EFFECT OF ALGINURE® (SEAWEED EXTRACT), MYCO-SIN®VIN (SULFURIC CLAY) AND POLYVERSUM® (PYTHIUM OLIGANDRUM DRECHS.) ON YIELD AND DISEASE CONTROL IN ORGANIC STRAWBERRIES

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Abstract

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In 2011 and 2012 three commercial products Alginure® (seaweed extract), Myco-Sin® VIN (sulfuric acid clay with yeast and plant extracts) and Polyversum® (spores of *Pythium oligandrum*) were tested in field experiment with strawberry cv. 'Induka' under the conditions of organic growing system. The aim of the study was to determine the effect of the preparations on yield, fruit quality and diseases control. The preparations were applied five times during flowering. We assessed total yield, marketable and unmarketable yields, average fruit weight, grey mould incidence on fruits caused by *Botrytis cinerea* and leaf spot severity caused by *Mycosphaerella fragariae*. Efficacy (%) of preparations was calculated. Preparations did not show positive effect on yields parameters. Total and marketable yields were not significantly different to untreated control in both years. Nevertheless considerable increase of yields from 2011 to 2012 was observed in strawberries treated by biofungicide Polyversum®. Preparations did not significantly reduce the incidence of grey mould. Alginure® showed the best results by efficacy 39.6% and 57.4%, respectively. All preparations provided very low, less than 10% efficacy to control leaf spot severity.

organic strawberry, Polyversum®, Alginure®, Myco-Sin®VIN, Botrytis cinerea

Organic fruit growing represents dynamically developing sector experiencing remarkable growth rates since the middle 1990's in Europe (Weibel et al., 2004). Organic growers overcome the restrictions of use synthetic pesticides by selection suitable resistant varieties but they may meet the problem to sell a new product (variety) on the market. Another problem is the possibility of development of a new pest or pathogen populations overcoming existing resistance. New races of Venturia inaequalis causing apple scab in resistant varieties carrying Rvi6 and Rvi7 genes represent a typical example (Gessler et al., 2006). Therefore besides of newly bred resistant cultivars, organic growers need innovative cultivation technologies to ensure good yield and

high quality of production. One approach is low input management using pests and disease self-regulatory mechanisms by supporting natural beneficial organisms in orchards (Laer, 2010; Weibel et al., 2010). However this management is still somewhat uncertain in intensive fruit plantations. Therefore artificial introduction and application of beneficial organisms (bioagens) are more widely used. Success of organic farming is highly encouraged by advances in the field of knowledge about plant defence mechanism which leads to using biocontrol agents and natural plant elicitors to enhance natural plant defence responses (Amil-Ruiz et al., 2011). Induced systemic resistance is a phenomenon whereby resistance to pathogen

is systematically induced by local infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds (Kuć, 2001).

In order to reduce or replace copper fungicides not only in organic production, sulfuric clays are tested to control pathogens (Dorn *et al.*, 2007; Michelante and Haine, 2004). In organic growing sulfuric clays are successfully introduced into the protection strategies, mainly in apples (Jamar and Lateur, 2007; Jamar *et al.*, 2010; Kelderer *et al.*, 2010; Kunz *et al.*, 2011; Falta and Vávra, 2010).

Natural compounds used as elicitors of induced systemic resistance offer new prospects for controlling diseases in organic production (Lateur, 2002; Kaufmann *et al.*, 2009). Seaweed extracts are tested in horticultural crops not only for control diseses (Washington *et al.*, 1999; Prokkola *et al.*, 2007; Loyola and Muñoz, 2009; Parikka *et al.*, 2009; Malusa *et al.*, 2010) but also as biostimulants and fertilizers (Crouch *et al.*, 1993; Paszt *et al.*, 2011; Malusa *et al.*, 2012).

Strawberry (*Fragaria* × *grandiflora* Ehr.) is a popular horticultural crop widely grown in organic system, although it can be challenged with many pests and pathogens. Grey mould caused by the fungus Botrytis cinerea Pers. ex Fr. is one of the most important disease of strawberries in humid climate. Therefore there is an effort to find out protection methods involving biological control using fungal or bacterial microorganisms (Peng and Sutton, 1991; Swadling and Jeffries, 1996; Adikaram et al., 2002; Donmez et al., 2011). Mycoparasitic fungus Pythium oligandrum Drechs. can be used for biological control in a wide spectrum of crops by its ability to attack fungal plant pathogens (Brožová, 2002) and to induce systemic resistance due to elicitinlike proteins POD-1, POD-2, POS-1 and oligandrin (Masunaka et al., 2010). Several studies proved oligandrin to be effective in pathogen control in strawberries and other horticultural crops (Picard et al. 2000; Benhamou et al., 2001; Mohamed et al., 2007; Wang et al., 2011). Lou et al. (2011) explain the ability of oligandrin to induce resistance against Botrytis cinerea in tomatoes by eliciting production of PR-2a and PR-3a (genes related to pathogenesis) and by increasing of defence enzymes POD, PPO and PAL in host plant.

This work is the output of a pilot project focused on the development of methods for commercial organic strawberry growing in the Czech Republic, where actually only hobby growers produce strawberries without chemical treatment. The aim of the present study was to test several bio preparations to find out their diseases control effectiveness and to assess their impact on yield and quality variables. The field experiment was conducted to prove efficacy of commercial plant protective agents Alginure® (seaweed extract), Myco-Sin®VIN (clay mineral with horsetail extract) and Polyversum® (spores of *Pythium oligandrum*) on yield, fruit quality

and health status of strawberry cv. 'Induka' under the organic system conditions.

MATERIALS AND METHODS

The experiment was carried out in fields of Faculty of Horticulture in Lednice (Mendel University in Brno). The locality is situated in Southern Moravia and is characterized by silty soils, altitude 179m, mean year temperature 9 °C, mean year precipitation 517mm. The soil was prepared two years according to the rules of organic agriculture using three kinds of green manure every year - phacelia, white mustard and mixture of field pea with oat, respectively. The experiment was established in the middle of September 2010 with seedlings of cv. 'Induka' (propagation station SEMPRA Turnov, Ltd., Czech Republic). Seedlings were planted in double row system on ridges covered by black polypropylene plastic mulch (nonwoven fabric). The distance of double rows was $0.8 \, \text{m}$, spacing of plants $0.35 \times 0.25 \, \text{m}$. Drop irrigation was installed above the plastic mulch immediately after planting. All agrotechnical operations were carried out according to the principles of organic agriculture. During the growing period the plantation was kept free of weeds by hand. In order to limit weed development, inter-rows were mulched with approximately 40mm thick layer of wheat straw in the beginning of April in 2011 and 2012, respectively.

The trials comprised of four treatments with three replicates with 20 plants (totally 60 plants). A randomised complete block design was used. Treatments were following.

1. Alginure®

Alginure® is a liquid substance containing seaweed extract (24%) from *Ascophyllum nodosum* and *Laminaria* sp., plant aminoacids (7%), phosphonates and other active substances. According to the producer and the supplier (Tilco Biochemie GmBH, Germany; Biocont Laboratories, Ltd., Czech Republic) the product stimulates innate and induced resistance by triggering production of H₂O₂, salicylic acid (SA), jasmonic acid (JA), ethylen, PR-proteins, stilben, resveratrol, lactone, saponine, lignine, phenylalanine ammonia lyase (PAL), lipoxygenase (LOX) and other substances in plants. The preparation shows preventive effect and should be repeated in 7–14 days intervals.

Application in the experiment: Five foliar sprays, each one in the dosage 3.5 l.ha⁻¹ in 600 l.ha⁻¹ spraying volume.

2. Myco-Sin®VIN

Myco-Sin*VIN is a powdery formulated product containing 75% of sulfuric acid clay $(Al_2(SO_4)_3)$, deactivated yeast components, extracts from plants – horsetail and sage, organic wetting and sticking components. According to the producer and the supplier (Dr. Schaette AG, Germany; Biocont

Laboratories, Ltd., Czech Republic) the product shows preventive effect against fungal pathogens attack. It cause hardening of leaf surface, inhibition of germination of spores and penetration of pathogens through cuticle by changing the pH and it supports production of phenolic compounds in treated plants.

Application in the experiment: Five foliar sprays, each one in the dosage 7.5 kg.ha⁻¹, in 600 l.ha⁻¹ spraying volume.

3. Polyversum®

Polyversum® is a biofungicide containing oospores of the fungus *Pythium oligandrum* at a number of 106–107.g⁻¹ of preparation. According to the producer (Biopreparaty Ltd., Czech Republic) it has three effects on the plants. i) *Pythium oligandrum* directly decays fungal pathogens (mycoparasitism), ii) inhibits the growth of phytopathogenetic microorganisms by stimulating morfological and biochemical barriers in a plant (induced resistance) and iii) influences the production of growth-stimulating substances in a plant and indirectly increases the intake of phosphorus and micronutrients by the plant (growth stimulation).

Application in the experiment: Five foliar sprays, each one in the dosage 0.1 kg.ha⁻¹, in 600 l.ha⁻¹ spraying volume.

4. Untreated control

All products were applied by foliar way with hand sprinkler five times according to phenological stages of strawberries. In 2011 the spraying schedule was following:

1st application: April 15 (BBCH 55 – first flowers at the bottom of the rosette).

2nd application: April 22 (BBCH 58 – early balloon stage).

3rd application: May 10 (BBCH 67 – flowers fading: majority of petals fallen).

4th application: May 17 (7 days after the end of flowering).

5th application: July 6 (two weeks after the end of harvesting).

In 2012 spraying schedule was analogous (in the same phenological stages) in following dates: April 20, April 27, May 7, May 14 and July 4.

During the harvest time we assessed the yield and quality of production. Data were recorded during each harvest, berries were picked twice a week. Cv. 'Induka' showed 8 harvests in both years. In 2011 the first harvest was conducted on the 26th May, the last one on the 22nd June. In 2012 harvest time began on the 22nd May and it finished on the 20th June. Fruits were weighed and sorted into qualitative classes according to the marketing standards of the European Union (commission regulation / EC/ No 843/2002): Extra class (regular shape and colour, diameter > 25 mm), Class I (slight defect of shape, a white patch max 1/10 of the surface, diameter 18–25 mm) and Class II (defect of shape, a white patch max 1/5 of the surface), respectively.

The marketable yield was expressed as the sum of weight of all qualitative classes. Berries with smaller diameter, diseased or showing deformations or other disorders formed unmarketable yield. Berries infected by Botrytis cinerea were counted and weighed separately and finally disease incidence (% of diseased berries) was determined. Mean fruit weight was calculated from weights of all berries regardless of the quality classes including unmarketable part. Cumulative marketable yield was finally expressed as the sum of marketable yields of both years. In the middle of July disease severity (index) of leaf spot (Mycosphaerella fragariae) was assessed using 5-point rating scale reflecting the intensity of diseases symptoms on leaves: 1 – no symptoms, 2 – rare spots (up to 10), 3 – spots on the area smaller than 1% of total leaf area, 4 – spots on 10–25% of total leaf area, 5 – spots covering more than 25% of the leaf area. We evaluated every plant in the trial repetition (total 60 plants). Subsequently disease severity (index) was expressed. Efficacy (%) of plant protective preparations on disease reduction was calculated according to Abbott (1925).

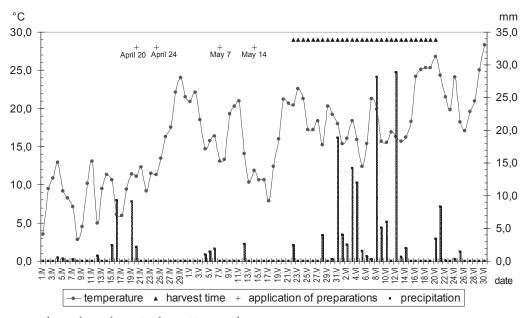
Data were statistically processed by ANOVA and Tukey's test (p < 0.05) using software Unistat version 5.1. Grey mould incidence is presented in percentage units, statistical analysis (ANOVA) was performed by using software UPAVplus, version 1.06 (Czech Phytosanitary Administration) taking into account arcsin data transformation. Assessed data are expressed as averages \pm standard deviation.

RESULTS

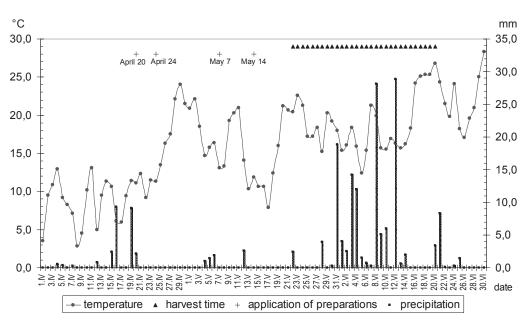
Late spring frosts negatively influenced the experiment in both years. In 2011 temperature dropped bellow 0 °C in four subsequent days during flowering. Critically low temperatures were recorded in the level of strawberries plants on the 6th May in 2011 (–4.1 °C), and on the 18th May in 2012 (–4.7 °C), respectively. Frosts caused significant flower and fruit set reduction (estimate up to 70% reduction in 2012) and resulted in very low total and marketable yields with increased ratio of Class II and unmarketable portion due to relatively high amount of malformed berries. Meteorological data of the period from April to June in 2011 and 2012 are presented in Fig. 1 and Fig. 2, respectively.

Analysis of variance did not show significantly effect of tested preparation on yield and fruit quality parameters in both years (Tab. I). Average fruit weight was significantly influenced by year.

Untreated control showed paradoxically the highest total yield (Fig. 3). In 2011 the control showed the highest weight of all quality classes, except of Class I, where application of Polyversum® resulted in the biggest amount. Generally the yields were very low and they did not reach 100 g.plant¹ (Fig. 3). Regarding preparations the highest total yield was found out in plots treated by Myco-Sin®VIN (83.0 g.plant¹) and Alginure® (78.8 g).



1: Weather conditions during April-June 2011 in Lednice

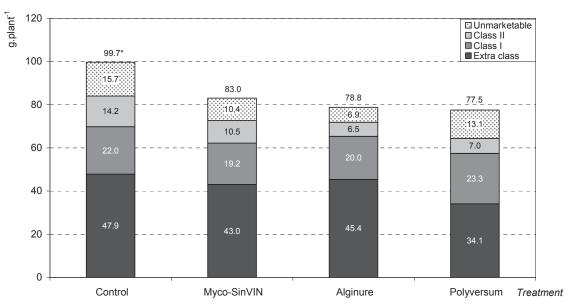


2: Weather conditions during April-June 2012 in Lednice

 $I:\ Analysis\ of\ variance\ for\ marketable\ yield\ (g.plant^{-1})\ and\ fruit\ weight\ (g)$

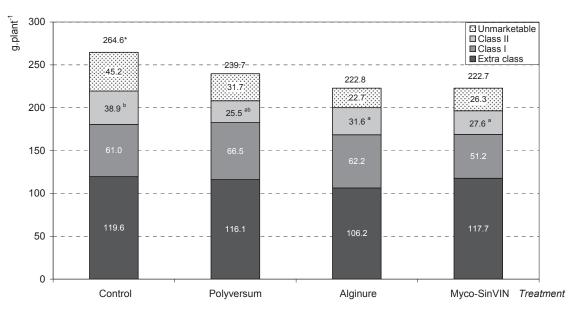
	Marketable yield (g.plant ⁻¹)				Fruit weight (g)			
Source of variability	df	2011	2012	Cumulative 2011+2012	2011	2012	Averag	ge 2011–2012
ar	aı	MS	MS	MS	MS	MS	df	MS
Treatment	3	195.485 ^{ns}	310.290ns	784.7 ^{ns}	$0.579^{\rm ns}$	$0.171^{\rm ns}$	3	0.254 ^{ns}
Residual	8	684.030	942.021	814.8	0.797	0.153	16	0.475
Year							1	17.105***
Year×Treatment							3	0.497

^{***} $p \le 0.001$, ns non-significant (p > 0.05)



*Referring to the total yield (the sum of Extra class, Class I, Class II and unmarketable)

3: Yield data in 2011



Different letters between the columns of Class II indicate significant differences (ANOVA, Tukey test, P<0.05) Referring to the total yield (the sum of Extra class, I. class, II. class and unmarketable yield).

4: Yield data in 2012

Application of Polyversum $^{\circ}$ resulted in the lowest total yield in 2011 (Fig. 3).

Classification of berries into quality classes in 2012 is presented in Fig 4. The highest total yield was 264.6 g.plant⁻¹ in untreated control. Comparing preparations, the highest total yield was found out in plots treated with Polyversum $^{\circ}$ and the lowest yields showed plants sprayed with Myco-Sin $^{\circ}$ VIN (Fig. 4). Application of Alginure $^{\circ}$ and Myco-Sin $^{\circ}$ VIN significantly (p < 0.05) decreased weight of berries in class II compared to control.

Marketable yield is the economically important parameter involving production which is realized on the market. Fruit weight is a quality parameter proving external attractiveness for consumers. As mentioned above, quantity and quality of production were strongly reduced by unfavourable weather conditions during flowering. The highest marketable yield belonged to untreated control in both years (84.4g and 219.4g, respectively). From all tested preparations Polyversum® showed considerable increase of marketable yield from the first year (64.4g) to the second year (208.1g) and thus

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Treatment	IV.	Tarketable yie	eld (g.plant-1)	Fruit weight (g)		
	2011	2011	Cumulative 2011+2012	2011	2012	Average 2011-2012
Alginure®	71.9 ± 25.6	200.1 ± 27.7	272.0 ± 7.5	9.9 ± 1.3	7.7 ± 0.6	8.8 ± 1.5
Myco-Sin*VIN	$\textbf{72.7} \pm \textbf{38.1}$	196.4 ± 43.9	269.1 ± 4.1	$\textbf{9.8} \pm \textbf{0.7}$	$\textbf{8.0} \pm \textbf{0.4}$	8.9 ± 1.1
Polyversum®	64.4 ± 16.3	208.1 ± 28.5	272.5 ± 43.9	9.0 ± 0.4	$\textbf{8.2} \pm \textbf{0.2}$	8.6 ± 0.5
Control	84.0 ± 19.1	219.4 ± 16.1	303.4 ± 35.2	10.0 ± 0.9	$\textbf{8.2} \pm \textbf{0.3}$	9.1 ± 1.2

III: Analysis of variance for grey mould incidence (%) and leaf spot severity (index)

		Grey mould inci	dence (%)	Leaf spot severity (index)		
Source of variability	16	IV.	IS	MS		
	ai -	2011	2012	2011	2012	
Treatment	3	10.313 ^{ns}	10.451 ^{ns}	0,008 ^{ns}	0.127 ^{ns}	
Residual	8	14.078	4.450	0.018	0.107	

ns non-significant (p > 0.05)

IV: Grey mould incidence (%) and efficacy of the products

Treatment	Grey mould i	incidence (%)	Efficacy (%)		
	2011	2012	2011	2012	
Alginure*	1.27+0.80	2.34+1.18	57.4	39.6	
Myco-Sin®VIN	2.69+1.21	2.04+1.43	9.9	47.2	
Polyversum [®]	1.53+1.33	4.08+1.08	48.6	-5.5	
Control	2.98+2.46	3.87+0.92	_	_	

V: Leaf spot severity (%) and efficacy of the products

Treatment	Leaf spot sev	verity (index)	Efficacy (%)		
	2011	2012	2011	2012	
Alginure®	1.20	2.38	4.0	0.8	
Myco-Sin®VIN	1.18	2.25	5.6	6.3	
Polyversum [®]	1.30	2.22	-4.0	7.5	
Control	1.25	2.40	_	_	

it was found out as the best preparation after two years of evaluation. Cumulative yields obtained by the sum of yields of both years were low and ranged from 269.1 g.plant⁻¹ in Myco-Sin®VIN and 303.4 g.plant⁻¹ in untreated control. Differences between treatments were statistically non-significant.

Preparations did not affect mean fruit weight of strawberries (Tab. I). Average value did not exceed 10 g.plant⁻¹. The largest fruits were in the control in both years (Tab. II). Comparing preparations application of Myco-Sin*VIN resulted in the highest fruit weight 8.9 g. Nevertheless the differences were very low and non-significant.

Average fruit weight was significantly influenced by year. Fruit weight was significantly higher in the first fruiting year (2011). Average fruit weight was 9.7 g in 2011 and 8.0 g in 2012, respectively.

The main object of the investigation was to determine the efficiency of tested preparations on disease control. Treatments did not affect the incidence of grey mould caused by *Botrytis cinerea* and severity of leaf spot (caused by *Mycosphaerella*

fragariae). Effect was not statistically significant (Tab. III).

Effect of the products on grey mould incidence was considerably different between years (Tab. IV). Alginure* showed quite stable efficacy in both years (39.6 and 57.4%, respectively). Myco-Sin*VIN and Polyversum* gave very different results in each year. While Polyversum* provide 48.6% efficacy in the first year, it did not proved it next year, when disease incidence was higher on berries harvested from plots treated by this preparation (4.08 %). On the contrary Myco-Sin*VIN showed better efficacy in 2012 compared to 2011. Generally efficacy of tested preparation was unsatisfactory.

Leaf spot severity was higher in 2012 compared to 2011. Application of preparation did not significantly affect severity of leaf spot caused by *Mycosphaerella fragariae*. Efficacy of preparations was very low (Tab. V) and did not exceed the level of 10%. While Myco-Sin*VIN showed low but relatively stable efficacy in both years (5.6% and 6.3%, respectively), Polyversum* provide highly different

values in each year. In 2011 disease severity index was lower in untreated strawberries compared to those sprayed by suspension of *Pythium oligandrum* (Polyversum*). Application of Alginure* did not affect leaf spot severity, the efficacy varied from 4.0% and 0.8%, respectively.

DISCUSSION

Tested preparations did not showed positive effect on yield and did not significantly decreased the incidence of fungal diseases in strawberries. Stephenson (1966) stated that treatment with seaweed extract increased yield and reduced the incidence of grey mould on strawberries in greenhouse experiments. On the contrary Washington et al. (1999), Prokkola et al. (2003) and Prokkola and Kivijärvi (2007) did not find significant differences in yields and grey mould incidence in strawberries after seaweed extract application. According to Prokkola and Kivijärvi (2007) general low incidence of grey mould occurrence may partly explain those small differences. Grey mould incidence was similarly very low in our experiment. That can be explained partly because of high resistance of the variety 'Induka', partly due to weather conditions unfavourable for pathogen development. Xu et al. (2000) indicate, that for Botrytis cinerea infection on strawberry the optimal temperature is 15-25 °C with >90% relative humidity, associated with rainy periods, when conidia are washed out. Especially spring 2012 was very dry, sum of precipitation during April - May was only 57.2 mm. Wilcox and Seem (1994) reported, that grey mould on strawberries fruits is positively correlated with rainfall, but Blanco et al. (2006) did not proved this correlation. In our experiment disease incidence was higher in 2012 (index 3.1) than in 2011 (index 2.1) in spite of opposite distribution of precipitation. It may be explained by higher density of plantings with limited airflow due to higher robustness of plants in the second productive year. The incidence of grey mould tended to increase during years in strawberry cultivation (Birkeland et al., 2002).

Differences between preparations efficacy on Botrytis cinerea in years could be partly explained by different weather conditions. We can see differences in daily temperatures and precipitation between both years (Fig. I and Fig. 2) during the flowering season, when the products were applied. While altogether 10 days with rainfall was recorded between the second and the third application of preparations in 2011 (Fig. 1), only 5 days with rainfall existed in 2012 during the period of all applications (Fig. 2). Furthermore the precipitation was very low (1.0–2.6 mm). Myco-Sin[®]VIN was ineffective to control grey mould in 2011, when rainfall period occurred in the end of April during strawberry flowering, perhaps because it could be washed away from the leaf surface. This assumption is based on the experience of Michelante and Haine (2004) and Dorn et al. (2007), who noted Myco-Sin (very similar product to Myco[®]Sin VIN) was effective to reduce Phytophthora infestans in potatoes in in vitro assay, but it showed significantly lower efficacy in field conditions. The authors supposed the failure under field conditions due to a lack of stability (sulfuric clay showed poor rainfastness) under prevailing environmental conditions and not to a lack of efficacy. Kloutvorová et al. (2011) tested efficacy of Myco-Sin®VIN on Venturia inaequalis in laboratory and field trials. They found out lower efficacy of Myco-Sin®VIN in field conditions, where this product showed 27.3% efficacy in 2009 but only 18.0% in extremely rainy year 2010. In our experiment the efficacy of Myco-Sin®VIN in 2012 was much higher than in 2011. Biofungicide Polyversum® provided completely opposite results. The efficacy was higher in the first year. It could be explained by more favourable weather condition in spring 2011, when more rainy and humid conditions probably supported development of mycoparasitic fungus Pythium oligandrum applied on strawberry leaves and allowed it to parasitize substrate.

These facts demonstrate the difficulty of testing products under common field conditions. That is one of the reasons why there are many discrepancies in published studies concerning effectiveness of biocontrol agents. While Nikolić at al. (2009) present Polyversum® as very effective disease control agent with routine use in strawberry production in Serbia, and Meszka and Bielenin (2010) find out high efficacy of Polyversum® not only on Botrytis cinerea (61.2–95.4%) but also on Mycosphaerella fragariae and Sphaerotheca macularis, Tanovic et al. (2008) desribe Polyversum® to be ineffective to control Botrytis cinerea in raspberries. They stated unsatisfactory efficacy (18.2% and 46.6%) which is quite similar to our results (48.6% efficacy in the second year of experiment). Tanovic et al. (2008) point out, that most of the studies reporting high efficacy of biocontrol agents were conducted under controlled environments (Peng and Sutton, 1991; Picard et al., 2000; Benhamou et al., 2001; Donmez et al., 2011; Lou et al., 2011). Highly effective antagonists in controlled environments can be moderately or totally ineffective when introduced to commercial fields because effectiveness of biocontrol agents is strongly influenced by weather conditions (Elad et al., 1994).

Further experiments are needed to focus on the optimization time for application of products with regard to the course of weather conditions.

CONCLUSION

Two year results of testing plant strengtheners Alginure® and Myco-Sin®VIN and biofungicide Polyversum® did not revealed their positive effect on yield traits and reduction of disease development. The field experiment was considerably negatively influenced by weather conditions during blossom, when late spring frosts caused pronounced

reduction of yields and negatively influenced the quality of fruits as well. Very dry spring in the second experimental year with extremely low precipitation did not allow significant development of fungal diseases either at untreated control.

Discrepancies in preparations efficacy between years raise questions for further research aimed to determine optimal time for application of these tested products. Therefore more investigations are needed.

SUMMARY

The aim of this study was to examine the effect of three commercial products: Alginure® (seaweed extract), Myco-Sin°VIN (sulfuric clay with yeast and plant extracts) and Polyversum® (spores of Pythium oligandrum) on disease control and yield parameters in organic strawberry cv. 'Induka'. In 2011 and 2012 the field experiment was established in Lednice (Czech Republic) in double row elevated beds with PP mulch. Spacing of plants was 0.35 × 0.25 m, spacing of double rows 0.8 m. Trial was set as randomized block design with three repetitions. All preparations were applied by foliar way five times according to phenological stages: BBCH 55, BBCH 58, BBCH 67, seven days after the end of flowering, two weeks after the end of harvesting. Harvested fruits were weighed and categorized into qualitative classes according to marketing standards of the European Union (commission regulation / EC/No 843/2002. In 2011 and 2012 we assessed following traits: total yield (sum of weight of all fruits), marketable yield (sum of weight of Extra Class, Class I and Class II), unmarketable yield (involving small, deformed of diseased fruits) and average fruit weight. Cumulative marketable yield was expressed as the sum of marketable yields of both years. Disease incidence (%) of grey mould (Botrytis cinerea) was expressed as percentage of rotten fruits. Severity of leaf spot caused by Mycosphaerella fragariae was evaluated using 5-point rating scale (1 - no symptoms, 5 - spots covering more than 25% of the leaf area). Finally efficacy (%) of plant protective preparations was calculated.

Yields were very low due to severe frost reduction of flowers and fruit set in spring in both years. Application of preparations did not bring expected positive effect on yields parameters. Nevertheless considerable increase of yield in 2012 was observed in strawberries treated by biofungicide Polyversum*. Preparations did not significantly reduce the incidence of grey mould caused by *Botrytis cinerea*. Regarding both years Alginure* showed the best results with 39.6% and 57.4% efficacy. Polyversum* and Myco-Sin*VIN provide highly different results in each year. The preparations showed very low efficacy to control leaf spot caused by *Mycosphaerella fragariae*. Disease severity was similar in all treatment. Myco-Sin*VIN showed low but relatively stable efficacy in both years (5.6% and 6.3%, respectively).

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REFERENCES

- ABBOTT, W. S., 1925: A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265–267. ISSN 0022-0493.
- ADIKARAM, N. K. B, JOYCE, D. C. and TERRY, L. A., 2002: Biocontrol activity and induced resistance as a possible mode of action for *Aureobasidium pullulans* against grey mould of strawberry fruit. *Australasian Plant Pathology* 31, 223–229. ISSN 1448-6032
- AMIL-RUIZ, F., BLANCO-PORTALES, R., MUÑOZ-BLANCO, J., CABALLERO, J. L., 2011: The strawberry plant defence mechanism: a molecular review, *Plant and Cell Physiology*, 52, 11: 1873–1903, ISSN 1471-9053.
- BENHAMOU, N., BÉLANGER, R. R., REY, P., TIRILLY, Y., 2001: Oligandrin, the elicitin like protein produced by the mycoparasite *Pythium oligandrum*, induces systemic resistance to Fusarium crown and root rot in tomato plants.

- Plant Physiology and Biochemistry, 39: 681–698. ISSN 0981-9428.
- BIRKELAND, L., DØVING, A. and SØNSTEBY, A., 2002: Yield and quality in relation to planting bed management of organically grown strawberry cultivars. *Acta Horticulturae*. 567: 519–522. ISSN 0567-7572.
- BLANCO, C., SANTOS, B., ROMERO F., 2006: Relationship between concentrations of *Botrytis cinerea* conidia in air, environmental conditions, and the incidence of grey mould in strawberry flowers and fruits. *European Journal of Plant Pathology*, 114: 415–425. ISSN 1573-8469.
- BROŽOVÁ, J., 2002: Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection. *Plant Protection Science* 38 (1): 29–35. ISSN 1212-2580.
- CROUCH, I. J. and STADEN, J. Van., 1993: Commercial seaweed products as biostimulants in horticulture. *Journal of Home & Consumer Horticulture*, 1, 19–76. ISSN 1054-4682.

- DONMEZ, M. F., ESITKEN, A., YILDIZ, H. and ERCISLI, S., 2011: Biocontrol of *Botrytis cinerea* on strawberry fruit by plant growth promoting bacteria. *The Journal of Animal & Plant Sciences*, 21, 4: 758–763. ISSN 1018-7081.
- DORN, B., MUSA, T., KREBS, H., FRIED, P. M., FORRER, H. R., 2007: Control of late blight in organic potato production: evaluation of copper-free preparations under field, growth chamber and laboratory conditions. *European Journal of Plant Pathology*, 119, 2: 217–240. ISSN 1573-8469.
- ELAD, Y., KŐHL, J. and FOKKEMA, N. J., 1994: Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprofitic bacteria and fungi. *European Journal of Plant Pathology*, 100: 315–336. ISSN 0929-1873.
- FALTA, V. and VÁVRA, R., 2010: Testování přípravků k ochraně proti strupovitosti jabloní pro integrovanou a ekologickou produkci. *Zahradnictví* 11: 10–13. ISSN 1213-7596.
- GESSLER C., PATOCCHI, A., SANSAVINI S., TARTARINI, S., GIANFRANCESCHI L., 2006: Venturia inaequalis resistance in Apple. Critical Review in Plant Sciences, 25: 473–503. ISSN 1549-7836.
- JAMAR, L., CAVELIER, M. and LATEUR, M., 2010: Primary scab control using a "during-infection" spray timing and the effect on fruit quality and yield in organic apple production. *Biotechnoogy, Agronomy, Society and Environment*, 14(3): 423–439. ISSN 1370-6233.
- JAMAR, L. and LATEUR, M., 2007: Strategies to reduce copper use in organic apple production. *Acta Horticulturae*, 737: 113–120. ISSN 0567-7572.
- KAUFFMANN, S., DOREY, S., BAILLIEUL, F., 2009: Towards an alternative strategy for crop protection. *Biofutur*, 296: 26–30. ISSN 0294-3506.
- KELDERER, M., CASERA, C., LARDSCHNEIDER, E, RAINER, A., 2010: Controlling Gloeosporium rot on Pinova apple fruits. Part 1: preharvest acid clay sprays versus postharvest hot water dipping treatments. In: 14th International Conference on Organic Fruit-Growing: Proceedings to the Conference from February 22 to February 24, 2010 at Hohenheim, Germany. Weinsberg: FOEKO, p. 78–85. ISBN 978-3-00-030214-5.
- KLOUTVOROVÁ, J., KNĚZÁČEK, L., KUPKOVÁ, J., NÁMĚSTEK, J., 2011: Evaluation of efficacy of some biocontrol agents against fungal diseases on apples. *Vědecké práce ovocnářské*, 22: 61–69. ISSN 0231-6900.
- KUĆ, J., 2001: Concepts and Direction of Induced Systemic Resistance in Plants and its Application. European Journal of Plant Pathology. 107, 1: 7–12. ISSN 1573-8469.
- KUNZ, S., SCHMITT, A., HAUG, P., SOBICZEWSKI, P., KALUZNA, M., PULAWSKA, J., 2011: International Development of strategies for fire blight control in organic fruit growing. *Acta Horticulturae*, 896: 431–436. ISSN 0567-7572.
- LAER, P. van, 2010: Importance of diversification in the variety assortment of apple, pear, cherry and

- plum orchards. *Obstbau und Früchteverwertung*, 60, 4: 458–462, ISSN 0007-5922.
- LATEUR M., 2002: Perspectives de lutte contre les maladies des arbres fruitiers à pépins au moyen de substances naturelles inductrices d'une résistance systémique. *Biotechnology, Agronomy, Society and Environment*, 6: 67–77. ISSN 1370-6233.
- LOU, B., WAN, A., LIN, CH., XU, T., ZHEN, X., 2011: Enhancement of defense responses by oligandrin against *Botrytis cinerea* in tomatoes. *African Journal* of *Biotechnology*, 10, 55: 11442–11449. ISSN 1684-5315.
- LOYOLA, N. and MUÑOZ, C., 2009: Effect of the biostimulant foliar addition of marine algae on 'O'Neal' production. *Acta Horticulturae*, 810: 709–722. ISSN 0567-7572.
- MALUSA, E., PASZT, L. S., TRZCINSKI, P., GÓRSKA, A., MOURÃO, I., AKSOY, U., 2012: Influences of different organic fertilizers and amendments on nematode trophic groups and soil microbial communities during strawberry growth. *Acta Horticulturae*, 933: 253–260, ISSN 0567-7572.
- MASUNAKA, A., SEKIGUCHI, H., TAKAHASHI, H. and TAKENAKA, S., 2010: Distribution and Expression of Elicitin-like Protein Genes of the Biocontrol Agent *Pythium oligandrum*. *Journal of Phytopathology*, 158, 6: 417–426. ISSN 1439-0434.
- MESZKA, B. and BIELENIN, A., 2010: Polyversum WP a new biological product against strawberry grey mould. *Phytopathologia*, 58: 13–19. ISSN 2081-1756.
- MICHELANTE, D. and HAINE, D., 2004: Evaluation of products for potato protection against late blight in order to replace copper based fungicides in organic farming. In: WESTERDIJK, C. E and SCHEPERS H. T. A. M. (eds.) *PPO-Special report No.* 10, 303–310. ISSN 1569-321X.
- MOHAMED N., LHERMINIER, J., FARMER, M. J., FROMENTIN, J., BENO, N., HOUOT, V., MILAT, M. L., BLEIN, J. P., 2007: Defense responses in grapevine leaves against *Botrytis cinerea* induced by application of a *Pythium oligandrum* strain or its elicitin, oligandrin, to roots. *Phytopathology*, 97: 611–620. ISSN 0031-949X.
- NIKOLIĆ, M., MILIVOJEVIĆ, J. and M. IVANOVIĆ, M., 2009: Strawberry Production in Serbia the State and Perspectives. *Acta Horticulturae*. 842: 615–618. ISSN 0567-7572.
- PARIKKA, P., KIVIJÄRVI, P., PROKKOLA, S. and KEMPPAINEN, R., 2009: Microbiological quality of organic strawberry fruit. *Acta Horticulturae*. 842: 377–380. ISSN 0567-7572.
- PASZT, L. S., SUMOROK, B., MALUSA, E., GŁUSZEK, S., DERKOWSKA, E., 2011: The influence of bioproducts on root growth and mycorrhizal occurrence in the rhizosphere of strawberry plants 'Elsanta'. *Journal of Fruit and Ornamental Plant Research*, 19, 1: 13–34. ISSN 1231-0948.
- PENG, G. and SUTTON, J. C., 1991: Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in

- strawberry. Canadian Journal of Plant Pathology, 13, 247–257. ISSN 0706-0661.
- PICARD, K., PONCHET, M., BLEIN, J. P., REY, P., TIRILLY, Y. and BENHAMOU, N., 2000: Oligandrin. A proteinaceous molecule produced by the mycoparasite *Pythium oligandrum* induces resistance to *Phytophthora parasitica* infection in tomato plants. *Plant Physiology*, 124, 1: 379–396. ISSN 1532-2548.
- PROKKOLA, S., KIVIJÄRVI, P. and PARIKKA, P., 2003: Effects of biological sprays, mulching materials and irrigation methods on grey mould in organic strawberry production. *Acta Horticulturae*, 626: 169–175. ISSN 0567-7572.
- PROKKOLA, S. and KIVIJÄRVI, P., 2007: Effect of biological sprays on the incidence of grey mould, fruit yield and fruit quality in organic strawberry production. *Agricultural and Food Science* 16: 25–33. ISSN 1795-1895.
- STEPHENSON, W. M., 1966: The effect of hydrolysed seaweed on certain plant pests and diseases. In: YOUNG, E. G. AND MCLACHLAN J. L. (eds.): Proceedings of international seaweed symposium 5, 1965. p. 405–415.
- SWADLING, I. R. and JEFFRIES, P., 1996: Isolation of microbial antagonists for biocontrol of grey mould disease of strawberries. *Biocontrol Science and technology*, 6, 1: 125–136. ISSN 1360-0478.
- TANOVIC, B., REKANOVIC, E., POTOCNIK, I., TODOROVIC, B., 2008: Effectiveness of fungicides and biofungicides in the control of grey mould of raspberry in Serbia. *Acta Horticulturae*, 777: 339–344. ISSN 0567-7572.
- VALLANCE, J., FLOCH, G. L., DÉNIEL, F., BARBIER, G., LÉVESQUE, C. A., REY, P., 2009:

- Influence of *Pythium oligandrum* biocontrol on fungal and oomycete population dynamics in the rhizosphere. *Applied and Environmental Microbiology*, 75, 14: 4790–4800. ISSN 1098-5336.
- WANG, A., LOU, B., XU, T. LIN, CH., 2011: Defense responses in tomato fruit induced by oligandrin against *Botrytis cinerea*. *African Journal of Biotechnology*, 2011, 10, 22: 4596–4601. ISSN 1684-5315.
- WASHINGTON, W. S., ENGLEITNER, S., BOONTJES, G. and SHANMUGANATHAN, N., 1999: Effect of fungicides, seaweed extract, tea tree oil, and fungal agents on fruit rot and yield in strawberry. *Australian Journal of Agriculture*, 39: 487–494. ISSN 0816-1089.
- WEIBEL, F. P., DANIEL, C., HAMMELEHLE, A., PFIFFNER, L., WYSS, E., 2010: Potential and Limits of Pesticide Free Apple Growing by a Self-Regulating Orchard Set-Up: Project Presentation and First Experience. In 14th International Conference on Organic Fruit-Growing: Proceedings to the Conference from February 22 to February 24, 2010 at Hohenheim, Germany. Weinsberg: FOEKO, p. 292–296. ISBN 978-3-00-030214-5.
- WEIBEL, F. P., HÄSELI, A., SCHMID, O. and WILLER, H., 2004: Present status of organic fruit growing in europe. *Acta Horticulturae*. 638: 375–385. ISSN 0567-7572.
- WILCOX, W. F and SEEM, R. C., 1994: Relationship between strawberry grey mould incidence, environmental variables and fungicide applications during different periods of the fruiting season. *Phytopathology*, 84: 264–270. ISSN 0031-949X.

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