Hungarian University of Agriculture and Life Sciences

ASSESSMENT OF ECOSYSTEM SERVICES PROVIDED BY TERRESTRIAL ISOPODS (ISOPODA: ONISCIDEA)

Theses of Doctoral (PhD) dissertation

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Anett Mészárosné Póss

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Doctoral School

name: Doctoral School of Plant Sciences

discipline: Plant Production and Horticulture

head of the Doctoral School:

Lajos Helyes DSc
Professor
Hungarian University of Agriculture and Life Sciences, Buda Campus
Institute of Horticulture

Supervisor:

Ferenc Tóth PhD
Horticultural team leader
Hungarian Research Institute of Organic Agriculture

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Approval by the Head of Doctoral School	Approval by the Supervisor

1. Scientific background and objectives

The role of terrestrial isopods in ecosystem services has not been sufficiently researched, although they are widespread in near-human habitats, including suburban areas (Figure 1), where they can reach a particularly large number of individuals (Vilisics et al. 2007). Isopods are involved not only in the breakdown of dead plant parts, but they are also important aliments for soil-dwelling predatory arthropods as well as vertebrates (Paoletti and Hassall 1999; Purse et al. 2012; Collison et al. 2013). In our studies, isopods were also involved as examinee because, as defined in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market (EC No. 1107/2009), the vulnerability of ecosystem services requires continuous testing for indirect human intervention and to monitor its direct effects.

Figure 1: Experiment about the microhabitat preference of isopods, Gödöllő (Photo: Anett Mészárosné Póss 2016)

The aims of my PhD work were the following:

A) Investigation of microhabitat preference in isopods in near-human habitats

B) Examination of ecosystem services provided by isopods

• Combined effect of organic mulching and *Porcellionides pruinosus* on the yield in tomato test plant

• Investigation of plant pathogen consumption of *P. pruinosus*

• Investigation of the role of *Armadillidum vulgare* in the spread of *Fusarium solani* as a storage pathogen

C) *P. pruinosus* as a non-target organism testing with biological plant protection products in laboratory

• Investigation of *P. pruinosus* as a non-target organism in the presence of insect pathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*

• Investigation of *P. pruinosus* as a non-target organism using neem

2. Materials and methods

The main characteristics of the experiments are summarized in the table below (Table 1).

Table 1 Detailed data of the experiments

	Microhabitat preference of isopods	**Changes in soil biological activity and its effect on yield**	**Consumption of plant pathogens by *Porcellionides pruinosus***	**Role of *Armadillidium vulgare* individuals in the spread of the storage pathogen *Fusarium solani***	**Laboratory testing of biological plant protection products: insect pathogenic fungi of *Beauveria bassiana a*nd *Metarhizium anisopliae***	**Laboratory testing of biological plant protection products: neem products**
Environment	Field	Pot, semi-open field	Laboratory	Cellars	Laboratory	Laboratory
Used organisms and substances	walnut, straw, compost, mixed litter	*Porcellionides pruinosus,* tomato plant, 40 dkg mixed litter/ sample,	*P. pruinosus, Gymnosporangium sabinae, Mycosphaerella pyri/Septoria*	*Armadillidium vulgare, Fusarium solani,* potato,	*Porcellionides pruinosus*, *Beauveria bassiana* and	*Azadirachta indica*, NeemAzal T/S,

		Florimo common potting soil	*pyricola, Macrophomina phaseolina, Sclerotinia sclerotiorum, Fusarium solani, Aspergillus niger*	*Aureobasidium pullulans*	*Metarhizium anisopliae*	*Porcellionides pruinosus*
Duration of the experiment	2016–2018 vegetation periods	2 growing seasons	first experiment 2 weeks, medium and propagation, then 2 weeks	2 months	first experiment 5 weeks, second experiment 6 weeks	120 hours
Experimental setup	two 12 × 12 meter blocks, 6 × 6, 2 × 36 plots per spatial repetition	control: Á-T-, only covered: Á-T +, only isopods: Á + T-, combined: Á + T +; 20–20 individuals / sample	15 pear-rusted and 15 rust-free leaves in a Petri dish, 15–15 leaves in a box filled with potting soil, 10–10 isopods; colony consumption: 40 samples, 7 replicates per fungal species	10 replicates at each of the three sites, ten (20–40 g) healthy potato tubers, infected tubers, tubers coated with Botector®, 10–10 isopods in 10/5 box	112 g of quartz sand and 40 g of potting soil, 20 isopods / sample, 10 repetitions of the two treatments from 5 suspensions of insect pathogens in 5 boxes and 25	2–2 ml of 0, 0,05, 0,1, 0,25, 0,5, 0,75 and 1% solutions, 5 isopods / sample in 13 cm diameter glass petri dishes 1 g of soil (pH = 7.0), 1 g of fresh potatoes as food source

					ml in a further 5 boxes; second experiment: 15 ml of suspension, 10 replicates, 20 g of soil, 5 isopods / sample; always 1% Tween 80	
Evaluation frequency, recorded indicators	every two weeks, 2 minutes per plot, ernight examination, handsorting	2016 FDA test, phenological examinations, root mass, isopod presence	leaf and ecidium consumption; survival rate; fungi depletion measurement for two weeks, twice a week	tuber condition examination every two weeks, number of individuals measured	2 times; weekly; survival	1, 24, 48, 72, 96, and 120 hours, survivals

3. Results

3.1. Investigation of microhabitat preference in isopods in near human habitats

Ascending species in the area in descending order: *Armadillidium vulgare, Cylisticus convexus, Porcellionides pruinosus, Porcellio scaber, Trachelipus rathkii, Hyloniscus riparius, Armadillidium nasatum, Porcellium collicola*. Most of the walnut litter, mixed litter then straw, and last the compost provided shelter for the observed animals, while no individuals were caught in the uncovered control plots.

3.2. Effect of combined application of organic soil cover and *Porcellionides pruinosus* on crop yield in tomato test plant

The combined treatment significantly increased the generative growth of the tomatoes. Significantly more flowers were evolved and significantly more tomatoes were harvested compared to the control treatment. Our preliminary results indicate a beneficial effect of *P. pruinosus*, as its presence had a beneficial effect on tomato yield.

3.3. The role of *Porcellionides pruinosus* in the consumption of plant pathogens and the spread of Fusarium solani as a storage pathogen

We found that: (1) The presence of *G. sabinae* resulted in decreased leaf consumption but did not affect the loss of mycospheric patches. (2) The most intense mycelium loss was observed in *S. sclerotiorum* samples, while *A. niger* was rejected. (3) The presence of isopods has reduced the spread of *F. solani* on potato tubers. Based on our results, it can be said that isopods are able to dispose infected plant parts.

3.4. *Porcellionides pruinosus* as a non-target organism in the presence of insect pathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*

Of the beet slices treated with fungal spores, isopods consumed significantly more per unit time compared with the untreated control. There was no significant difference in mortality.

3.5. Porcellionides pruinosus as a non-target organism with neem

Our results are consistent with previous results with other non-target organisms, i.e., none of the tested neem products has affected the death of *P. pruinosus* individuals.

4. Conclusions and suggestions

4.1. Microhabitat preference of terrestrial isopods

Fewer isopods were observed in the compost than in the litter types. This is because compost is a degraded, porous material with limited water retention. As a result, isopods preferred wallnut litter, straw and mixed litter, which retained more moisture and contained more nutrients. In our experience, compost is not recommended as mulch in itself, but rather as a valuable nutrient supplement for crop production. It is a good idea to use the compost with litter or straw, which protects both the compost and the soil against sunlight and dehydration.

During 2017, we monitored the isopod-fauna in the experimental area and found a higher density of generalist species (*Armadillidium vulgare, Cylisticus convexus*) that were more tolerant of disturbance (Farkas and Vilisics 2013).

We have found that we created microhabitats by leaflitters where the number, biomass, and activity of terrestrial isopods can be at a level that they can already make a significant contribution to ecosystem service.

4.2. Ecosystem services provided by terrestrial isopods

4.2.1. Effect of the presence of *Porcellionides pruinosus* on soil biological activity and yield of tomato test plant

Regarding the relationship between the yield, root weight and the presence of terrestrial isopods, the following conclusions were drawn: In the 2016 study of the dry root weight of the tomato plant, no significant difference was detected. As the plant aged, the decomposition of the root has begun, so it is likely that the root weight value measured at harvest is not related to the health status of the plant.

Studying the total berry weight of the tomato plant (2016), terrestrial isopods had a greater effect than mulch. However, this difference was not apparent in the second year. Without mulch, woodlice survived less. They being found in the combined treatment in the highest number.

Based on the results obtained using the two types of growing media, it can be concluded that pure flower soil is more favorable for the tomato plant, while 1: 1 flower soil and sand proved to be more suitable for isopods.

Based on our results, it is recommended to repeat the experiment using both media, i.e. 1: 1 potting soil and sand, and potting soil only. Furthermore, it may be more effective for the experiment to increase the number of terrestrial isopods and to apply a properly controlled amount of irrigation water. Last but not least, it is recommended to extend the applied treatments to other crops in the future.

4.2.2. Consumption and distribution of plant pathogens by *Porcellionides pruinosus* and *Armadillidium vulgare*

Pathogen consumption is a little researched topic. It expands the range of ecosystem services, especially the consumption of mycelium and reproductive formulas of various pathogenic fungal species by terrestrial isopods.

In the potato storage experiment, tuber infestation caused by fusarium in the presence of the *Armadillidium vulgare* was much less than in the absence of isopods. From this it can be concluded that isopods likely consume different formulas of the pathogen, thereby preventing its spread.

No infection was observed at all on the tubers dressed with *Aureobasidium pullulans*, so it proved to be effective against potato diseases during storage.

Based on our experimental results, it is possible to promote the establishment and breeding of terrestrial isopods, which provide ecosystem services, and to create a favorable environment for them by certain agrotechnical method. Following our experiments, we can state that it is worthwhile to perform these woodlice-supporting operations in home gardening and small farming conditions.

4.3. Porcellionides pruinosus as a non-target organism

Examining the effect of the two insect pathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) and neem extracts on isopod species, we found that the range of commercially available biological plant protection products is still less than in conventional farming. In our studies, we observed that the strains of the two used fungal species did not affect the individuals. These entomopathogenic fungi can be safely applied in practice. It is recommended to investigate more soil

types, isopod species, and spore suspensions with similar methodologies for further researches.

Among the species, *Porcellionides pruinosus* is a suitable test animal, which, despite its fragility, is an ideal test animal that is widely tolerated and does not require soil and food. Strict monitoring of humidity and humidity is recommended. Potted soil mixed with quartz sand retains less soil moisture and therefore requires either more wetting of the crop or a reduction in the quartz sand content of the soil or its replacement with compost.

It can be stated that NeemAzal T / S and homemade neem leaf extract do not differ in their effect on *P. pruinosus*. From our results, it can be concluded that neither NeemAzal T / S nor neem leaf extracts pose a risk to the studied terrestrial isopods at the concentrations studied. However, further research is needed to monitor the potential impact of different neem products on soil fauna.

5. New scientific results

5.1. We found that as a result of mulching terrestrial isopods appear in larger numbers of species and individuals on the experimental plots every year.

5.2. We have confirmed that mulching favors the introduction and reproduction of more tolerant, generalist species (*Armadillidium vulgare, Cylisticus convexus*).

5.3. We have shown that terrestrial isopods prefer walnut and mixed litter over straw and compost mulch as well as uncovered soil surface.

5.4. The combined presence of *P. pruinosus* and organic soil cover helps to preserve the microbial activity of the soil.

5.5. The presence of *P. pruinosus* individuals in the soil under favorable conditions has a favorable effect on the yield of the crop.

5.6. We were the first to document that *P. pruinosus* individuals consume infected leaves with pear rust and mycospherella, thereby eliminating pathogens on the leaves.

5.7. We found that *P. pruinosus* individuals prefer to eat leaves without pear rust than leaves with pear rust, however, the presence of pear rust does not affect the rate of loss of microspheric spots.

5.8. *P. pruinosus* are the most likely to consume the mycelium of *Sclerotinia sclerotiorum* and the least likely to consume the colonies of Aspergillus niger.

5.9. Overall, it can be concluded that the colony consumption of isopods is higher in *S. sclerotiorum, Macrophomina phaseolina, Fusarium solani* than the regeneration of the colonies. In the case of *A. niger*, the opposite is true.

5.10. We found that *Fusarium solani* infestation was reduced during potato storage in the presence of *Armadillidium vulgare*. Isopods consume the mycelium of the pathogen and the plant tissues destroyed by the pathogen, thereby preventing the spread of Fusarium.

5.11. We have shown that the insect pathogenic fungal species *Metarhizium anisopliae* and *Beauveria bassiana* are harmless to *P. pruinosus*.

5.12. Neither NeemAzal T / S nor neem leaf extracts pose a risk to *Porcellionides pruinosus* in the concentration range commonly used in practice. NeemAzal T / S and homemade neem extract do not differ in their effect on *P. pruinosus*.

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7. Scientific publications related to the topic of the dissertation

7.1 Publications in foreign languages in peer-reviewed, scientific journals

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TDK supervisor:

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Supervisor:

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