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COLONIZATION BY MITES (ACARI) OF WOOD CHIPS FOR USE IN MULCHING ORGANIC FRUIT CROPS

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Summary

The study was conducted in 2011-2012 by using litter bags on microplots in a forest soil under a canopy of trees, in optimal environmental conditions for most mites. The aim of the study was to analyze the colonization by mites of wood chips after application of two biopreparations containing cellulose-degrading bacteria. The experiment was conducted in the following variants: WC - control wood chips, WB I - chips after application of bacterial inoculum I (an unidentified G(-) rod-bacterium, *Bacillus* sp.) and WB II - chips after application of bacterial inoculum II (Streptomyces sp.). To maintain the optimum moisture level, the microplots were irrigated by means of microsprinklers. The highest average population density of mites in the two-year series of tests was found in the control chips: 42.28 individuals per 50 cm³. In the chips treated with the biopreparations, the density of these arthropods was lower, but the differences were not statistically significant. Dominant among the mites were mostly oribatid mites. Altogether, 34 species of oribatid mites were found in all the experimental variants. The most species (30) were found in the control variant, and fewer in the chips treated with the biopreparations -27-26. Among the oribatid mites, Tectocepheus velatus was dominant, and quite numerous were such species as: Suctobelba sp., Oppiella nova, Gymnodamaeus bicostatus, Metabelba pulverulenta, Oribatula tibialis, Eniochthonius minutissimus. The study shows that the wood chips were colonized by oribatid mites gradually - in the first year there were variations in the size of individual

populations, mostly at a low level. The process of colonization was accelerated considerably in the second year of the study, especially in the summer.

Key words: litter bags, species diversity, oribatid mites

INTRODUCTION

An important agrotechnical procedure affecting the quality of fruit production is the mulching of plantations (Kęsik and Maskalaniec 2005, Ochmian *et al.* 2007). Long-term research conducted at the Institute of Horticulture in Skierniewice has shown very high effectiveness of mulches from fragmented branches – wood chips (Treder *et al.* 2004, 2009). Such mulches have been found to maintain higher moisture levels and uniform soil temperature for long periods of time. They can also play an important role in the organic production of fruit because they inhibit the growth of weeds and reduce water consumption in a natural way.

Research conducted in forest nurseries has shown that mulching soils with forest ectohumus has a positive effect on the occurrence of soil mites (*Acari*) (Klimek *et al.* 2008, 2009, 2013a,b). The main objective of that procedure was revitalization and biological enrichment of the nursery soil system. Mulching of the soil creates optimal conditions for the development of microorganisms and small soil fauna (Forge *et al.* 2003). A similar conclusion was reached by the authors of this study after conducting experiments on a strawberry plantation mulched with wood chips (Klimek *et al.* 2014a,b).

Small soil arthropods – including mites, and in particular oribatid mites (*Oribatida*) – are known to have very important functions in terrestrial ecosystems: they have a positive effect on soil-forming processes, the spread of bacteria and fungi, and indirectly on the formation of endo – and ectomicorrhizas (Klironomos and Kendrick 1996, Behan-Pelletier 1999, Remén *et al.* 2010, Schneider *et al.* 2005). In addition, they are good bioindicators of the biological activity of soils (Behan-Pelletier 1999, 2003, Gulvik 2007).

As part of the project 'Development of innovative products and technologies for the environmentally-friendly cultivation of fruit plants', co-financed by the European Union through the European Regional Development Fund, research is being conducted at the Institute of Horticulture in Skierniewice to develop new biopreparations and technologies to apply bacterial and mycorrhizal inocula in horticulture (Sas-Paszt L. *et al.* 2014). These studies include the use of beneficial soil microorganisms, such as mycorrhizal and filamentous fungi, and bacteria isolated from the rhizosphere of different fruit plant species, as biostimulators in organically grown horticultural crops. The aim of this study was to analyze the colonization by mites of wood chips treated with two biopreparations containing cellulose-degrading bacteria. The wood chips had been prepared for use in mulching organic fruit crops.

MATERIALS AND METHODS

The study was conducted in 2011-2012 using litter bags in a forest soil under the canopy of a tree stand, in the optimum environmental conditions for most mites. Bags filled with wood chips were laid out on microplots in the Białe Błota Forest Nursery (Forest Inspectorate in Bydgoszcz). The experiment was established in an uncut strip of forest stand (53°06'13.2"N, 17°55'46.6"E) with a width of 20 m, which was to mitigate the impact of weather conditions, such as excessive sunlight, temperature fluctuations, and heavy precipitation. The stand consisted of pine (*Pinus sylvestris* L.), oak (*Quercus* L.) and ash (*Fraxinus excelsior* L.), and the undergrowth consisted of maple (*Acer platanoides* L.), ash, birch (*Betula pendula* Roth) and oak. The soils were classified as the sub-type: rusty podzolic (data from the Forest Inspectorate in Bydgoszcz).

The wood chips were produced from branches of roadside deciduous trees and shrubs with a disc chipper in the autumn of 2010. They were stored in piles outdoors over winter. At the beginning of March 2011, they were transferred in bags to a greenhouse, where, after two weeks, they were put into boxes and treated with aqueous biopreparations (the control chips were treated with pure water in the same amount).

For the experiment, bacteria that had the ability to degrade cellulose had been isolated from soil using the serial dilutions method C09EX – an unidentified G(-) rod-bacterium, C7D11 – *Bacillus* sp., and 7GII – *Streptomyces* sp. Inoculated petri dishes with CMC agar medium were incubated for 14 days at 28°C (Hankin and Anagnostakis 1977). Strains showing the degradation of carboxymethylcellulose were transplanted onto the Dubos agar medium with the filter paper. Three strains which discolored the filter paper and produced the biggest amount of biomass were selected to the next phase of the experiment. Next, the bacteria were multiplied in Tryptic Soy Broth for 48 hours (C09EX, C7D11) or 148 hours (7GII). The experiment was conducted in the following variants: WC – control wood chips, WB I – chips treated with bacterial inoculum I (C09EX – an unidentified G (-)rod-bacterium, C7D11 – *Bacillus* sp.), and WB II – chips treated with bacterial inoculum II (7GII – *Streptomyces* sp.).

Litter bags measuring 15×20 cm were made from nylon mosquito net with a mesh size of 2 mm. This enabled the migration of species belonging to the soil mesofauna. Just before the chips were put into the litter bags, they had been additionally fragmented twice with a VIKING GE 25 garden shredder. Each bag was filled with 1.5 dm³ of chips, which were weighed and laid out on the microplots.



Photo 1. Irrigated microplots with litter bags under the canopy of a tree stand in the forest nursery Białe Błota (photo by Andrzej Klimek)

The microplots, with an area of 1 m^2 each, were set up in two rows (Photo 1). The distance between the individual microplots was approx. 1 m. For each of the three variants of the experiment, 4 microplots were established. On each microplot, 6 bags of one variant of the experiment were laid out, assuming that sampling would be carried out 6 times. The bags were laid out on a mineral soil and covered with a 5 cm layer of ectohumus.

To maintain the optimal moisture level, the microplots were irrigated in accordance with the guidelines and irrigation schedule for forest nurseries by means of microsprinklers, maintaining the average soil moisture at a level of 5.1-9.9%.

To conduct acarological examinations, samples were collected three times in each year of the experiment, in the spring, summer and autumn: 24 May 2011, 20 July 2011, 27 October 2011, 19 May 2012, 19 July 2012, 16 October 2012. Each time, 10 samples were taken from each variant: from 4 litter bags from 4 consecutive microplots: 2+3+2+3. Altogether, over the two years, 60 samples of 50 cm³ each were collected from each variant of the experiment. Extraction of mites was carried out for 7 days in Tullgren funnels. Then, the mites were preserved in 70% ethanol. All the mites were classified into orders and the oribatid mites into species or genera, including the juvenile stages. A total of 6,779 mites were classified, including 3,090 oribatid mites. The average population density (*N*) of the mites was expressed per 50 cm³ of substrate, and the species dominance ratio (*D*) in %. Species diversity was determined by the average number of species in the sample (*s*). Prior to statistical analyses, numerical data were subjected to a logarithmic transformation – ln (x+1) (Berthet and Gerard 1965). The statistical analyses were performed using Statistica 6.0 software: assessment of the goodness of fit of the distribution of measurable parameters with a normal distribution were performed with the Kolmogorov-Smirnov test; because there was no distribution fitting the normal distribution, nonparametric analysis of variance (Kruskal-Wallis test) was carried out; for statistically significant differences (p<0.05) an analysis for each pair (Mann-Whitney U rank sum test) was carried out to select significantly different means.

The samples of the tested substrates for chemical analyses were collected from the material prepared for exposure in the field on 31 March 2011 and on the first and last test dates – 24 May 2011 and 16 October 2012. The analyses were conducted in the Laboratory of Chemical Contaminants of the Research Institute of Horticulture in Skierniewice. The following methods were used: pH was determined by the electrochemical method; N-NO₃ and N-NH₄ contents were determined by the electrochemical method after extraction with 0.03N acetic acid; Nog. and Corg. were determined according to Dumas using a TruSpec CNS apparatus; the amounts of phosphorus, potassium, magnesium and calcium were determined by atomic emission spectrometry with inductively coupled plasma (ICP-OES); air-dry weight was determined by the gravimetric method.

RESULTS

Meteorological conditions.

In 2011-2012, the growing season (April-October) was characterized by higher values of average air temperatures and higher precipitation totals (Table 1) compared with long-term averages. The growing season in 2011 was characterized by an air temperature of 14.2° C and the total precipitation of 403.1 mm. In 2012, the average air temperature was lower -13.6° C, and precipitation higher – 418.5 mm.

In 2011, the highest air temperatures occurred in the months of June, July and August, reaching 17.7°C, 17.5°C and 17.7°C, respectively. The following year, June was colder (15.2°C), and July proved to be hotter (18.8°C). The highest total precipitation, exceeding 100 mm, was recorded in June and July of both years. In 2011, precipitation was more abundant in July (132.5 mm), while in the following year – in June (133.8 mm). Much less precipitation occurred in April and May and in September and October of both years.

| Securification | Months | | | | | | | |
|----------------------|--------|------|-------|-------|------|------|------|-------|
| Specification | IV | V | VI | VII | VIII | IX | Х | IV-X |
| Air temperature (°C) | | | | | | | | |
| 2011 | 10.5 | 13.5 | 17.7 | 17.5 | 17.7 | 14.3 | 8.4 | 14.2 |
| 2012 | 8.4 | 14.5 | 15.2 | 18.8 | 17.6 | 13.3 | 7.4 | 13.6 |
| Long-term value | 8.0 | 13.0 | 16.3 | 18.5 | 17.8 | 13.1 | 7.8 | 13.5 |
| Precipitation (mm) | | | | | | | | |
| 2011 | 13.5 | 38.4 | 100.8 | 132.5 | 67.7 | 37.0 | 13.2 | 403.1 |
| 2012 | 26.5 | 25.4 | 133.8 | 115.6 | 51.8 | 25.1 | 40.3 | 418.5 |
| Long-term value | 29.0 | 61.2 | 48.8 | 87.7 | 68.6 | 45.6 | 36.2 | 377.1 |

 Table 1. Air temperature and precipitation in 2011-2012

Source: own research data

| Table 2. Physico-chemical | narameters prepare | ed substrates of wood | chins |
|----------------------------------|---------------------|-----------------------|-------|
| Labic 2. Thysico chemical | parameters, prepare | a substrates of wood | unps |

| | | | | Variant | of the exp | periment | | | |
|-------------------------------|---------|---------|---------|---------|------------|----------|---------|---------|---------|
| Parameters | | WC | | | WB I | | | WB II | |
| | 03.2011 | 05.2011 | 10.2012 | 03.2011 | 05.2011 | 10.2012 | 03.2011 | 05.2011 | 10.2012 |
| pH H ₂ O | 6.8 | 6.8 | 6.9 | 7.0 | 6.8 | 7.0 | 6.6 | 6.6 | 7.0 |
| N-NO3 [mg/kg] | 43.6 | 20.6 | 63.4 | 46.0 | 21.9 | 91.0 | 33.7 | 21.7 | 43.0 |
| N-NH _{4 [mg/kg]} | 116.6 | 77.7 | 39.8 | 142.6 | 77.9 | 68.0 | 154.6 | 70.3 | 40.6 |
| N [% p.s.m.] | 1.3 | 1.3 | 1.3 | 1.4 | 1.4 | 1.2 | 1.3 | 1.2 | 1.1 |
| $C_{[\% \ p.s.m.]}$ | 37.5 | 34.8 | 24.2 | 38.4 | 37.6 | 23.6 | 47.8 | 45.2 | 21.5 |
| Organic matter | 64.5 | 59.9 | 41.7 | 66.1 | 64.6 | 40.7 | 82.2 | 77.8 | 36.9 |
| P _[% p.s.m.] | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| K _[% p.s.m.] | 0.4 | 0.7 | 0.1 | 0.5 | 0.3 | 0.1 | 0.5 | 0.1 | 0.1 |
| $Mg_{[\% \ p.s.m.]}$ | 0.2 | 0.7 | 0.1 | 0.2 | 0.3 | 0.1 | 0.1 | 0.5 | 0.1 |
| Ca _[% p.s.m.] | 2.1 | 2.1 | 1.9 | 2.5 | 2.3 | 2.0 | 1.4 | 1.7 | 1.6 |
| Air-dry weight [%] | 79.4 | 41.3 | 66.2 | 67.2 | 37.8 | 56.5 | 74.6 | 36.6 | 64.0 |
| Average sample Weight loss | | - | 33.3 | - | - | 41.6 | - | - | 26.2 |

Source: own research data

Chemical analysis of substrates.

The pH in all variants of the experiment was neutral; it changed only slightly after exposure on the microplots (Table 2). The N-NO, nitrogen content after the chips had been laid out on the microplots decreased and then increased markedly in all the variants. The situation was different in the case of N-NH₄, whose amount before exposure in the field was 116.6-154.6 mg/kg, but decreased considerably after the bags had been placed on the microplots, reaching a minimum value (39.8-68.0 mg/kg) on the last test date of the experiment. As a result of the mineralization process taking place during the experiment, the organic matter content decreased in all the variants, which was most pronounced in variant WB II, where less than 45% of its original amount was left at the end. The process of mineralization also changed the C/N ratio, reducing the percentage of carbon in variant WC to 64%, in WB I to 61%, and in WB II to 44%. The litter bags had been weighed before exposure on the microplots and after they had been collected from the microplots. On the basis of the dry matter content, the average percent weight loss of the sample was calculated. The largest weight loss was recorded in variant WB I, and the smallest in WB II; the difference between them was 15.4%.

Size and structure of mite populations.

The highest average population density of mites in the two-year series of tests was found in the control wood chips: 42.28 individuals per 50 cm³ (Table 3). In the chips treated with biopreparations, the density of these arthropods was lower, but the differences were not statistically significant. In 2011, mite density was similar in all the experimental variants – 16.23-17.53 individuals per 50 cm³. In the second year of the study, this density increased 3-4-fold. The differences between the first and second year of the study were statistically significant for all the variants. In 2011, the density of mites on consecutive test dates (from spring to autumn) increased slightly – WC, or fluctuated – WB I and WB II (Figure 1). In the same seasons in 2012, the rate of colonization of chips by mites increased considerably.

The control chips were clearly dominated by oribatid mites – they represented, on average, 49% of all the *Acari* (Table 3). By comparison, in 2011, the chips treated with biopreparations were dominated by the *Actinedida*. These mites were most abundant in variant WB I. The population density of the *Actine-dida* in the chips treated with biopreparations increased approx. 2-fold during the experiment, but in 2012 these mites were dominated by the *Oribatida*. In both years of the study, the oribatid mites occurred most abundantly in the control chips, but the differences in the population density of these arthropods among the individual variants were not statistically significant. However, a significant

increase in the density of these mites over the consecutive years was recorded in all the experimental variants.

| Taxon | Veen | Variar | t of the expe | Kruskal-Wallis test | | |
|---------------|------|----------------------|----------------------|----------------------|-------|--------|
| | Year | WC | WB I | WB II | Н | р |
| | 2011 | 0 | 0 | 0.10 | | |
| Acaridida | 2012 | 0 | 0.10 ^A | 0.13 ^A | 10.95 | 0.0524 |
| | Mean | 0 | 0.05 ^A | 0.12 ^A | | |
| | 2011 | 5.70 ^A | 9.47 ^в | 5.90 ^A | | |
| Actinedida | 2012 | 22.03 ^A * | 25.40 ^A * | 10.00 ^A * | 28.73 | 0.0000 |
| | Mean | 13.87^{AB} | 17.43 ^A | 7.95 ^B | | |
| | 2011 | 3.40 ^A | 2.90 ^A | 4.97 ^A | | |
| Mesostigmata | 2012 | 11.03 ^A * | 8.67 ^A * | 10.77 ^A | 25.47 | 0.0001 |
| | Mean | 7.22 ^A | 5.78 ^A | 7.87 ^A | | |
| | 2011 | 8.17 ^A | 3.07 ^A | 5.03 ^A | | |
| Oribatida | 2012 | 33.67 ^A * | 25.67 ^A * | 27.40 ^A * | 58.67 | 0.0000 |
| | Mean | 20.92 ^A | 14.37 ^A | 16.22 ^A | | |
| | 2011 | 0.27 ^A | 1.20 ^B | 0.23 ^A | | |
| Tarsonemida | 2012 | 0.30 ^A | 0.20 ^A * | 0.20 ^A | 22.31 | 0.0005 |
| | Mean | 0.28 ^A | 0.70 ^B | 0.22 ^A | | |
| Acari (Total) | 2011 | 17.53 ^A | 16.63 ^A | 16.23 ^A | | |
| | 2012 | 67.03 ^A * | 60.03 ^A * | 48.50 ^A * | 52.75 | 0.0000 |
| | Mean | 42.28 ^A | 38.33 ^A | 32.37 ^A | | |

Table 3. Abundance of mites (N in 50 cm³ of substrate) in the studied variantsof the experiment

Explanations: A, B – the same letter denotes insignificant difference – a post hoc Mann-Whitney U test at p < 0.05. * – significant difference between 2011 and 2012– a Mann-Whitney U test at p < 0.05. Source: own research data

In addition to the orders of mites mentioned above, the tested material was found to contain fairly large numbers of the *Mesostigmata*, classified as predators. The average size of their populations was similar in the individual experimental variants. As in the case of the *Actinedida* and *Oribatida*, the population density of the *Mesostigmata* increased over the duration of the experiment, but the increase was smaller. Much less abundant orders of mites in the tested material were the *Tarsonemida* and *Acaridida*.

| Index | Veer | Varian | t of the expe | Kruskal-Wallis test | | |
|-----------------|-------|---------------------|---------------------|---------------------|-------|--------|
| | Year | WC | WB I | WB II | Н | р |
| | 2011 | 19 | 18 | 18 | | |
| S of Oribatida | 2012 | 27 | 25 | 20 | - | - |
| | Total | 30 | 27 | 26 | | |
| | 2011 | 2.23 ^A | 1.60 ^A | 1.63 ^A | | |
| s of Oribatida | 2012 | 5.47 ^A * | 5.00 ^A * | 4.47 ^A * | 54.81 | 0.0000 |
| | Mean | 3.85 ^A | 3.30 ^A | 3.05 ^A | | |
| % juv Oribatida | 2011 | 16 | 9 | 17 | | |
| | 2012 | 57 | 35 | 45 | - | - |
| | Mean | 49 | 32 | 41 | | |

Table 4. Number of species (S), average number of species (s), and % of juvenile Oribatida forms in the studied variants of the experiment

Explanations: see table 3.

Source: own research data

Species diversity and age structure of oribatid mites.

In all the experimental variants, a total of 34 species of oribatid mites were identified. The most species (30) were found in the control variant, and fewer in the chips treated with the biopreparations -27-26 (Table 4). Analysis of the average number of species in the sample (*s*) did not show significant differences in species diversity among the different variants of the experiment. There was, however, a statistically significant increase in this index in all the variants over the course of the study.

For the two-year study period, the average percentage of juvenile forms of the *Oribatida* in the control wood chips was markedly higher in comparison with the chips treated with the biopreparations. In the first year of the experiment, the percentage share of larvae and nymphs was low -9-17% of all oribatid mites. In the second year, the proportion of these forms increased substantially to 35-57%.

Analysis of the occurrence of selected species of oribatid mites.

The oribatid mite that was found in the largest numbers in the tested wood chips was *Tectocepheus velatus* (Table 5) – D from 32.3 to 55.2% of all oribatid mites. In 2011, the population density of this species was low, especially in the variants with the addition of biopreparations. In 2012, a multiple increase in the number of *T. velatus* was recorded. A marked increase in the size of this population in variants WC and WB II occurred in July 2012, and in WB I not until

October (Figure 1). For the whole test series, the average number of *T. velatus* in variants WB I and WB II was markedly lower compared with the control – the differences between WC and WB II were statistically significant (Table 5).

In the second place of the dominance hierarchy of the *Oribatida* in all the variants were minor mites of the genus *Suctobelba* (*D*=13.5-27.4%). The density of their populations was uniform. In 2011, the number of these mites ranged from 1.37 to 2.40 individuals per 50 cm³. The following year, a statistically significant increase in the density of these mites was recorded in all the variants.

A high place (3rd or 4th) in the dominance hierarchy of oribatid mites was taken by *Oppiella nova*. The density of this mite in the first year of the study ranged from 0.07 to 0.87 individuals per 50 cm³. At that time, the species preferred the control chips. In the second year, the size of *O. nova* populations increased substantially – especially in the variants with the application of biopreparations.

The changes in the abundance of *Ramusella mihelcici* did not follow the same pattern recorded for most species of oribatid mites. This species occurred in large numbers in the control chips on the first test date - 6.1 individuals per 50 cm³ (Figure 1). In this variant, only 0.7 individuals per 50 cm³ were recorded already in July 2011, and in subsequent seasons the mite was not found at all.

In addition to the above species, the wood chips were also colonized in fairly large numbers by other oribatid mites such as: *Gymnodamaeus bicostatus*, *Metabelba pulverulenta*, *Oribatula tibialis*, *Eniochthonius minutissimus* and mites of the genus *Brachychthonius* (Table 5). Most of them did not show a clear preference for a particular variant of the experiment. Only *Eniochthonius minutissimus* showed some preference for the WC variant. The above analysis shows that the chips were colonized by oribatid mites gradually – in the first year there were variations in the size of individual populations, mostly at a low level. The colonization process was greatly accelerated in the second year of the study, especially in the summer.

DISCUSSION

The use of organic mulches in organic farming is justified because of the prevention of water erosion and land degradation (Smets *et al.* 2008), slow release of nutrients, natural inhibition of weed growth, and better use of water by plants (Treder *et al.* 2004, 2009). Organic mulches are increasingly used in orchards and on berry plantations. It is suggested to use the branches remaining in the orchard after pruning trees and shrubs as organic mulch in the form of chips. This type of mulch significantly increases the effectiveness of taking advantage of precipitation and limits evaporation of water from the soil, which reduces the water needs of the orchard.

Unfortunately, little is known about the influence of mulching on the presence of beneficial soil fauna, which is essential for the proper functioning of entire orchard or garden ecosystems. The few literature references on this subject suggest a positive impact of mulching on the development of soil meso – and macrofauna (Brévault *et al.* 2007, Forge *et al.* 2003). In the spring of 2011, parallel experiments began in an experimental field in Dąbrowice belonging to the Research Institute of Horticulture in Skierniewice and in a strip of uncut forest stand in the Białe Błota Forest Nursery near Bydgoszcz. The two experiments were conducted on similar substrates – wood chips without additives and with the addition of bacterial inoculants. In Skierniewice, the experiment was carried out with the strawberry cultivar 'Elsanta' on a plantation mulched with wood chips and not irrigated (Klimek *et al.* 2014a). The mulched surfaces were found to contain large numbers of mites (about 21,000 individuals \cdot m⁻²), of which 87-90% were oribatid mites.

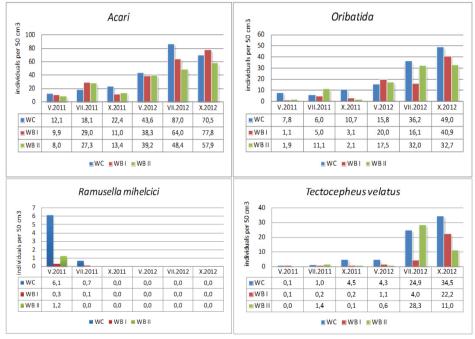
The experiment described here, with the use of litter bags on microplots, was set up on a forest soil under the canopy of a forest stand, ensuring optimum habitat conditions for most mites. In addition, the microplots were irrigated during periods of moisture deficiency. While conducting the experiment in these conditions, efforts were made to reduce the unfavourable impact of drought and strong solar radiation on the soil mesofauna, which is particularly sensitive to these factors (Lindberg and Bengtsson 2005).

In forest soils in the early stages of succession – after stand clearing and forest renewal – the population density of mites can be from 7,700-9,300 (Klimek and Kowalska 2013) to as many as 23,300 individuals \cdot m⁻² (Klimek and Rolbiecki 2011). In the first year of the experiment presented here, the recorded density of mites in wood chips – expressed per surface area of 1 m² and depth of 3 cm – was about 10,000 individuals. By comparison, the density of these arthropods recorded in the autumn of the second year of the experiment (Figure 1) corresponded to 35,000-47,000 individuals \cdot m⁻².

The colonization by mites of wood chips in litter bags in the first year of the study proceeded quite slowly: in the spring the number of them was 13-17% of the number on the last test date, and in the autumn it only increased to a level of 14-32% of the final number (Figure 1). However, already in the spring of the following year there was a significant progress in the rate of colonization of wood chips – 49-68% of the final count. In the summer of 2012, the number of *Acari* reached a level of 82-123% of the density on the last test date. By comparison, the number of mites on the strawberry plantation mulched with wood chips was already high on the first test date in the spring of 2011 – 26,910 individuals \cdot m⁻² (Klimek *et al.* 2014b). In that experiment, the population density of mites reached a maximum during the summer.

Oribatid mites were a little slower at colonizing the litter bags compared with other mites. At the beginning of the study, the number of these mites in

the control chips constituted 16% of the number on the last test date, and in the autumn that proportion increased only to 22%. In the spring of the following year, there was a slight increase in that proportion (32%), and it was only in the summer that this level reached 74% of the final count. In the variants with the application of biopreparations, the colonization proceeded differently: the initial density level was extremely low (3-6%), then it increased (12-34%), and in the autumn it fell to 6-8% of the final count. However, already in the spring of the following year, oribatid mites colonized these variants of the experiment quite extensively (49-54%). This pattern of changes in the number of oribatid mites may be due to the effects of the biopreparations used in the experiment. Cellulolytic bacteria may have created competition and inhibited the growth of fungi in the first year of the study before they were displaced by the latter. The literature says that a large part of the *Oribatida* prefer fungi in their diet (Luxton 1972, Ponge 1991, Schneider *et al.* 2005, Remén *et al.* 2010).



Source: own research data

Figure 1. The dynamics in the number of groups of mites and selected species of oribatid mites in the studied variants of the experiment

| Spacing | Vaar | Variant of the experiment | | | Kruskal-Wallis test | | |
|--|-------|---------------------------|---------------------|----------------------|---------------------|--------|--|
| Species | Year | WC | WB I | WB II | Н | р | |
| Brachychthonius sp. | 2011 | 0.83 ^A | 0.23 ^A | 0.30 ^A | | | |
| | 2012 | 0.53 ^A | 2.60 ^A * | 2.73 ^A * | 22.48 | 0.0004 | |
| | Mean | 0.68 ^A | 1.42 ^A | 1.52 ^A | | | |
| | 2011 | 0 | 0.03 | 0 | | | |
| Eniochthonius minutissimus (Berlese 1903) | 2012 | 2.03 ^A | 0.03 ^B | 0.10 ^B | 33.16 | 0.0000 | |
| (Benese 1903) | Mean | 1.02 ^A | 0.03 ^B | 0.05 ^B | | | |
| | 2011 | 0.17 ^A | 0.10 ^A | 0.13 ^A | | | |
| Gymnodamaeus bicostatus (C.L. Koch) | 2012 | 0.70 ^A | 0.87 ^A * | 1.03 ^A * | 14.28 | 0.0139 | |
| (U.L. KOCII) | Mean | 0.43 ^A | 0.48 ^A | 0.58 ^A | | | |
| Metabelba pulverulenta (C.L. Koch) | 2011 | 0.10 ^A | 0.10 ^A | 0.13 ^A | | | |
| | 2012 | 0.30 ^A | 0.73 ^A | 1.10 ^A | 5.99 | 0.3063 | |
| | Mean | 0.20 ^A | 0.42 ^A | 0.62 ^A | | | |
| <i>Oppiella nova</i> (Oudemans) | 2011 | 0.87 ^A | 0.07 ^B | 0.50 ^{AB} | | | |
| | 2012 | 1.63 ^A | 2.20 ^A * | 4.33 ^A * | 32.47 | 0.0000 | |
| | Mean | 1.25 ^A | 1.13 ^A | 2.42 ^A | | | |
| | 2011 | 0.03 ^A | 0.03 ^A | 0 | | | |
| Oribatula tibialis (Nicolet) | 2012 | 0.90 ^A * | 1.00 ^A * | 0.80 ^A | 44.36 | 0.0000 | |
| | Mean | 0.47 ^A | 0.52 ^A | 0.40 ^A | | | |
| | 2011 | 2.27 ^A | 0.13 ^A | 0.40 ^A | | | |
| Ramusella mihelcici (Pérez-Íñigo 1965) | 2012 | 0 | 0 | 0 | 24.02 | 0.0002 | |
| (Felez-Illigo 1903) | Mean | 1.13 ^A | 0.07 ^A | 0.20 ^A | | | |
| | 2011 | 1.37 ^A | 1.43 ^A | 2.40 ^A | | | |
| Suctobelba sp. | 2012 | 4.30 ^A * | 6.43 ^A * | 4.17 ^A * | 23.36 | 0.0003 | |
| | Total | 2.83 ^A | 3.93 ^A | 3.28 ^A | | | |
| Tectocepheus velatus (Michael) | 2011 | 1.87 ^A | 0.17 ^{AB} | 0.50 ^B | | | |
| | 2012 | 21.23 ^A * | 9.10 ^A * | 13.30 ^A * | 54.56 | 0.0000 | |
| | Mean | 11.55 ^A | 4.63 ^{AB} | 6.90 ^B | | | |

 Table 5. Density of some oribatid species (in 50 cm³ of substrate) in the studied variants of the experiment

Explanations: see table 3.

Source: own research data

Among the oribatid mites found in the tested wood chips the dominant species was *Tectocepheus velatus* (Table 5, Figure 1). It is a common soil oribatid mite found in different biotopes (Weigmann and Kratz 1981); it is characterized by a high reproduction rate and high capacity for colonizing new environments. The species is classified as mycophages (Luxton 1972, Ponge 1991). A fairly similar pattern of changes in population size was recorded for another common mycophage – *Oppiella nova*. According to the literature, *O. nova* is parthenogenetic, has a short development cycle (20 days), and its population can develop very quickly once it has found favourable environmental conditions (Siepel 1994, Skubała and Gulvik 2005, Klimek 2013). However, in this experiment, the population of *O. nova* – although present from the beginning of the test series – developed rather reluctantly in the first year, especially in the WB I variant. In the second place in the *Oribatida* hierarchy (*D*=16%). In oribatid mites, successful colonization is associated with the immigration strategies of the species, although it also depends on the reproductive ability (eg. parthenogenesis), trophism, and resistance to dehydration (Lehmitz *et al.* 2012).

Only one species – *Ramusella mihelcici* – occurred in large numbers at the beginning of the study, and then it could not be found. This oribatid mite was relatively abundant in May 2011 in the control chips, but already in July its population declined to 0.7 individuals per 50 cm³. *R. mihelcici* also dominated at the beginning of the experiment on the strawberry plantation, and its population density decreased sharply on the last two test dates (Klimek *et al.* 2014b). It should be added that in the ectohumus derived from the soil of the forest stand (surrounding the microplots) no evidence of the presence of this species was found (unpublished data). On the basis of the population size dynamics of this species in both experiments, it can be concluded that *R. mihelcici* had already colonized the wood chips before they were placed on the experimental plots. It is interesting that the same species in the laboratory, at the optimum temperature and humidity, on a substrate of compost produced from municipal sewage sludge with the addition of 20% straw could reach population density at a level of 100,000 individuals \cdot m⁻² (Klimek *et al.* 2011).

The average number of species (*s*) of oribatid mites, like their density, increased in the tested wood chips gradually: in the first year the observed species diversity was at a level of 32-41% of the level in the second year of the experiment. By comparison, in the wood chips on the strawberry plantation, oribatid mites had already reached in the first year a diversity level of 78% of the next year's level (Klimek *et al.* 2014b). It should be remembered, however, that in the present experiment, the chips had been additionally shredded twice with a garden shredder immediately before exposure in litter bags, which undoubtedly had a negative effect on the mites that had already populated the chips during storage. Other studies with fresh pine chips, in which mites did not occur before the experiment, and which were isolated from the forest soil by means of Cellfast garden edging, found in the first year only 4% of the population density and 8% of the species diversity recorded in the second year of the experiment.

The study presented here and other experiments conducted by the authors of this paper indicate that wood chips can be a good substrate for mulching and revitalizing soils of organic orchards and berry crops. The rate of colonization of chips by beneficial mesofauna can depend on many factors, and a high level of abundance and species diversity can already be reached in the second year after application. This study, however, has failed to demonstrate statistically significant effects of the biopreparations containing cellulose-degrading bacteria on the overall abundance and species diversity of soil mites.

CONCLUSIONS

The population density of mites in all the variants of the experiment in the first year of the study was uniform – 16.23-17.53 individuals per 50 cm³. In the second year, the density of these arthropods increased 3-4-fold, and the differences between the first and second year were statistically significant. In both years of the study, oribatid mites occurred in the largest numbers in the control chips, but the differences in the density of these arthropods among the individual variants were not statistically significant. Statistical analysis of the average number of the *Oribatida* species (*s*) did not show significant differences in species diversity among the different variants of the experiment. However, there was a statistically significant increase in this index in all the variants over the course of the study. Wood chips were colonized by oribatid mites gradually – in the first year there were variations in the size of individual populations, mostly at a low level. The process of colonization was accelerated significantly in the second year of the study, especially in the summer.

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