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THE INFLUENCE OF PESTICIDES ON THE GROWTH OF FUNGUS *Hirsutella nodulosa* (PETCH) – ENTOMOPATHOGEN OF STRAWBERRY MITE (*Phytonemus pallidus* ssp. *fragariae* ZIMM.)

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A B S T R A C T

The influence of different pesticides used in strawberry cultivation on the growth of the fungus *Hirsutella nodulosa*, a natural pathogen of strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.), was evaluated under the laboratory conditions. The fungicides studied were dichlofluanid (Euparen 50 WP) and iprodione (Rovral 50 WP). The insecticides studied were endosulfan (Thiodan 350 EC), amitraz (Mitac 200 EC), hexythiazox (Nissorun 050 EC) and fosalone (Zolone 350 EC). The herbicide studied was pendimethalin (Stomp 330 EC). The insecticides and the herbicide were applied at the following rates: 0.1, 1 and 10 times the rate recommended by the manufacturer. The fungicides were applied at the following rates: 0.01, 0.1 and 1 times the rate recommended by the manufacturer. These pesticides were toxic to *Hirsutella nodulosa*. Iprodione, endosulfan and fosalone had the strongest inhibitory action. The fungicide dichlofluanid and the acaricide hexythiazix had the weakest inhibitory action.

Key words: strawberry mite, *Hirsutella nodulosa*, pesticides

INTRODUCTION

The strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.) is one of the most destructive strawberry pests in Poland (Łęska, 1964; Łabanowska, 2000). The mite can reduce yields by up to 70% (Alford, 1972). It feeds on leaves from early spring to September, reducing their

size and number. In Poland, from 4 to 5 generations develop each year, depending on the weather. Endosulfan (Thiodan 350 EC and Thionex 350 EC) has long been used to control the mite, but is being withdrawn from use because of concerns about its toxicity. Some new acaricides have been evaluated for use against this pest (Łabanowska, 1992). Biological agents, including viruses, bacteria and entomopathogenic fungi, have also been studied for use in accordance with the guide-lines of Integrated and Organic Fruit Production.

Research carried out since 1995 by the Chair of Plant Protection of the University of Podlasie has demonstrated that the entomopathogenic fungus, *Hirsutella nodulosa* (Petch), can infect the strawberry mite under natural conditions (Miętkiewski et al., 2000). The effects of agrochemicals, particularly pesticides, have to be taken into account whenever biological control agents such as entomopathogenic fungi are to be used. Pesticides should be highly selective against pests and pathogens, but should not inhibit useful microorganisms.

In vitro studies have demonstrated that some pesticides can inhibit growth, sporulation and germination of entomopathogenic fungi (Ignoffo et al., 1975; Majchrowicz and Miętkiewski, 1976; Keller, 1978; Bajan and Kmitowa, 1982; Vänninen and Hokkanen, 1988; Miętkiewski et al., 1997). Only a few papers have been published on the effects of pesticides on the growth and germination of *Hirsutella* species, specifically *H. thompsonii* (Sosa Gomez, 1991; Sosa Gomez et al., 1984; Tkaczuk, 2001) and *H. aphidis* (Petch) (Tkaczuk and Miętkiewski, 2001).

The aim of this study was to determine the in vitro effects of different pesticides on the growth of the fungus *H. nodulosa* – a pathogen of the strawberry mite.

MATERIAL AND METHODS

Preliminary estimates of strawberry mite mortality caused by *H. nodulosa* were carried out in 1997 at plantations near Skierniewice and Siedlce in central Poland. At each site, 50 of the youngest and still furled leaves were randomly collected. The percentage of mites infected by the fungus was estimated in the laboratory. Leaf samples were collected from the beginning of May until the end of October.

The strain of *H. nodulosa* used in this study was isolated from strawberry mites from the plantation near Skierniewice. The pesticides studied were selected from a wide spectrum of the pesticides commonly used in strawberry cultivation. The characteristics of the pesticides evaluated are presented in Table 1.

The inscecticides and the herbicide were applied at the following rates: 0.1, 1 and 10 times the rate recommended by the manufacturer. The fungicides were applied at the following rates: 0.01, 0.1 and 1 times the rate recommended by the manufacturer.

Pesticides were added to sterilised Sabouraud medium after cooling to approximately 60°C. Plates were incubated at 21°C. Colony diameter was

T a b l e 1. Pesticides used in this study

Commercial name	Active ingredient and content [% or g l^{-1}]	Active ingredient [in g or ml per liter of medium]
Fungicides		
Euparen 50 WP	dichlofluanid – 50%	1.5
Rovral 50 WP	iprodione – 50%	1.5
Insecticides		
Mitac 200 EC	amitraz – 200 g	0.6
Nissorun 050 EC	hexythiazox – 50 g	0.5
Thiodan 350 EC	endosulfan – 350 g	0.5
Zolone 350 EC	fosalone – 350 g	0.1
Herbicide		
Stomp 330 EC	pendimethalin – 330 g	0.2

measured every five days for 25 days. The experiment was repeated four times.

Analysis of variance and Duncan's t-test at P = 0.05 were used to estimate the differences between means of fungal colony growth on Day 25.

RESULTS AND DISCUSSION

Under natural conditions, *Hirsutella nodulosa* infectes strawberry mite larvae and adults, especially those feeding on the youngest and still furled leaves. Mites infected by the fungus were found in commercial strawberry plantations near Siedlce and Skierniewice from the middle of June until the end of October. Mortality of mites due to *H. nodulosa* ranged from 0.6% to 13.0%, reaching a maximum in September and October (Fig.1).

Of the pesticides used in this experiment, iprodione (Rovral 50 WP) inhibited the growth of *H. nodulosa* the most. The fungus was unable to grow on medium containing iprodione at a concentration equivalent to the recommended rate. At 0.1 times the recommended rate, iprodione inhibited fungus growth until Day 20; at 0.01 times the recommended rate, until Day 25. Colonies which started to grow toward the end of observation period reached only 18-19% of the control value (Tab. 2).

The fungicide dichlofluanid (Euparen 50 WP) was considerably less toxic to the fungus. When added to the medium at the recommended field rate, Dichlofluanid inhibited fungal growth equally or less than the insecticides endosulfan (Thiodan 350 EC) and fosalone (Zolone 350 EC). Colonies growing on medium containing dichlofluanid at the 0.1 times the recommended rate reached about 50% of the control size. Dichlofluanid did not significantly affect the growth of *H. nodulosa* at 0.01 times the recommended rate (Tab. 2).

Tkaczuk and Miętkiewski (2001) reported that fungicides strongly inhibited the growth of *Hirsutella aphidis*., a pathogen of aphids. Fluzilazole (Punch 400 EC) completely suppressed mycelial growth of

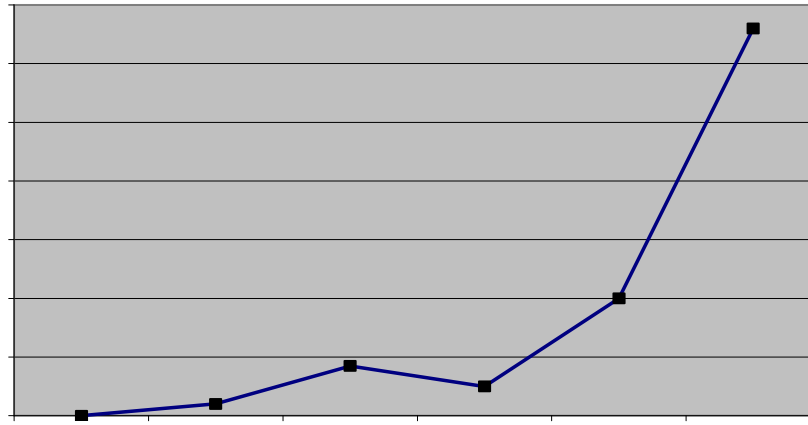

Figure 1. Dynamic graph of mortality of strawberry mite caused by fungus *Hirsutella nodulosa*

Table.2. Colony size of the fungus *Hirsutella nodulosa* growing on media containing pesticides (expressed)

Active ingredient and commercial name of pesticide	Dose rate	Colony size [as percentage of control value]				
		day 5	day 10	day 15	day 20	day 25 *
Iprodione (Rovral 50 WP)	A	0	0	0	0	0 cd
	B	0	0	0	24.1±	19.5 cd
	C	0	0	0	0	18.4 cd
Dichlofluanid (Euparen 50 WP)	A	0	37.3± 1.5	37.2± 1.3	27.6± 1.4	26.8 cd
	B	62.5± 0.0	44.8± 0.0	49.0± 0.0	51.7± 0.7	51.9 a-d
	C	100.0± 0.3	100.0± 0.6	95.1± 0.8	89.7± 0.5	92.2 ab
Amitraz (Mitac 200 EC)	A	0	0	0	0	0 cd
	B	0	41.8± 1.1	32.3± 0.4	24.1± 0.0	26.8 cd
	C	87.5± 0.4	59.7± 1.4	66.7± 1.1	67.6± 1.3	69.8 a-d
Hexythiazox (Nissorun 050 EC)	A	0	0	0	0	0 cd
	B	0	28.6± 0.2	38.3± 0.1	38.0± 0.1	39.7 bcd
	C	86.5± 0.4	62.5± 0.7	60.2± 0.5	65.4± 0.5	74.1 a-c
Endosulfan (Thiodan 350 EC)	A	0	0	0	0	0 cd
	B	0	0	0	0	0 cd
	C	71.9± 0.4	67.2± 1.4	71.6± 0.8	73.8±1.5	71.5 a-c
Fosalone (Zolone 350 EC)	A	0	0	0	0	0 cd
	B	0	20.0± 0.0	12.8± 0.2	13.8± 0.0	14.3 cd
	C	80.0± 0.6	47.0± 0.4	44.1± 0.4	45.0± 0.2	40.5 b-d
Pendimethalin (Stomp 330 EC)	A	0	0	0	0	0 cd
	B	62.5± 0.0	37.5± 0.7	34.3± 0.7	29.7± 0.4	29.1 cd
	C	62.5± 0.0	47.8± 0.3	42.2± 0.6	43.4± 1.4	44.7 b-d
Control	-	100	100	100	100	100 a

*The means followed by the same letter in columns do not differ significantly Duncan's multiple range t-test at P = 0.05 level of significances
Fungicides: A – 1 concentration, B – 0.1, C – 0.01
Herbicides and insecticides: A – 10, B – 1, C – 0.1

his fungus at the 1 and 0.1 the recommended rate. Mancozeb also strongly inhibited fungal development, but chlorothalonil (Bravo 500 SC) was less toxic. Fluzilasole (Punch 400 EC) and dichlofluanid (Euparen 50 WP) strongly reduced the growth of *Hirsutella thompsonii* var. *synnematosa* (Tkaczuk, 2001). According Sosa Gomez (1991) and his earlier investigation copper oxychloride reduced mycelial growth and completely suppressed sporulation of *Hirsutella thompsonii.* Sulphur also inhibited
conidiogenesis and mycelial growth, but less so than copper oxychloride.

Hirsutella nodulosa was unable to grow on media containing insecticides at 10 times the recommended rate. Endosulfan (Thiodan 350 EC) was the most toxic and prevented the growth of the fungus also at the recommended rate. The other insecticides strongly inhibited the growth of *H. nodulosa* at the recommended field rate. Fosalone (Zolone 350 EC) and amitraz (Mitac 200 EC) were the most toxic, reducing colony size by 85.6% and 73.2%, respectively. Hexythiazox (Nissorun 050 EC) was less toxic, reducing colony size by 60.3%.

Fosalone is highly toxic to entomopathogenic fungi such as *Metarhizium anisopliae*, *M. flavoviride* and *Paecilomyces fumosoroseus* (Mietkiewski and Sapieha 1995), as well as to the aphid pathogen, *H. aphidis* (Tkaczuk and Mietkiewski, 2001). Fosalone is one of the phosphoroorganic insecticides; this class of insecticide is highly toxic to entomopathogenic fungi (Bajan et al., 1977; Olmert and Kenneth 1974; and Vänninen and Hokkanen 1988). Methidathion, another phosphoorganic insecticide, was highly toxic to *H. thompsonii* (Sosa Gomez et al., 1990). These same authors observed a considerable reduction in the growth and sporulation of *H. thompsonii* on media containing the acaricides chlorobenzilat, dicofol and tetradifon.

The herbicide pendimethalin (Stomp 330 EC) prevented the growth of *Hirsutella nodulosa* at 10 times the recommended rate; even at 1 and 0.1 times the recommended rate, colony diameter on Day 25 reached only 29.1% and 44.7% of the control values, respectively.

Pendimethalin (Stomp 330 EC) and glifosat (Roundup 360 SL) completely inhibited the growth of *H. aphidis* at 10 times the recommended rate; pendimethalin was more inhibitory than glifosat at 0.1 times the recommended rate (Tkaczuk and Miętkiewski, 2001).

H. nodulosa isolated from the strawberry mite was sensitive to the majority of the pesticides used in the strawberry cultivation. Of the entomopathogenic fungi *B. bassiana*, *M. anisopliae*, *P. fumosoroseus* and *H. thompsonii* var. *synnematosa*, the most sensitive to toxic effects of pesticides was *H. thompsonii* var. *synnematosa* (Tkaczuk, 2001).

CONCLUSIONS

1. Pesticides used in strawberry cultivation are be toxic to the fungus *Hirsutella nodulosa* –

a natural pathogen of the strawberry mite.

2. Of the pesticides tested, iprodione (Rovral 50 WP), endosulfan (Thiodan 350 EC) and fosalone (Zolone 350 EC) inhibited fungal growth the most.
3. Of the pesticides tested, dichlofluanid (Euparen 50 WP) and hexytiazox (Nissorun 050 EC) seem to be the least toxic to *H. nodulosa*, and might be suitable for use in strawberry cultivation in accordance with the guidelines of Integrated Fruit Production.

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WPŁYW ŚRODKÓW OCHRONY ROŚLIN NA WZROST GRZYBA *Hirsutella nodulosa* (Petch) – PATOGENA ROZTOCZA TRUSKAWKOWCA (*Phytonemus pallidus* ssp. *fragariae* ZIMM.)

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S T R E S Z C Z E N I E

W doświadczeniu oceniano wpływ dwóch fungicydów: Euparen 50 WP (dichlofluanid) i Rovral 50 WP (iprodione), czterech zoocydów: Thiodan 350 EC (endosulfan), Mitac 200 EC (amitraz), Nissorun 050 EC (hexythiazox) i Zolone 350 EC (fosalone) oraz jednego herbicydu – Stomp 330 EC (pendimethalin) na wzrost grzyba *Hirsutella nodulosa.* Grzyb został wyizolowany z roztocza truskawkowca pochodzącego z plantacji produkcyjnej truskawki w okolicach Skierniewic. Fungicydy dodawano do podłoża hodowlanego w trzech dawkach: zalecanej, 10-krotnie niższej od zalecanej i 100-krotnie niższej od zalecanej; zoocydy i herbicyd zastosowano w dawkach: 10-krotnie wyższej od zalecanej, zalecanej i 10-krotnie niższej od zalecanej. Stwierdzono, że testowane środki ochrony roślin charakteryzowały się małą selektywnością w stosunku do entomopatogenicznego grzyba *Hirsutella nodulosa*. Najsilniej wzrost kolonii patogena hamowały: Rovral 50 WP, Thiodan 350 EC i Zolone 350 EC. Euparen 50 WP i Nissorun 050 EC w najmniejszym stopniu ograniczały rozwój kolonii grzyba entomopatogenicznego, co wskazuje na możliwość ich wykorzystania do ochrony roślin w integrowanej produkcji owoców.

Słowa kluczowe: roztocz truskawkowiec, *Hirsutella nodulosa*, środki ochrony roślin