Organic farming research project report submitted to:

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Project title: Evaluation of the Efficacy of Predatory Mites in Controlling Pests of Cultivated Mushrooms in Organic Mushroom Houses

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I. Species composition of mite predators and pests in mushroom houses with oyster mushrooms

The following species are the most common predatory mites in Polish mushroom houses, in which *Agaricus bisporus* is cultivated: *Arctoseius semiscissus, A. cetratus, Dendrolaelaps fallax* (Kropczyfiska-Linkiewicz 1984), and *Parasitus* sp. (Lewandowski, unpubl.). Species composition of mite predators of mushroom houses with oyster mushroom *(Pleurotus ostreatus)* is not known yet. Predatory mites are obviously of the most interest in mushroom pest control in the organic mushroom houses.

The aim of this part of the study was to identify the species composition of mite predators and pests in mushroom houses with oyster mushrooms *(Pleurotus ostreatus).*

METHODS

Samples of substrate for oyster mushroom cultivation were collected in 11 mushroom houses located near Warsaw, Kock, Lodz and Ostrow Wlkp. From each mushroom house 4-5 samples were taken. One sample consisted of 30-40 g material collected from 10-15 sacs. The substrate was mixed thoroughly in a laboratory and 200 cm^3 (okolo 100 g) of it was separated for mite extraction with the Berlese funnel. Mites were collected in 75% ethyl alcohol. The permanent slides for microscopic studies were prepared using the Heinz medium. Mites were identified to the species and counted. Results obtained are presented in Table I and Figure 1.

RESULTS

Mites belonging to several species were found in the substrate used for cultivation of the oyster mushroom:

* *Tyrophagus putrescentiae* (Schrank 1781);
* *Histiostima feroniarum* (Dufor 1839);
* *Siteroptes mesembrinae* (Canestrini 1861);
* *Siteroptes kneeboni* (Wicht 1970);
* *Arstoseius semiscissus* (Berlese 1892);
* *Arctoseius cetratus* (Sellnick 1940);
* *Dendrolaelaps rectus* (Karg 1965);
* *Dendrolaelaps fallax* (Leitner 1949);
* *Ameroseius imparasetasus* (Westerboer 1963);
* *Lasioseius furcisetus* (Athias - Henriot 1959).

Of mites, *Histiostoma feroniarum, Siteroptes kneeboni and S. mesembrinae* were the most numerous in the oyster mushroom houses. In some mushroom houses, the copra mites, *Tyrophagus putrescentiae,* pests of oyster mushroom mycelium, were found in the substrate. All these mites were accompanied by several predatory mites *(Dendrolaelaps fallax, D. rectus, Arctoseius semiscissus,* *Ameroseius imparsetasus)* (Table 1, Fig. 1). The predatory mites feed on other mites and/or fly eggs and larvae, and may play an important role as biological agents for controlling mushroom pests.

Table 1. Species composition of mite predators and pests in mushroom houses with oyster mushrooms.

Place	Date	T.p.	H.f.	S.k.	S.m.	A.s.	A.c.	D.f.	D.r.	A.i.	L.f.	Nymphs	Larvae	Hypopi	Dipteran larvae	Total
Ozarow	May 7	3	1					11						5		20
	May 21							5						5		10
	June 4		26											1		27
	June 18	2	11	2		1		1				1		1		19
	July 2		18	2		16		2	2	2		1	5		13	61
	July 15	5	13	3	4	44	1	36	4	3		25	1	3		142
	July 28		12	14	383	17	3	16	10	6	6	22	1	97	8	589
	Aug 18	4		3		1								1		9
Kock-Ia	June 4	1	1	1	44	4				2		1		1		55
Kock-Ib	June 4	5	55	8	157	35	4	1	7	71		37		3	11	394
Kock-II	June 4		6	3	446	2				2				1		460
Kock-III	June 4	4														
Grabow	Oct 29															
TOTAL		25	144	36	1034	120	8	72	23	123	6	87	7	118	47	1850

II. Predatory efficacy of *Arctoseius semiscissus* (Berlese) (Acari, Ascidae) against eggs of the sciarid flies (Sciaridae)

Mites of the genus *Arctoseius* are very common in soil and humus where they feed on springtails and mites inhabiting dead and decaying plant materials (Lindquist 1961, 1975). Nfites of two related species, *A. semiscissus* (Berlese) and *A. cetratus* (Sellnick), are phoretic on mushroom sciarid flies (Binns 1972, 1974; Dmoch 1995), and are known to prey on their eggs and first larval instars (Dmoch 1995).

The purposes of the study were (a) to find out if *A. semiscissus* mites could complete their development under laboratory conditions when fed on sciarid *(Lycoriella solani)* eggs with an addition of nematodes, and (b) to provide life table parameters for these predatory mites.

METHODS

Flies of *L. solani* were collected in a mushroom house near Warsaw, Poland. Sciarid flies with phoretic mites were placed in Petri dishes (9 cm diam.) filled with moist peat. Mites that left their carriers were collected and placed into new Petri dishes half-filled with moist peat. The mites were kept at 200 degrees C and fed nematodes, which were extracted from mushroom compost.

For extracting the nematodes, compost samples from a mushroom house were placed in Baermann funnels for 24 hours. The extract was added to a Petri dish that contained 2% agar medium with a 0.5 cm pieces of cheese as food for nematodes. Petri dishes were left for one week at a room temperature. Drops containing a nematode-rich mixture were added into the mite-rearing dish every other day.

For biological observations, the mites were kept in glass rearing cages (3 x 4 cm) provided with a 0.5 cm conical opening (4), filter paper on the bottom and covered with microscopic glass. Cages were placed in Petri dishes filled with pasteurized peat and, to prevent moisture loss, they were covered with self-shrinking film. *A. semiscissus* mites were fed with the sciard *(L. solani) eggs,* nematodes and both sciarid fly eggs and nematodes.

To determine the duration of developmental stages, 45 one-day-old eggs of *A. semiscissus* were placed individually in rearing cages for daily inspections. Duration of egg, larval, protonymphal, deutonymphal stages were recorded until the last molting.

Survival data were obtained as follows. About 100 eggs of *A. semiscissus* were placed into rearing cages, 5 eggs per cage. All eggs were observed daily to determine their hatchability, mortality of emerged mites at different developmental stages. Fecundity of *A. semiscissus* was determined for 20 mite pairs. Eggs laid by each female were counted and recorded.

RESULTS AND DISCUSSION

Populations of *A. semiscissus* developed well when mites were kept in Petri dishes half-filled with moist peat, in a room temperature (22-24 degrees C). Predatory mites were fed with the sciard eggs, nematodes and both sciarid fly eggs and nematodes. However, the best diet were eggs of the sciarids.

This was confirmed by the food preference studies. Predatory mites preferred eggs of the sciarids and nematodes, but they attacked also larvae of sciarids and cecids *(Heteropeza pygmaea)* and the mold mites *(Tyrophagus putrescentiae).* It was found that the former food determined the preference of predators (Figure 2).

The presence of nematodes in food of immatures accelerated their development (Table 2). Development period of *A. semiscissus* mites fed on sciarid eggs and nematodes at 20 degrees C was very short, and it lasted only 6-8 days, i.e., shorter than for *A. cetratus, Hypoaspis miles* and *Proctolaelaps deleoni* (Binns 1974, Enkegaard et al. 1997, Nawar 1992).

There was no significant difference (p>0.05) in the total time of juvenile development for females and males (Kruskal-Wallis test). None of postembryonic stages exceeded two days (Table 3).

Survival of *A. semiscissus* up to the adult stage was limited, but a high mortality rate was observed only for eggs (39%). Out of 61 mites that reached the adult stage, females outnumbered the males (39:22). The sex ratio found for *A. semiscissus* corresponds with this parameter found for *H. miles* mites (0.66) when fed sciarid larvae (Enkegaard et al. 1997), and for *P. deleoni* (Nawar 1992).

Fecundity of *A. semiscissus* females ranged from 9 to 131 eggs per female and averaged 55 (SE $\pm$ 5.23). The highest oviposition activity of females was between the 9th and 19th day of their lifespan. An average female lifespan was found to be 24 days (SE $\pm$ 1.3 1), with a range of 17-44 days. Fecundity of *A. semiscissus* females was found to be lower than that of *P. deleoni* females (Nawar 1992).

The following life-history parameters were determined for *A. semiscissus* mites fed on sciarid eggs and nematodes at 20 degrees C under laboratory conditions: net reproductive rate (R_o) - 21.55, generation time (T) - 14.42 days, intristic rate of increase (r_m) - 0.21 days, finite rate of increase (λ) - 1.24, and sex ratio - 0.64. These demographic parameters shows that the high finite rate of increase (λ) of *A. semiscissus* mites results from both short generation time (T) and high reproductive rate (R_o) (Table 4).

The net reproductive rate of increase (R_o) indicates that *A. semiscissus* populations can increase about 22 times during one generation, feeding on sciarid eggs and nematodes. Having a similar mean generation time (T) at the same temperature (20 degrees C), a population of *P. deleoni* mites can multiply 18 times when feeding on free-living nematodes (Nawar 1992). *P. deleoni* mites show also a lower intrinsic rate of natural increase (r_m). A high finite rate of increase (λ) found for *A. semiscissus mites,* as compared to *Hypoaspis miles* (λ=1.08), a biological control agent (Enkegaard et al. 1997), reflects a potential use of this species for suppressing sciarid flies in mushroom houses.

Table 2. Developmental period (days) of *A. semiscissus* females fed on different food.

Developmental stage	Food					
	Sciarid eggs only		Sciarid eggs + nematodes		Nematodes only	
	Mean	SE	Mean	SE	Mean	SE
Eggs	3.31	0.14	3.16	0.07	2.96	0.04
Larvae	2.00	0.09	1.34	0.08	1.11	0.06
Protonymphs	1.38	0.11	1.40	0.09	1.41	0.10
Deutonymphs	1.15	0.08	1.16	0.07	1.33	0.09
Total	7.85	0.18	7.06	0.08	6.81	0.15

Table 3. Duration (days) of different developmental stages of *A. semiscissus* mites fed on sciarid eggs and nematodes at 200C under laboratory conditions.

Developmental stage	Females (n=32)			Males (n=13)		
	Mean	SE	Range	Mean	SE	Range
Egg	3.16	0.07	3-4	3.38	0.14	3-4
Larvae	1.34	0.08	1-2	1.31	0.13	1-2
Protonymph	1.4	0.09	1-2	1.15	0.11	1-2
Deutonymph	1.16	0.07	1-2	1.23	0.12	1-2
Total	7.06	0.08	6-8	7.08	0.16	6-8

Table 4. Life-history parameters determined for *A. semiscissus* mites fed on different food at 20'C under laboratory conditions.

Life-history parameters	Food		
	Sciarid eggs only	Sciarid eggs + nematodes	Nematodes only
Intrinsic rate of increase (r_m)	0.23	0.21	0.19
Generation time (T)	13.85	13.40	11.38
Net reproductive rate (R_o)	24.50	21.55	8.79
Finite rate of increase (λ)	1.26	1.24	1.21
Sex ratio	0.62	0.64	0.50

III. Use of predators for controlling the sciarid flies

The aim of this part of study was (a) to determine incidence of phoretic relationships between *A. semiscissus* and the sciarid adults, (b) to determine methods of use of the predators for controlling the sciarid flies, and (c) to describe relationships between these predators and other mite predators occurring in the same substrate.

METHODS

Species composition of mites found on the sciarid adults *(L. solani)* was determined. The numbers of mites found on sciarid females were counted and the cases of phoretic relationships between mites and sciarid females were recorded during the crop season.

Experiments were set up in a climatic chamber to determine methods of use of the predators for controlling the sciarid flies. The method of Binns (1982) was adopted and used throughout these treatments. The substrate was prepared as follows: peat moss was sieved through a sieve with a 0.8 mm mesh, and mixed with 2% of ground soybean seeds and 4% of ground chalk. The substrate was then thoroughly mixed and placed into plastic boxes (200 mL). Then, adults of *L. solani* flies and then *A. semiscissus* mites (3, 6, 9 and 12 mites) were introduced to the substrate, and each box was closed by a glass vessel with the ventilation opening at the top covered with bolting cloth. Containers with infested substrate were stored at 19-20 degrees C. Three weeks later, the numbers of emerging flies that stuck to yellow sticky traps were recorded and compared with the control (no predatory mites). Dead flies found on the surface of the substrate were also counted. Each treatment comprised 2 replicates.

In a separate experiment, the relationships between *A. semiscissus and Dendrolaelaps fallax* mites and other mite predators *(Hypoaspis miles, H. aculeifer)* occurring in the same substrate were described.

RESULTS

It was found that females of *A. semiscissus* were more often phoretic on the sciarid adults than males. At the same time, some adults of sciarid flies were occupied by both males and females of *D. fallax* (Fig. 3). Phoretic mites *(A. semiscissus, D. fallax)* were found on the sciarid adults since the beginning of the mushroom cultivation to the end of the cultivation cycle. The number of phoretic mites per the sciarid fly increased with the age of substrate (Fig. 4).

Results of laboratory and field experiments to determine efficacy of the predators in controlling the sciarid flies indicate that *A. semiscissus* is able to reduce the population of *L. solani flies.* Predatory mites introduced into the substrate with *L. solani* larvae reduced their number, and the number of emerged adult flies (Fig. 5).

A. semiscissus mites are attacked by both *Hypoaspis miles and H. aculeifer,* and by *D. fallax* (Fig. 6). Therefore, *A. semiscissus* mites cannot be used for controlling the sciarid flies in a combination with other mite predators.

CONCLUSIONS

1. Predatory mites, *A. semiscissus,* can be easily reared in a laboratory, in Petri dishes filled with the substrate for mushroom cultivation.
2. The best food for *A. semiscissus* are eggs of *L. solani.* The highest life-history parameters are obtained by those predatory mites that are fed on both the sciarid eggs and nematodes.
3. *A. semiscissus* is able to reduce the population of *L. solani* flies in a laboratory trials.
4. *A. semiscissus* mites cannot be used for controlling the sciarid flies in a combination with other mite predators

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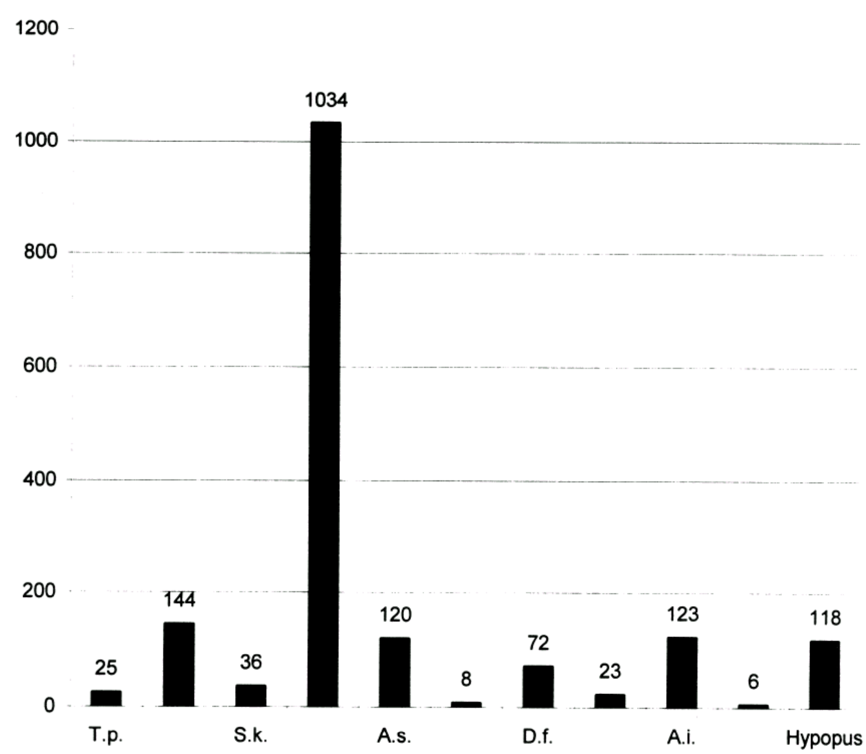
Fig. 1. Mite predators and pests in mushroom houses with oyster mushrooms



T.p. - *Tyrophagus putrescentiae* (Schrank);

H.f. - *Histiostoma feroniarum* (Dufor);

S.k. - *Siteroptes kneeboni* (Wicht);

S.m. - *Siteroptes mesembrinae* (Canestrini);

A.s. - *Arctoseius semiscissus* (Berlese);

A.c. - *Arctoseius cetratus* (Sellnik);

D.f. - *Dendrolaelaps fallax* (Leitner);

D.r. - *Dendrolaelaps rectus* (Karg);

A.i. - *Ameroseius imparsetasus* (Westerboer);

L.f. - *Lasioseius furcisetus* (Athias-Henriot);

Hypopus *Histiostoma feroniarum*

Fig. 2. Preference of predatory mites determined by the former food

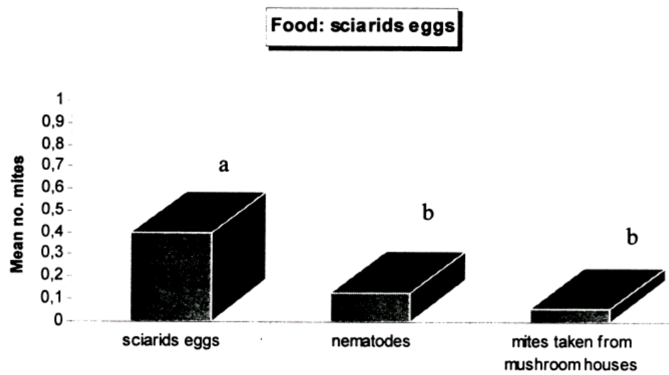


Fig. 3. Mean number *A. semiscissus* and *D. fallax* mites phoretic on the sciarid flies

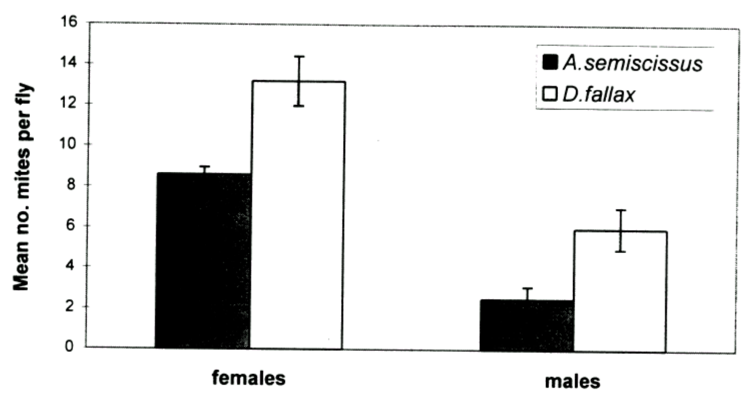


Fig. 4. Mean number of mites phoretic on sciarids flies during the cultivation cycle

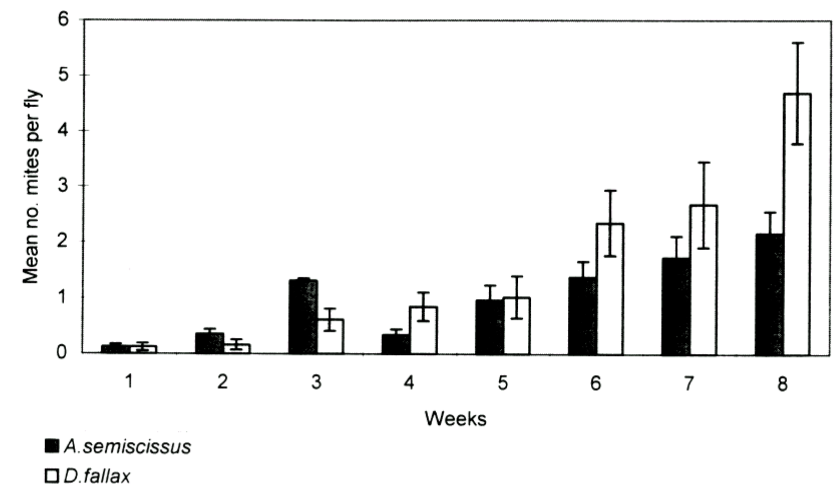


Fig. 5. Efficacy of mites predators in controlling sciarid flies

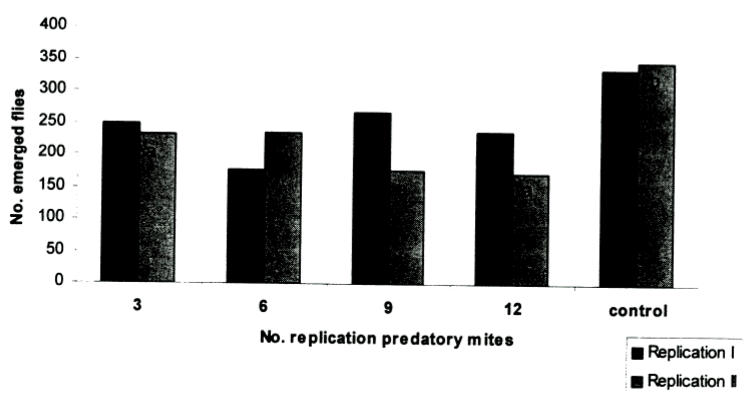
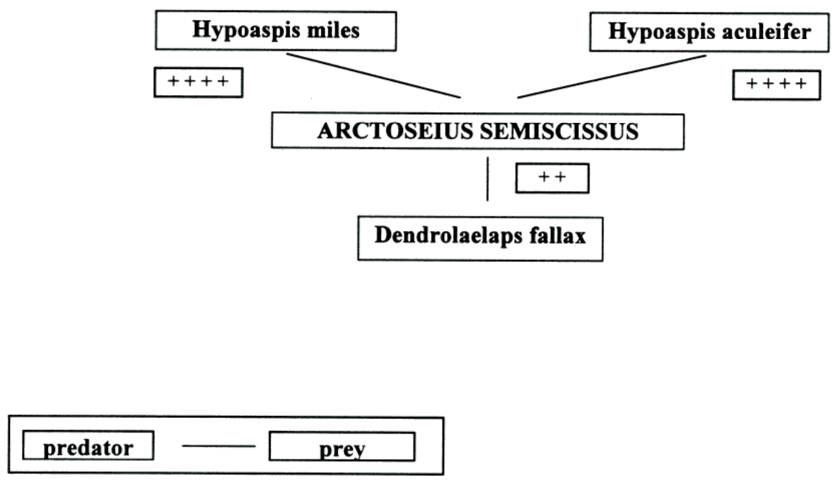


Figure 6. Interactions between *A. semiscissus* and other predatory mites



0 - no predation
+
++
+++
++++
} degree of predation (+ - low, ++++ - high)