Testing of varieties, mulch materials and biofungicides Supresivit (*Trichoderma harzianum* Rifai aggr.) and Polyversum (*Pythium oligandrum* Drechs.) in organic strawberries

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Abstract

In 2008–2009 experimental plots with organically grown strawberries cv. 'Elsanta', 'Honeoye' and 'Symphony' were conducted in Southern Moravia in the Czech Republic. The effect of two mulch materials (wheat straw, PE plastic) and two biofungicides (Supresivit – Trichoderma harzianum Rifai aggr., Polyversum – Pythium oligandrum Drechs.) on yield and health condition were assessed. 'Elsanta' reached the best yields, 'Symphony' proved high resistance to root rots. 'Honeoye' showed remarkable resistance to grey mould but it was quite susceptible to root rots as well as 'Elsanta'. Biofungicides decreased the number of necrotic plants due to root rot complex and decreased the number of berries infected by grey mould, nevertheless the differences were not statistically significant from untreated control. Application of Supresivit resulted in the highest marketable yields. Straw mulch significantly decreased the number of necrotic plants, but it increased grey mould occurrence on fruits.

Keywords: strawberries, *Pythium oligandrum*, *Trichoderma harzianum*, PE plastic mulch, straw mulch

Introduction

In the Czech Republic there does not exist verified organic strawberry mass production even thought there are many organic hobby growers. The most important premise of success is a selection of proper varieties with high degree of resistance to pest and diseases and providing adequate growth conditions. There are many studies referring cv. 'Honeoye' and 'Symphony' as very suitable for organic cultivation (Daugaard & Lindhard, 2000, Rhainds *et al.*, 2002, Barth *et al*. 2002). Plant diseases caused by fungal pathogens can be controlled with mycoparasitic fungi. Preparations Supresivit (active substance *Trichoderma harzianum* (Rifai aggr.) and Polyversum (*Pythium oligandrum* Drechs.) may be used for biological control of soil-borne fungal pathogens such as *Verticillium dahliae* Kleb, *Pythium spp., Phytophthora spp., Rhizoctonia spp.* as well as against other pathogens such as *Botrytis cinerea* Pers. (Ricard & Ricard, 1997, Benhamou *et al*. 1999, Brožová, 2002, Juhásová & Bernadovičová, 2004). The aim of this study was to verify recommended cv. 'Honeoye' and 'Symphony' under the conditions of the Czech Republic and to check the efficacy of the biofungicides Supresivit (*Trichoderma harzianum*) and Polyversum (*Pythium oligandrum*) in plant protection. In addition we examined the possibilities of using wheat straw mulch as an alternative to black PE plastic mulch.

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Material and Methods

Experimental plots were repeatedly established at Faculty of Horticulture in Lednice (Mendel University of Agriculture and Forestry Brno) in spring 2008 and 2009. The locality is situated in Southern Moravia (180 m above sea level, 9 °C average temperature, 517 mm average year precipitation, soils are silty). Conventionally grown pea was the preceding crop in 2008, while green manuring (white mustard – phacelia – white mustard) went in 2008 in the field assigned for experiments in 2009. Horse farmyard manure was applied (40 $t.ha^{-1}$) in the beginning of April in 2008. No other fertilizers were added.

We used cv. 'Honeoye' and 'Symphony', both recommended for organic system abroad, comparative cv. 'Elsanta' was added because it is most widespread strawberry in Europe. Planting system represented double rows hills mulched with black PE plastic mulch (50 μm). The distance between double rows was 0.8 m, spacing of plants 0.30×0.25 within the double row. Drop irrigation was installed under the plastic mulch. Two weeks after planting the furrows were mulched with wheat straw (approximately 0.03 m thick layer) originated from conventional agricultural system (The training agriculture enterprise of Mendel University in Brno, Lednice). Conventional frigo plants (originated from Goossens Flevoplant B.V., Netherlands) were planted at the end of April. The experiments were set as randomized block design with four replications of 20 plants. We used the following treatments:

1. Supresivit

Supresivit is a biogical fungicide with spores of the fungus *Trichoderma harzianum* Rifai aggr. at a number of 1.4×10^{10} spores per gram. (Fytovita, Ltd., Czech Republic). The roots of frigo plants were dipped in Supresivit 5% water suspension just before planting. Two weeks after planting each plant was watered with 150 ml of 5% Supresivit solution.

2. Polyversum – soil application

The biofungicide Polyversum contains oospores of *Pythium oligandrum* at a number of 10^6–$10^7.g^{-1}$ of preparation (Biopreparaty Ltd., Czech Republic). The roots of frigo plants were soaked in 0.05% suspension of Polyversum for 6 hours before planting. Two weeks after planting each plant was watered with 150 ml of Polyversum suspension (0.5 $g.l^{-1}$)

3. Polyversum – foliar application

The roots of frigo plants were soaked in suspension of Polyversum in the same way as described in previous treatment. The foliar applications were made three times: two weeks after planting, in the beginning of flowering (BBCH 61) and after flowering (BBCH 68), respectively. We use recommended dose of 0.1 $kg.ha^{-1}$ in spray liquid volume 400 $l.ha^{-1}$.

4. Straw mulch

Frigo plants were set in bare hills and wheat straw mulch was spread around the plants (0.03 m thick layer) two weeks after planting. This treatment represents alternative to black PE mulch.

5. Control: PE plastic mulch only.

During the vegetation season we observed the health condition of plant and kept the plots free of weeds. Drop irrigation was operated by automatic system according to soil humidity measured with VIRRIB sensor. During the harvest time we assessed the yield and quality of production. The marketable yield consisted of berries assigned to 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) according to the marketing standards of the European Union – commission regulation (EC) no 843/2002. All other berries including those ones infected with grey mould constructed unmarketable yield. Infestation of fruits with grey mould and the infestation of plants with soil-borne pathogens (root rot complex caused by *Phytophthora*, *Pythium*, *Rhizoctonia* and *Verticillium* species) were assessed according to number of diseased fruits or plants, respectively. Data were statistically processed by ANOVA and LSD test (P<0.05) using software Unistat version 5.1.

Results

In 2008 the harvest of 'Honeoye' began on the 12th of June and after 6 harvests (3 days interval) it finished on the 1st of July. The highest marketable yield was observed in plots treated with Supresivit (Table 1). Supresivit and foliar application of Polyversum significantly increased marketable yield compared to control. The lowest yield was found in plots with straw mulch. In 2009 we carried out 8 harvests from June 10th until July 17th. Similarly to preceding year we got the highest marketable yield at Supresivit treatment, but the yield was significantly higher than this one in plot treated with Polyversum, not at control. Straw mulch resulted in higher unmarketable yield mainly due to high number of fruits infected with grey mould (Table 4).

Table 1: Yield data ($g.plant^{-1}$) for 'Honeoye' in 2008 and 2009

Treatment	Marketable yield ($g.plant^{-1}$)		Unmarketable yield ($g.plant^{-1}$)	
	2008	2009	2008	2009
Supresivit	66.8 d	62.6 b	3.9 a	7.3 ab
Polyversum – soil	53.8 bc	39.8 a	2.6 a	6.4 ab
Polyversum – leaf	59.9 cd	42.7 a	2.8 a	4.6 a
Straw mulch	35.8 a	31.0 a	2.2 a	9.4 b
Control	42.2 ab	50.4 ab	2.4 a	6.5 ab

Different letters between rows indicate significant differences for P<0.05

In 2008 the first harvest of 'Elsanta' were done on the 16th of June and after 6 harvests it finished on July 7th. Biofungicides did not significantly influence marketable and unmarketable yield (Table 2). The highest yields were observed in plots with Polyversum applied on root system. In 2009 the berries were picked up during 8 harvests from June 18th until July 15th. Due to visually stronger frigo plant materials the yields were approximately twice higher as in 2008. Supresivit significantly increased marketable yield compared to control (Table 2).

Table 2: Yield data ($g.plant^{-1}$) for 'Elsanta' in 2008 and 2009

Treatment	Marketable yield ($g.plant^{-1}$)		Unmarketable yield ($g.plant^{-1}$)	
	2008	2009	2008	2009
Supresivit	62.5 a	145.9 b	4.6 a	11.4 a
Polyversum – soil	66.6 a	120.6 ab	4.7 a	7.9 a
Polyversum – foliar	58.9 a	101.2 a	5.8 a	9.7 a
Straw mulch	60.5 a	116.7 ab	5.3 a	9.9 a
Control	62.2 a	100.7 a	3.6 a	11.9 a

Different letters between rows indicate significant differences for P<0.05

The harvest of 'Symphony' was carried out firstly on the 19th of June and finally on the 7th of July (total five harvests) in 2008. All biofungicides applications increased marketable yield compared to untreated control, but not significantly (Table 3). In 2009 the results were rather similar to 'Elsanta'. The highest marketable yields gave Supresivit and Polyversum applied on root system. A late variety 'Symphony' began to ripe on the 22nd of June and finished on the 20th of July.

Table 3: Yield data (g.plant^{-1}) for 'Symphony' in 2008 and 2009

Treatment	Marketable yield (g.plant^{-1})		Unmarketable yield (g.plant^{-1})	
	2008	2009	2008	2009
Supresivit	45.9 a	75.5 b	4.3 a	15.2 a
Polyversum – soil	43.0 a	67.6 ab	3.6 a	9.9 a
Polyversum – foliar	48.1 a	57.9 a	4.2 a	12.0 a
Straw mulch	46.4 a	57.9 a	5.0 a	12.1 a
Control	38.9 a	63.9 ab	6.1 a	13.8 a

Different letters between rows indicate significant differences for P<0.05

We did not meet meaningful problem with *Botrytis cinerea* probably due to dry and hot climate of Southern Moravia. The weather was warm and very dry in June and July in 2008 so the infestation of berries was minimal in all varieties. In 2009 the course of weather was very unbalanced, hot and dry spring season took turns strongly rainy season from the middle of June to the middle of July, just in the peak of the strawberry harvest time. Despite of this situation the damage of berries by grey mould did not exceed 3 %. The differences between treatments were not generally significant, but mostly Polyversum applied by foliar way resulted in neglectable grey mould occurrence. On the contrary straw mulch showed to be somewhat unsuited because of keeping humidity and slowing down leaf drying. From the middle of July we observed sudden plant decline due to root rot complex infestation. 'Honeoye' showed to be very sensitive to soil-borne pathogens. In 2008 we found more then 50% drop out of plants after application of Polyversum on their roots, similarly as at control (Table 4). Biological control agents did not provide effective control of soil-borne pathogens.

Table 4: Necrotic plants (%) due to soil-borne pathogens (root rot complex) and grey mould occurrence on fruits (% of diseased fruits) – 'Honeoye'

Treatment	Necrotic plants (%)		Grey mould (%)	
	2008	2009	2008	2009
Supresivit	34.4 a	40.7 a	0.14 a	0.18 a
Polyversum – soil	55.2 a	44.8 a	0.16 a	0.19 a
Polyversum – foliar	44.5 a	28.9 a	0.00 a	0.00 a
Straw mulch	18.6 a	27.1 a	0.88 a	2.26 b
Control	50.5 a	34.5 a	0.18 a	0.42 a

Different letters between rows indicate significant differences for P<0.05

'Elsanta' was similarly sensitive to soil-borne pathogens. In 2009 Supresivit significantly decreased the number of necrotic plant compared to control (Table 5). Also straw mulch showed to be suitable for limiting root rot complex in this variety in 2009.

Table 5: Necrotic plants (%) due to soil-borne pathogens (root rot complex) and grey mould occurrence on fruits (% of diseased fruits) – 'Elsanta'

Treatment	Necrotic plants (%)		Grey mould (%)	
	2008	2009	2008	2009
Supresivit	27.9 a	21.8 ab	0.20 a	2.6 ab
Polyversum – soil	36.4 a	37.3 bc	0.00 a	1.6 a
Polyversum – foliar	42.5 a	39.9 bc	0.67 a	2.0 ab
Straw mulch	39.8 a	10.7 a	2.01 a	2.3 ab
Control	56.7 a	55.6 c	0.36 a	3.2 b

Different letters between rows indicate significant differences for P<0.05

We found distinct differences in treatment incidence on root rots between years in the case of cv. 'Symphony'. While in 2008 both Polyversum application decreased the number of necrotic plants and Supresivit plot had the biggest infestation, in 2009 the situation was converse (Table 6). Anyway 'Symphony' showed significantly high resistance to soil-borne pathogens (Table 8), so it seem to be the best choice for growing in silty clay soils.

Table 6: Necrotic plants (%) due to soil-borne pathogens (root rot complex) and grey mould occurrence on fruits (% of diseased fruits) – 'Symphony'

Treatment	Necrotic plants (%)		Grey mould (%)	
	2008	2009	2008	2009
Supresivit	22,9 a	2.7 a	0.12 ab	0.69 a
Polyversum – soil	7,3 a	14.6 a	0.16 ab	1.09 a
Polyversum – foliar	7,3 a	10.4 a	0.00 a	1.02 a
Straw mulch	8,9 a	3.8 a	0.00 a	1.74 a
Control	17,9 a	5.6 a	0.80 b	1.27 a

Different letters between rows indicate significant differences for P<0.05

Table 7 shows the summarized data collected from both years and all evaluated varieties. Biofungicides did not significantly influenced yield but Supresivit apparently amended marketable yield which was nearly about 17 g per plant higher than at the control. The lowest marketable yield was found using straw mulch but it practically did not differ from the control PE plastic mulch. The positive effect of straw mulch was found in reducing the risk of soil-born pathogens causing plant decline in summer. Straw mulch significantly decreased the number of necrotic plants compared to the PE plastic mulch (control), on the contrary it intensified the occurrence of grey mould on berries (Table 7). The effect of the biofungicides on decreasing soil-borne pathogens and *Botrytis cinerea* was not significant nevertheless we found lower infestation in plots treated by Supresivit and Polyversum (Table 7).

Table 7: The effect of treatment on marketable yield, percentage of necrotic plants and grey mould infestation of fruits (average data 2008–2009, all varieties)

Treatment	Marketable yield ($g.plant^{-1}$)	Unmarketable yield ($g.plant^{-1}$)	Necrotic plants (%)	Grey mould (%)
Supresivit	76.5 a	7.8 a	25.1 ab	0.65 a
Polyversum – soil	66.3 a	5.9 a	32.4 ab	0.57 a
Polyversum – foliar	61.5 a	6.5 a	28.9 ab	0.62 a
Straw mulch	59.2 a	7.2 a	17.0 a	1.50 b
Control	59.7 a	7.4 a	36.8 b	1.03 ab

Different letters between rows indicate significant differences for P<0.05

We also compared the varieties. 'Elsanta' proved high productivity, however low resistance to root rots. In our experiment 'Elsanta' had mostly infected berries with grey mould (Table 8). 'Symphony' was also very productive nevertheless it tended to make small berries in late harvest season. This fact resulted in quite high unmarketable yield. A great virtue of 'Honeoye' is its ability to keep good size of berries till the end of the crop and thus the rate of unmarketable yield is low. 'Honeoye' showed the best resistance to *Botrytis cinerea* but it was in the same way sensitive to root rots like 'Elsanta' (Table 8).

Table 8: Comparison of varieties – average data (2008–2009)

Variety	Marketable yield ($g.plant^{-1}$)	Unmarketable yield ($g.plant^{-1}$)	Necrotic plants (%)	Grey mould (%)
Honeoye	49.2 a	4.6 a	37.5 b	0.40 a
Elsanta	90.5 b	7.5 b	37.2 b	1.53 b
Symphony	54.5 a	8.6 b	10.1 a	0.69 a

Different letters between rows indicate significant differences for P<0.05

Discussion

There are many studies focused on testing varieties potentially suitable for organic production. Even though the variety 'Honeoye' is known to be susceptible to crown rot (Parikka, 2003), it is generally often recommended for organic growers due to its attractive appearance and high degree of resistance to *Botrytis cinerea* (Daugaard H. & Lindhard H. 2000, Barth *et al*., 2002). Our results confirmed these experiences and that is why we can recommend 'Honeoye' for growers farming in humid climate conditions but not in areas with heavy dense soils with bad water drainage. 'Symphony' proved good resistance to soil-borne pathogens. Kerby & McNicol (1997) declared strong resistance of this variety to *Phytophthora fragariae* var. *fragariae* since the variety was selected on fields heavily infected with several races of this pathogen. Biological fungicides Polyversum and Supresivit did not provide effective control of soil-borne pathogens. High percentage of rotted plants in our experiment could be explained by poor suppressive soil ability. In 2008 plots were established after conventionally managed pea crop. According to Sullivan (2004) introducing a single antagonistic organism to soil seldom achieves disease suppression for long time because the new organism may not be competitive with existing microorganisms. If soil conditions are inadequate, the introduced beneficial organism will not survive. Furthermore fresh plant residues (intensive green manuring before experiment repetition in 2009) as well as raw insufficiently decomposed farmyard manure could support development of pathogenic *Pythium* and *Rhizoctonia* species (Manici *et al*., 2004, Sullivan, 2004). The best health condition regarding low number of necrotic plants was found in plots with straw mulch. Ellis *et al*. (1998) showed that straw mulch between strawberry rows was equally or even more effective than fungicides for controlling *Phytophthora cactorum*. Due to generally high drop-out caused by root rots just in the year of setting frigo plants, we present data only from one year old plantation (2008 and 2009, respectively), that is why the yields are relatively low. Two year old strawberry plantation (established in 2008) gave on the average 441 g.plant^{-1} (Honeoye 348.7 g, Elsanta 481.0 g, Symphony 492.4 g).

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