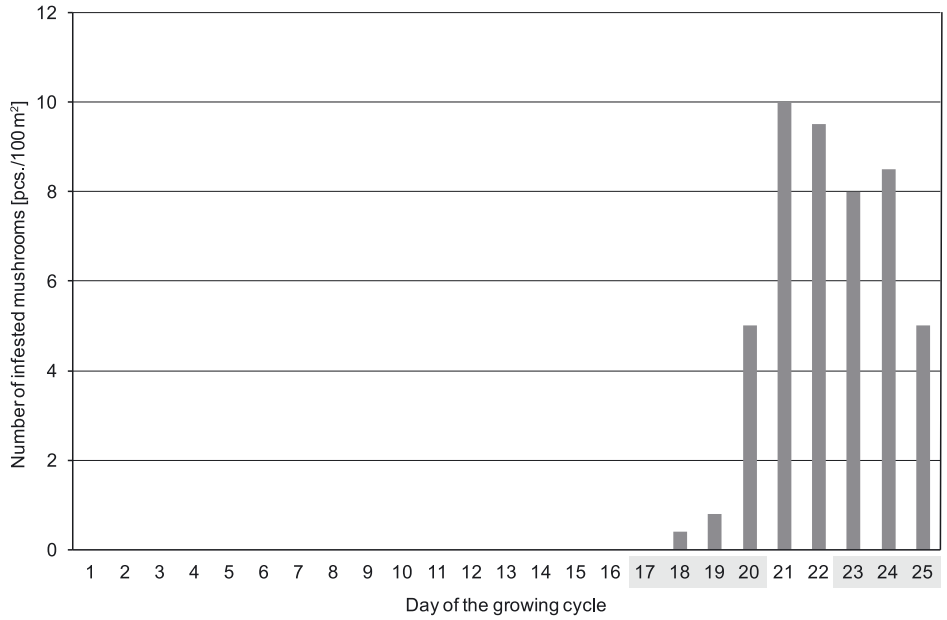
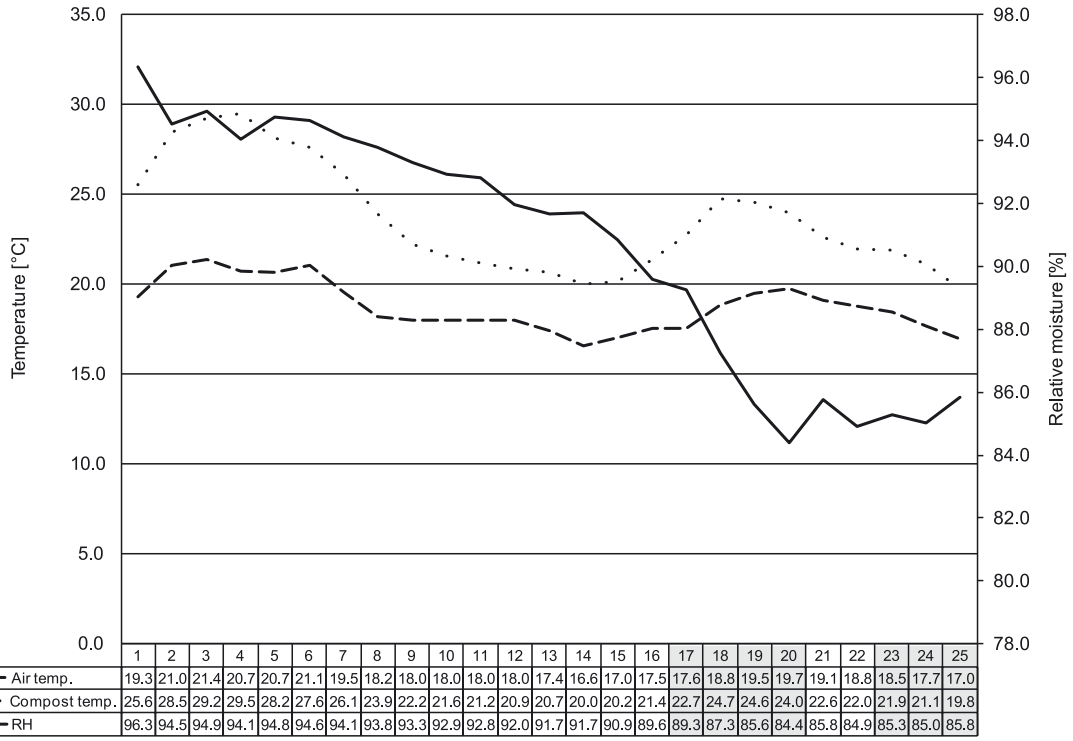


Fig 1. Number of mushrooms with advanced-stage, soft rot symptoms (pcs./100 m²). Days of 1st and 2nd break are indicated with grey color respectively, days 21 and 22 – period between full harvests (between break)



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Air temp.	19.3	21.0	21.4	20.7	20.7	21.1	19.5	18.2	18.0	18.0	18.0	18.0	17.4	16.6	17.0	17.5	17.6	18.8	19.5	19.7	19.1	18.8	18.5	17.7	17.0
Compost temp.	25.6	28.5	29.2	29.5	28.2	27.6	26.1	23.9	22.2	21.6	21.2	20.9	20.7	20.0	20.2	21.4	22.7	24.7	24.6	24.0	22.6	22.0	21.9	21.1	19.8
RH	96.3	94.5	94.9	94.1	94.8	94.6	94.1	93.8	93.3	92.9	92.8	92.0	91.7	91.7	90.9	89.6	89.3	87.3	85.6	84.4	85.8	84.9	85.3	85.0	85.8

RH – relative humidity

Fig. 2. Air and compost temperature and relative moisture during mushroom cultivation. Grey color indicates full harvest of 1st and 2nd break, respectively

were observed at an RH higher than 89% at the beginning of the first break. The relatively late onset of disease symptoms in the growing cycle and disease development with reduced humidity, indicate crop contamination through employee contact. Such contact takes place during technical procedures, like crop watering, mushroom picking, *etc*. Desremaux *et al*. (2004) showed that the development of disease during mushroom cultivation depends on the moment of infection. Slow development of the disease was observed in the early stages of cultivation (filling with the compost, loading the casing). The first symptoms were visible at the beginning of the first break of mushrooms. However, when the infection took place while mushrooms were growing on shelves (regardless

of the stage of development) the disease was very active. Complete rotting of the infested fruiting bodies was observed within 48 h after infection.

The spread of the infection during the harvest

Infections during mushroom cultivation can be spread: by air movement, and when watering the crops, or by contact with employees and insects. In the presented study, cultures were spread from the air in the growing hall, and air in the air-conditioning systems. As a result, the presence of *J. agaricidamnosum* was not recorded in the air. These observations confirm the previous assumptions that spread of soft rot through the air is unlikely (Desrumaux *et al.* 2004). In this study, the location of the lesions were analyzed in the cultivation room. The occurrence of infected fruiting bodies as it spread across the growing hall is shown in figure 3.

During the early stages, infested fruiting bodies were sparse at random locations (Fig. 3A). However, the lesions showed a pattern which could be seen during the development of the disease in the growing hall. There were clearly more diseased fruiting bodies on the edges of the cultivation shelves (zones marked with a dashed line in figure 3B). The distribution of lesions indicates that a human factor was involved in the spreading of soft rot. It seems that during harvest operations, workers moving along the shelves contributed to the spread of disease. Previous studies also pointed to a contact route for the spread of infection (Desrumaux *et al.* 2004). In some cases, the pathogen remained on workers' hands after touching 40 fruiting bodies within a row.

Infection under the experimental conditions

Later steps focused on the analysis of the disease development and its stages. For this purpose, surfaces of randomly selected mushrooms growing in the cultivation room were infected with 100 µl of previously prepared inoculum. Development of soft rot symptoms is shown in figure 4.

As a result of artificial infection, 86.7% of fruiting bodies showed signs of infestation. The remaining 13.3% of mushrooms did not show disease symptoms and did not differ from the untreated control treatments. In the case of infection, distinct soft rot symptoms were observed and the symptoms progressed very rapidly (Fig. 4 I, J). Symptoms were usually visible and continued to progress after 48 hours and faster than 60 hours after inoculation. In the first 24 hours, darkening discolorations (Fig. 4 A–E) were

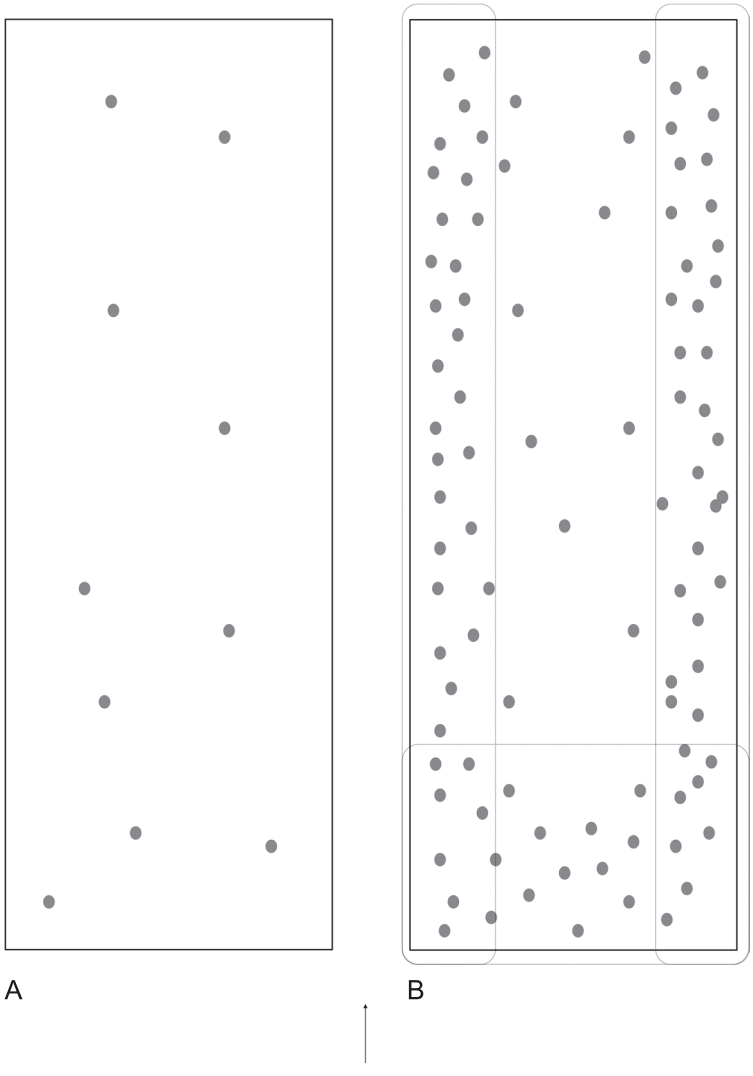


Fig. 3. Summary of the observed lesions' locations in the cultivation room. A – beginning of the infection, B – the period of maximum severity. Arrow indicates the location of the entrance to the hall

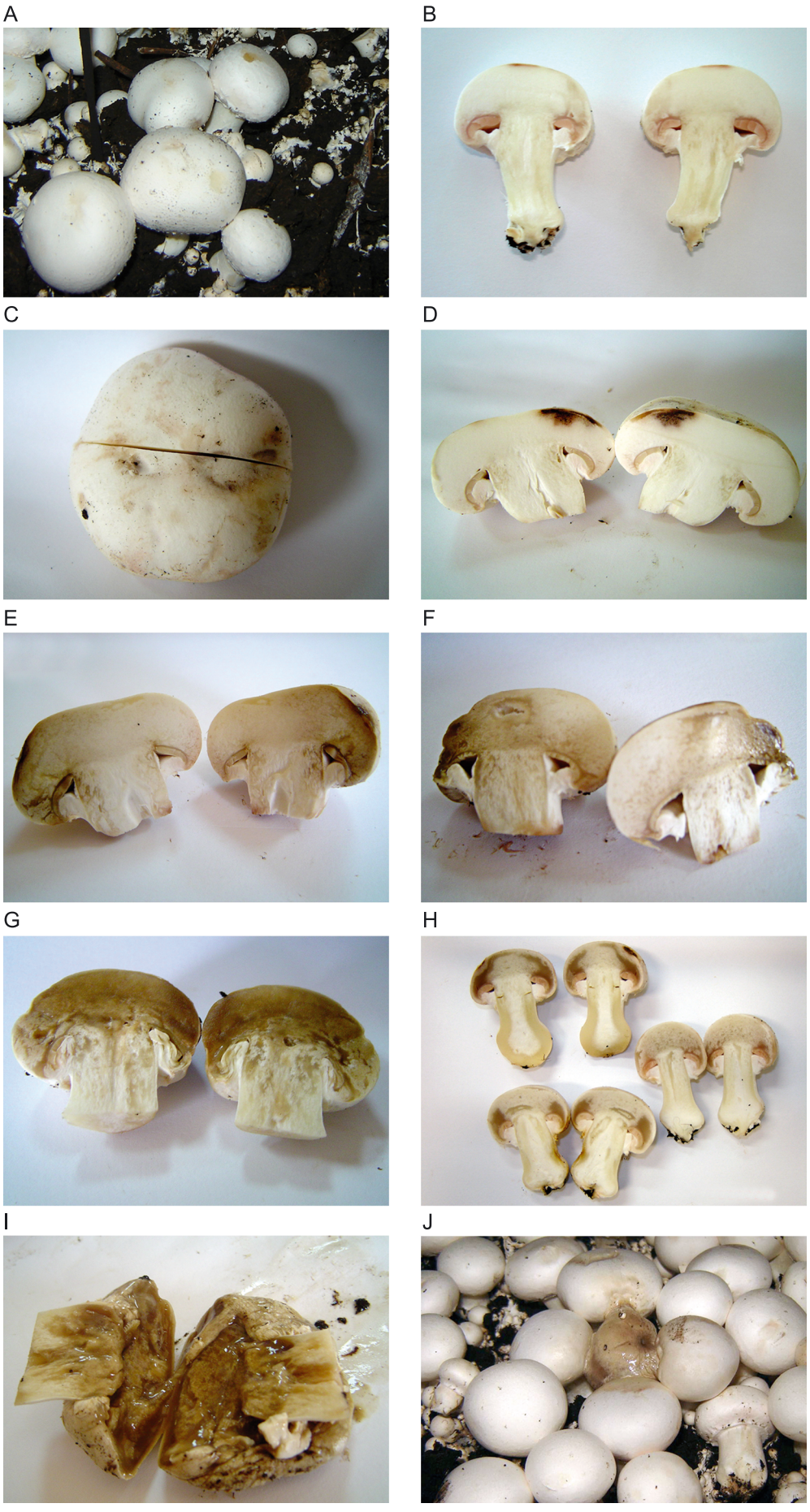


Fig. 4. Symptoms of mushrooms soft rot during the development of the disease. Further description in the text

visible on mushroom caps. Over the next 12–24 hours discolored spots developed into either a partial loss of the cap structure (Fig. 4 F), or substantial damage to the internal structure of the fruiting body, with no apparent change in the fruiting body shape (Fig. 4 G, H). Fruiting bodies showed a slight change in color – from white to a bright shade of gray. These observations confirmed that it is surface contact which is the most likely way that soft rot is spread during mushroom cultivation. It should be noted, that despite the fact that the disease developed quickly, during the first several hours after infection, the external appearance of the diseased mushrooms was only marginally different from the healthy fruiting bodies grown on the same shelves. Under industrial conditions, diseased fruiting bodies are collected together with the highest quality mushrooms, and then divided into separate containers.

Post-harvest disease development

These observations of soft rot development during mushroom cultivation, verify and supplement the limited information from previous studies carried out in similar conditions (Desrumaux *et al.* 2004). However, no published studies are available on the development of soft rot when the infection occurs during harvest. An attempt was made to analyze the development of soft rot after mushrooms harvesting, during the storage stage of the mushroom.

The experiment was carried out with 6 variants. Healthy and good-looking fruiting bodies were selected immediately after harvest, on the second day of the 1st break. After infecting them with a previously prepared 100 µl inoculum, mushrooms were placed in containers along with healthy mushrooms. This was designed to simulate industrial conditions. Variants of the experiment were: 1) mushrooms packed in 3 kg openwork open plastic crates and stored at 4°C (Sk4) and room temperature (Sk20), 2) mushrooms packed in 250 g plastic boxes tightly wrapped in stretch film and stored at 4°C (Pu4) and at room temperature (Pu20), and 3) identical crates and boxes used but mushrooms stored at 4°C till the 5th day after collection and then transferred to a room with room temperature (denoted Sk4-20 and Pu4-20, respectively). The results of the experiment suggest that late infection, even at the time of harvest, can have a significant commercial impact (Table 2). This disease can also develop under refrigerated conditions. It seems that only the packaging of mushrooms in well-ventilated openwork boxes may help minimize the risk of the disease spread on the final product (Sk4). The present observations indicate a high rotting of mushrooms packed in tightly sealed containers.

Table 2. Symptoms of soft rot during storage of infected mushrooms

Experimental variant	Percentage of symptomatic mushrooms during storage [%]	The time from infection to the first obvious symptoms [h]
Sk4	0	nd
Sk20	60	72
Sk4-20	30	144
Pu4	20	168
Pu20	100	60
Pu4-20	100	144

CONCLUSIONS

1. Soft rot of mushrooms, caused by *J. agaricidamnosum*, is present in Polish mushroom farms.
2. Soft rot spreads by contact, especially by the contact by employees of the mushroom farm.
3. Infection of fruiting bodies during harvest leads to development of disease infections during the cold storage of mushrooms.
4. Well-ventilated containers stored at 4°C reduced the risk of soft rot in harvested mushrooms.

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