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MICROBIOLOGICAL CHARACTERISTICS OF THE WĘGIERKA ZWYKŁA PLUM ORCHARD IN SUBMONTANE REGION

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Key words: orchard, plum, microflora of trees, air and soil

Study samples were collected at six sites in the Węgierka Zwykła plum orchard at five different time points (from April to October 1999). Quantitative and qualitative composition of microflora inhabiting plum trees significantly depended on their occurrence in soil and air. Qualitative microbial analysis revealed the presence of 30 species of bacteria, 20 species of yeasts and 60 cultures of moulds. The highest counts in the samples were found for microorganisms of the genera *Micrococcus*, *Staphylococcus*, *Rhodotorula*, *Cladosporium*, *Penicillium* and *Trichoderma*. Yeast strains belonging to the genera *Rhodotorula*, *Candida* and *Debaryomyces*, *Aerobasidium*, and filamentous fungi of *Rhizopus nigricans* species, characterised by relatively good ability of fermentation of many sugars, dominated on tree surface.

INTRODUCTION

Soil and water are the main natural environmental habitats of microorganisms, in which they find adequate supply of nutrients to survive disadvantageous external conditions, such as low temperature in winter [Conclin, 2002].

The highest counts in the atmosphere have been observed for moulds (up to 20 000 cfu/m^3), mostly of the genera *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* [Stępalska *et al.*, 1999], and yeasts (up to 60 000 cfu/m^3), with the prevalence of *Candida* strains incapable of fermentation, besides less abundant yeasts possessing fermentation ability. Air usually contains also bacterial cells (up to 10 000 cfu/m^3) and actinomycetes with counts from 1 to 2 000 cfu/m^3 [Krzysztofik, 1992]. Sporogenous genus *Bacillus* and aerobic micrococci were the most common groups among aerobacteria.

Microorganisms are transmitted to tree and fruit surface by air or insects, in which they play a role of symbionts in their alimentary tract [Morais *et al.*, 1995]. Microbiological contamination of fruits occurs also by their direct contact with the ground (grass, soil) during harvest.

Composition of microflora inhabiting the surface of vegetative plant parts (leaves, bark) depends on air contamination, particularly at the beginning of spring, when leaves start to develop. Subsequently, strains adapted to utilisation of cellulose, the main carbon source in this environment, begin to dominate. Qualitative and quantitative analyses have indicated high diversity of microorganisms, dependent on plant species. In general, filamentous fungi (genera *Cladosporium*, *Alternaria*, *Fusarium*, *Epcioccum*) and yeast-like organisms (genus *Aureobasidium*) prevail, and their counts range between 500–10 000 cfu/cm^2, with bark showing much higher colonisation [Davenport, 1974; Buck, 1998]. Frequently, both yeast strains capable and incapable (genus *Rhodotorula*) of fermentation appear also on plates.

Similar microflora to that occurring on the surface of vegetative plant parts can be isolated from flowers and fruits. Yeasts and yeast-like organisms, usually with good fermentative ability, are the most widespread groups in this environment. The studies on cherries demonstrated the highest counts of the genera *Alternaria*, *Aureobasidium* and *Cladosporium*, constituting 87–100% of all filamentous fungi [Olszak, 1994 a, b]. On the other hand, representatives of the genera *Kloeckera* and *Hanseniaspora* were reported to make up 50–75% of the whole yeast population on grapes [Davenport, 1974]. These tendencies were also confirmed by analysis of other fruits [Dugan & Roberts, 1994; Morais *et al.*, 1995].

Hitherto conducted studies have indicated that quantitative and qualitative composition of microorganisms inhabiting air, soil and trees in orchards is determined by a number of ecosystem characteristics, local atmospheric conditions, fruit species and degree of their ripeness [Davenport, 1974; Krzysztofik, 1992; Hasnain, 1993; Spotts & Cervantes, 1994].

The aim of the present analyses was to establish quantitative and qualitative characteristics of soil, air, tree and fruit microflora in the Węgierka Zwykła plum orchard located in the area of Łącko, Poland. The chosen study area is famous of production of original plum brandy by the method of spontaneous fermentation. The samples were collected at five different time points (from April to October 1999), each time from six sites (designated with letters A – F). Bacteria, yeasts and filamentous fungi isolated from plates were identified and preserved for further studies.

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MATERIALS AND METHODS

Microbiological analysis was carried out in many-years--old unmanaged Węgierka Zwykła plum orchard (about 1 hectare), not subjected to any chemical protection or other agrotechnical procedures, which is located in submontane region (Łącko municipality) in the area of the Beskid Wyspowy mountains on the ridge of a local hill (526 m). There are farm buildings to the north of the orchard and mixed forest with spruce dominance to the south. The area is open to the east and west, so usually E or SE air currents prevail.

Method of isolation of the study material was adjusted to its type and site of its origin (Figure 1). Soil samples for microbiological analysis were collected from a depth of 10 cm. Approximately 1 g of soil was transferred to a mortar and gently mixed with fine quartz sand, and the obtained mixture was plated on plates filled with Martin-Johnson medium (fungi) or nutritional agar for bacterial cultures [Mańka, 1974].

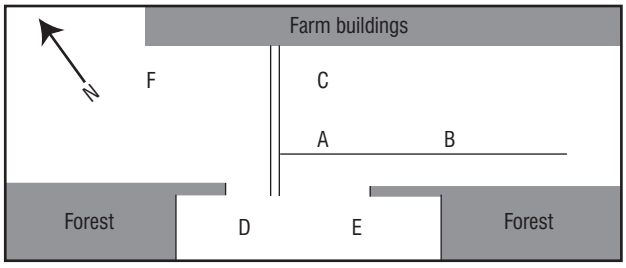

FIGURE 1. Schematic representation of location of study sites in the orchard.

Microorganisms were isolated from air by sedimentation method, exposing horizontally-placed Petri dishes with appropriate medium (nutritional agar for bacteria and actinomycetes or wort agar for moulds and fungi) to the air for 5 min [Krzysztofik, 1992].

Microflora from fruits, leaves, bark and flowers (organic matter) was isolated by rinsing their surface with sterile physiological saline, and these solutions, after appropriate dilution, were plated on plates with the medium suitable for microorganisms under investigation.

All microbial cultures were incubated at 28°C for 72 h, successively diagonally cut as colonies appeared, and then monocultures were identified on the basis of their morphological (macro- and microscopic) and biochemical features using appropriate microbiological keys [Buchanan & Gibbon, 1974; Barnett *et al.*, 1983; Fassatiova, 1983]. The results presented in the figures are arithmetic means of two parallel examinations.

RESULTS

Soil microflora

Qualitative analysis of soil microflora revealed its wide diversity dependent on both, study period (Figure 2) and sample collection site (Figure 1).

Bacterial microorganisms dominated in terms of counts with minimum value reaching 7.2×10^6 cfu/g of soil in June and maximum of 26.9×10^6 cfu/g of soil observed in July.

Among the study sites, those designated as D and E (Figure 1), located on the slope close to the forest were

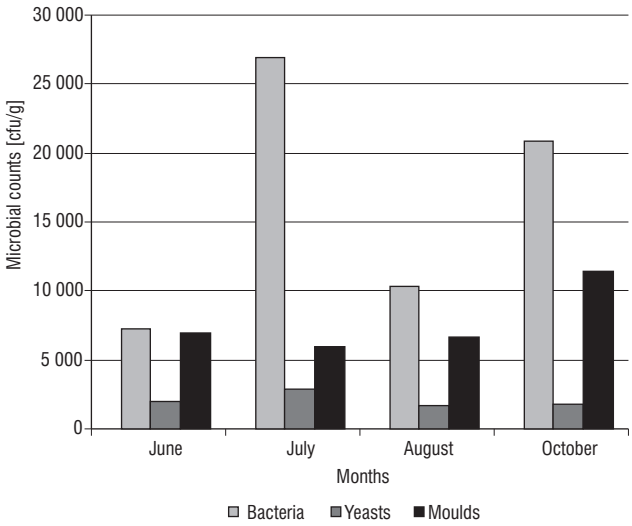

FIGURE 2. Average microbial counts in soil samples collected during vegetative season (bacteria $\times 10^3$; yeasts and moulds $\times 10^2$).

distinguished by relatively high bacterial counts (from 21.1×10^6 to 30.0×10^6 cfu/g of soil) while only 5.9×10^6 cfu were isolated at site F. Considerable variations in counts of bacterial cells inhabiting surface soil layers at different sites in the orchard could be caused by such factors as the influence of the adjacent forest, not uniform composition of grass cover and different pH values of the samples (Table 1).

TABLE 1. Soil pH at the selected study sites in the orchard.

Study site	pH			
	June	July	August	October
A	5.53	5.68	5.98	6.38
E	5.60	5.63	5.58	6.40
F	6.10	6.20	6.62	6.68

The results of qualitative analysis of bacterial microflora confirmed its high diversity in the orchard. Among 17 species of bacteria belonging to 8 genera, Gram-positive micrococci of family *Micrococcaceae* were the most abundant while bacteria of genus *Bacillus* were classified the second in terms of counts. Probably pH fluctuations precluded development of nitrobacteria, which are very demanding with regard to growth conditions and do not occur in environment whose pH value drops below 6.0.

It is worth noting that there is a relationship between counts of representatives of *Micrococcaceae* and *Bacillus* families in soil and air. In soil, an increase in counts of microorganisms of *Micrococcaceae* family was accompanied by a decrease in *Bacillus* counts while parallel proportion in the air was opposite.

In contrast to bacteria, yeast counts were less diversified. The highest yeast counts were noted in the samples collected in July (2.8×10^5 cfu/g) and remained at a similar level throughout the study period, averaging about 1.7×10^5 cfu/g of soil.

Similarly as in the case of bacteria, yeast were also most abundant at sites D and E (approximately 2.8×10^5 cfu/g of soil). In total, 10 yeast species were identified, with the dominance of strains of the genera *Rhodotorula* and *Candida* lacking fermentative ability.

Moulds were relatively less abundant group of microorganisms, and their counts were usually inversely proportional to bacteria and yeast counts. Maximum concentration of filamentous fungi was observed at the beginning of autumn, in October (1.1×10^6 cfu/g of soil) while the lowest soil fungi content was noted in July amounting to 0.6×10^6 cfu/g. It should be noted that the study sites where bacteria and fungi dominated were characterised by lower mould counts. The highest numbers of filamentous fungi were found in soil in the orchard section bordering the forest (sites D and E) and at sites exposed to wind (site F, Figure 1).

Isolation procedure allowed to obtain from soil 289 pure fungal cultures, which were classified by further studies as 50 species belonging to 27 genera, including 2 fungi defined as asporogenous (*Dematiaceae* and *Mucedinaceae*).

In addition, wide qualitative differences were revealed between fungal communities inhabiting different study sites.

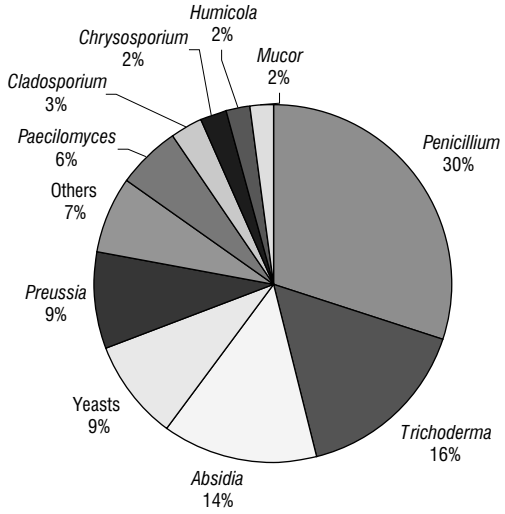

FIGURE 3. Soil fungal microflora in the studied orchard.

Microbiological analysis indicated that spores of four fungi genera prevailed in soil (about 60%): *Penicillium*, *Trichoderma*, *Absidia* and *Preussia*, while remaining genera constituted less than 7% of the population. Genus *Penicillium* (about 45% of the population) was identified at site F (Figure 1) mostly due to very high counts of *P. aurantiogriseum*.

The following species of filamentous fungi were observed at all three sites: *Absidia spinosa*, *Chrysosporium pannorum*, *Mucor piriformis*, *Paecilomyces carneus*, *Penicillium aurantiogriseum*, *Penicillium fellutanum*, *Preussia aemulans*, *Preussia fleischhakii,* and *Trichoderma koningii*.

Analysis of the combined results of isolation of microorganisms from all study sites revealed domination of fungi of genus *Penicillium* (about 30% of the population) species of family *Mucoraceae* (17%) and genus *Trichoderma* (16%), while contribution of yeasts was much lower amounting to about 9% of fungal population (Figure 3).

Air microflora

Air is an environmental buffer zone between soil and plant organisms, such as trees. However, very low amount of nutrients in air hinders reproduction of microorganisms. Hence, air microflora counts are much lower in comparison with soil and trees, while qualitative composition of air microflora is a resultant of both these habitats.

Relatively the highest counts of microorganisms were observed in the samples collected at site B located between farm buildings and the forest (5 200 cfu/m^3).

Bacterial counts significantly varied between the sites (from 255 to 4 345 cfu/m^3) and sampling periods (from 1 500 in April and October to more than 6 000 cfu/m^3 in June) (Figure 4).

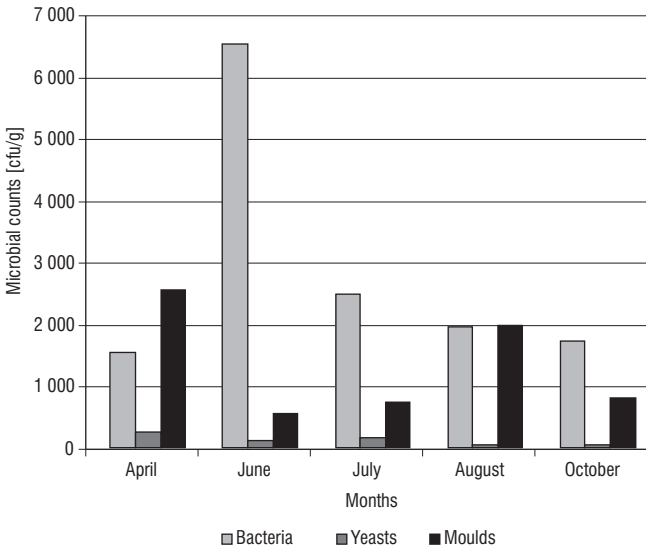

FIGURE 4. Average counts of microorganisms in air during vegetative season.

Among bacteria, microorganisms of genera *Micrococcus*, *Staphylococcus* and *Bacillus* dominated, being observed on almost all plates. Remaining groups of bacteria were present in the samples collected in April and October, but their contribution to the isolated bacterial microflora was minimal.

The contents of filamentous fungi at different study sites were highly diversified. Their highest numbers (1 500–1 800 cfu/m^3) were isolated from the samples taken in the zone shielded by clumps of forest trees, which corresponds to sites D and F. It should be emphasised that counts of fungi in atmosphere were inversely proportional to bacterial counts.

The samples collected in April, August and September contained from 1 700 to 2 500 cfu/m^3 of air. In subsequent months counts of filamentous fungi remained at much lower level (450–600 cfu/m^3).

In total, about 30 species of different filamentous fungi were classified, with their presence depending principally on sampling period.

The genus *Cladosporium* definitively prevailed (41% of the whole fungal population), followed by *Rhizooctonia* (13%), *Alternaria* (9%), *Aspergillus* (7%), and *Botrytis* (Figure 5).

Yeasts occurred in the air sporadically (up to 160 cells/m^3). Plate analysis did not show domination of any genus, but the presence of four species was demonstrated, which were characterised by the lack of fermentation ability and belonged to three genera: *Candida*, *Lipomyces* and *Rhodotorula*.

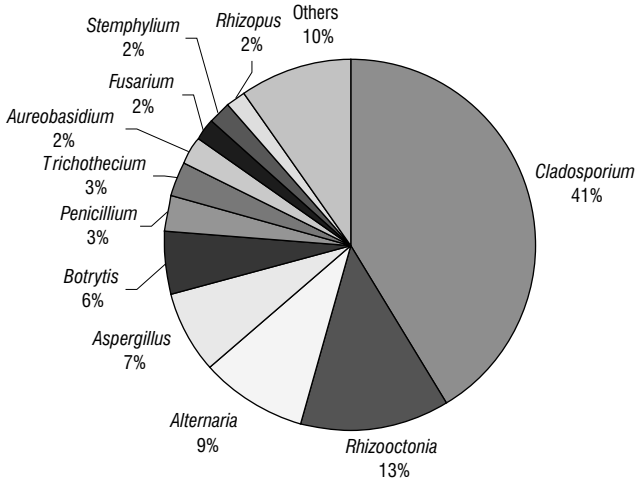

FIGURE 5. Fungal microflora in the air in the studied orchard.

Microflora inhabiting fruits, bark and leaves of the Węgierka Zwykła plum tree

Bacteria were the most abundant group in plum microflora.

Their counts in 1 g of organic material averaged about 2.72×10^7 cfu (Figure 6). The largest numbers of bacterial colonies were observed in June (4.87×10^7 cfu/g). Subsequent measurements demonstrated their gradual decrease, which can be explained by a drop in mean daily temperature.

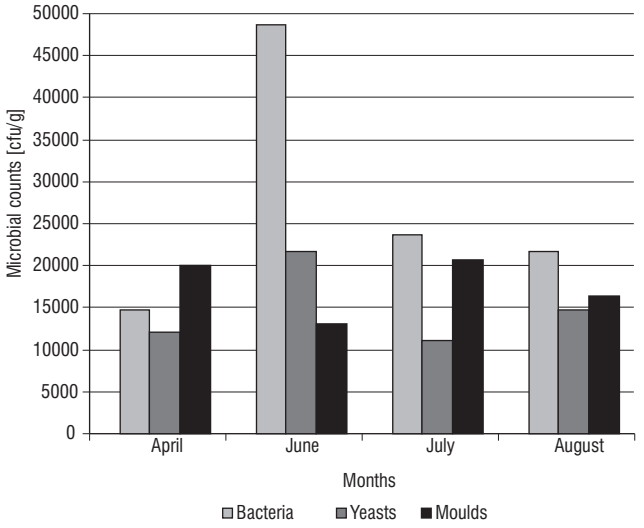

FIGURE 6. Average counts of microorganisms on the Węgierka Zwykła plum trees in vegetative period (bacteria × 10^2, yeasts and moulds × 10).

The most advantageous conditions for growth of these microorganisms were found at site B, most exposed to blows of wind, from where twice as much bacteria were isolated on the average as from other study sites.

Yeast microflora was also an abundant group inhabiting both fruits and bark. Similarly as in the case of bacteria, an increase in yeast counts was observed in June (2.2×10^5 cfu/g), while minimum was reached in July (1.1×10^5 cfu/g). The increased temperature in July (above 30°C) probably hindered development of some genera of yeasts [Barnett *et al.*, 1983]. Site B proved the most beneficial for yeast growth, similarly as for bacteria (Figure 1).

Qualitative characteristics of the strains isolated from plums indicated relatively high contribution of yeasts possessing fermentative ability, with the domination of *Debaromyces hansenii*, which constituted over 30% of all yeast colonies. Yeasts of the genus *Rhodotorula*, producing pink carotenoid dyes, were the second most abundant group.

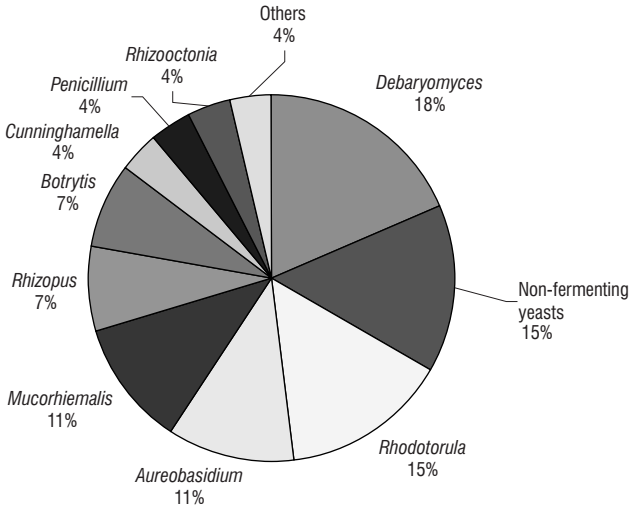

FIGURE 7. Fungal microflora on the Węgierka Zwykła plum trees in the studied orchard.

The content of mould spores on plums and bark of plum trees substantially differed from remaining microflora. Maximum mould counts were observed in April and July, amounting each to about 2.0×10^5 cfu/g of the studied material, while the value estimated in June was as low as 1.3×10^5 cfu/g.

In comparison with other sampling sites, the most abundant not only bacterial and yeast microflora but also moulds were isolated from site B.

Microbiological analyses of trees in the Węgierka Zwykła plum orchard allowed to isolate a total of 20 mould species, belonging mostly to the order *Mucorales* (*Mucor* and *Rhizopus*), and genera *Botrytis*, *Fusarium*, *Aspergillus* and *Penicillium* (Figure 7).

DISCUSSION

The studies conducted in the Węgierka Zwykła plum orchard demonstrated wide diversity of microflora both in terms of its quantitative and qualitative characteristics. The observed diversity resulted from features of the environment at sampling sites (soil, air, trees), period of sample collection and location of sampling site.

Significant factors determining composition of microflora in soil, air and on trees, included sampling period, with essential contribution of weather and environmental conditions (mostly temperature, humidity, insolation, pH), and degree of fruit ripeness.

Soil in the orchard under examination was characterised by acid pH values (Table 1), which is the most optimal for development of moulds, thus their counts remained at relatively high level (from 0.6×10^6 to 1.1×10^6 cfu/g). The obtained picture was similar to the results of microbiological studies of soil in apple orchard [Mazzola, 1998]. Parasitic fungi counts were very low, while saprophytic species of genera *Penicillium*, *Trichderma* and *Absidia* were

in abundance. Low soil pH value probably limited growth of some bacteria (genus *Azotobacter*), so they were not observed on plates [Buchanan & Gibbon, 1974].

The locations exposed to blows of wind and air turbulence in the vicinity of site B were characterised by very high microbial counts, and this relationship was observed both in air and on plum fruits. It can be expected that higher abundance of microorganisms at certain locations in the studied orchard resulted from environmental conditions, mainly topographic features and shielding by the forest. Numbers of mould and bacterial spores, resistant to aforementioned factors, increased in the periods with higher temperature and insolation (July, August), while counts of viable bacterial and yeast cells decreased, as they are usually destroyed by UV radiation [Krzysztofik, 1992].

Immediate neighbourhood of the forest directly influenced qualitative composition of microflora, causing significant enhancement of bacterial, mould and yeast counts in the samples from sites D and E. Similar situation occurred at site C, located the closest to farm buildings. At this location, numbers of bacteria of genus *Streptococcus*, accompanying human activity and capable of parasitising on farm animals [Petrycka *et al.*, 1995], were increased.

As fruits ripen, contribution of yeasts and yeast-like organisms, e.g. *Aureobasidium sp*., to microflora inhabiting their surface rises. Counts of these microorganisms on the studied plums was several times higher (from 10 000 to 20 000 cfu/g) than those found on grapes [Davenport, 1974] and cherries [Olszak, 1994 a, b]. In our studies, we found only negligible counts of pathogenic microorganisms, such as genera *Taphrina*, *Podosphaera*, *Sphaerotheca*, *Monilia* and others, which usually dwell on plants [Ale-Agha, 1997].

When we compare three studied environments (soil, air and trees), we should bear in mind that they are closely interrelated. One of indications of such relationship was appearance of maximum counts of microflora on trees and in the air, with their concomitant minimum counts in soil, which can be noticed in the figures presenting total microbial counts (Figure 2 and 6). Drop in total counts of microorganisms in soil was usually accompanied by their increase in the air and on fruits. Similar relationship was found during quantitative analysis of bacteria and moulds. Yeast counts in soil were proportional to their numbers in the air.

The Węgierka Zwykła plums from the studied orchard were destined for production of plum brandy (Śliwowica Łącka). It is a product of fermentation of plum mush by autochtonous microflora inhabiting fruits. Microorganisms isolated from the surface of plums, flowers and bark of plum trees and from the air directly influence chemical composition of the manufactured spirits. We did discover in these environments also microorganisms characterised by strong fermentative (genera *Debaromyces*, *Aureobasidium*, *Rhizopus*, *Hansenula*) and enzymatic (amylases, pectinases, cellulases, esterases) capability, which, after mashing fruit flesh, may have very beneficial effect on fermentation process [Madamwar & Patel, 1992; Eleis, 1995; Rana *et al.*, 1996; Blanco *et al.*, 1999; Barbosa *et al.*, 2001]. Further experiments will provide detailed characterisation of fermentative and macerative capabilities of the isolated strains and will assess their contribution to development of flavour and aroma.

CONCLUSIONS

1. The studied Węgierka Zwykła plum orchard was characterised by relatively high diversity of microorganisms both in terms of their quantitative and qualitative features. The highest counts of bacteria, yeasts and moulds were observed in summer.

2. There were specific relationships between microflora inhabiting plum trees and microorganisms present in soil and air. Representatives of the family *Micrococcaceae*, genera *Bacillus*, *Rhodotorula*, *Candida*, *Trichoderma*, *Penicillium* and order *Mucorales* were the groups dominating in the studied environment.

3. Strains of the genera *Debaryomyces*, *Aureobasidium* and species *Rhizopus nigricans*, characterised by good fermentative utilisation of many sugars, prevailed on the surface of fruits, flowers, leaves and bark of plum trees.

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CHARAKTERYSTYKA MIKROBIOLOGICZNA SADU ŚLIWY WĘGIERKI ZWYKŁEJ Z REJONU PODGÓRSKIEGO

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Badania obejmowały analizę mikrobiologiczną gleby, powietrza oraz drzew (liście, kora, kwiaty, owoce) w sadzie śliwy Węgierki Zwykłej, zlokalizowanym w rejonie podgórskim (okolice Łącka, 526 m n.p.m.). Próby do oznaczeń pobierano z sześciu punktów sadu (rys. 1), w pięciu różnych terminach (kwiecień – październik 1999). Mikroorganizmy namnażano na odpowiednich podłożach mikrobiologicznych i identyfikowano na podstawie cech morfologicznych oraz testów biochemicznych.

Największą liczbę kolonii bakterii, drożdży i pleśni stwierdzono w okresie letnim (rys. 2, 4 i 6). Skład ilościowy i jakościowy mikroflory bytującej na drzewach śliw był istotnie uwarunkowany ich obecnością w glebie i powietrzu. Najbardziej zróżnicowaną grupę mikroorganizmów stanowiły grzyby strzępkowe (wyizolowano około 60 gatunków). Najliczniej w próbkach identyfikowane były drobnoustroje z rodzajów *Micrococcus*, *Staphylococcus*, *Rhodotorula*, *Cladosporium*, *Penicillium* i *Trichoderma* (rys. 3, 5 i 7). Na powierzchni drzew dominowały szczepy drożdży z rodzajów *Rhodotorula*, *Candida* oraz *Debaryomyces*, *Aureobasidium* i pleśnie z gatunku *Rhizopus nigricans*, które z reguły, charakteryzują się stosunkowo dobrymi właściwościami fermentacyjnymi w stosunku do wielu cukrów.