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Evaluation of spruce biomass extract for control of grey mould (*Botrytis cinerea*) in field-grown strawberries

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Abstract

The aim of the present study was to evaluate the efficacy of spruce biomass extract to control grey mould (*Botrytis cinerea*) development in field-grown strawberries. The study was performed in a commercial strawberry plantation for two years and also according to EPPO guidlines. In contrast to the laboratory investigation, the efficiency of spruce biomass extract for grey mould control on strawberries in field conditions was not convincing, possibly due to weather conditions that did not favour mould development. A better level of control was observed when lower concentrations of spruce biomass extract were used. The only treatment that significantly reduced the amount of rotted fruits, compared to untreated control was 0.5% spruce biomass extract used once a week in 2013. However, significant reduction of leaf spot diseases by the extract applications in 1 and 2% concentrations was observed.

Key words: *Botrytis cinerea, Fragaria* × *ananassa*, fruit rot, spruce, strawberry yield.

Introduction

Numerous studies have described the importance of *Botrytis cinerea* Pers. as a pathogen on field-grown strawberries (Bulger et al. 1987; Sosa-Alvarez et al. 1995; Berrie et al. 1998). This disease can significantly reduce both yield and postharvest quality of fruits (Ceredi et al. 2009). Since most strawberry cultivars are not highly resistant to grey mould, control of the disease is mainly based on fungicide sprays during the flowering period. However, *B. cinerea* forms conidia and causes secondary infection of berries also during harvest (Bulger et al. 1987; Sosa-Alvarez et al. 1995) when fungicide use is limited. Another problem is that most of the fungicides recently developed for control of *B. cinerea* are site specific inhibitors with a high risk of resistance development in pathogens, especially, with intensive use (Leroux et al. 2002).

As research in general aims at limiting the input of fungicides, alternative measures to control grey mould are desirable. It is generally considered in the literature that there is a lack of environmentally- and consumer-friendly plant protection products that could be used in organic farming or during the harvest period (Capieau 2004; Freeman et al. 2004; Laugale et al. 2012). In this respect, natural plant products represent a useful resource for development of new means of plant protection. Coniferous plants produce a large diversity of chemical compounds, such as an enormous array of terpenoids and phenolics with a potential to protect against variety of herbivores and microorganisms (Keeling, Bohlmann 2006). In particular, several studies have been performed on antifungal effects of different components from coniferous trees (Kozlowski, Métraux 1999; Hong et al. 2004; Ludley et al. 2008; Laugale, Daugavietis 2009; Zarins et al. 2009).

Forest industry provides wood residues potentially useful as a rich source of natural products. Therefore, development of new environmentally friendly plant protection products from coniferous tree biomass has begun. Several preparative forms of products from coniferous biomass have been developed, and in preliminary investigations spruce (*Picea abies* L.) biomass extract has shown promising results for grey mould control (Laugale et al. 2013). The aim of the present study was to evaluate the efficacy of spruce biomass extract to control grey mould development in field-grown strawberries.

Materials and methods

Spruce biomass extract

Grinded spruce biomass was extracted with 96% ethanol with Universal Extraction System (Büchi, Switzerland) in Soxhlet regime. Spruce biomass extract was formulated to improve adhesion to plant, including water, ethanol, sticking agent, emulsifier, pH stabilizer. Before use, 0.5, 1 and 2% dilutions of the extract formulation were prepared using clean, warm tap water.

Trials in commercial strawberry plantations

In 2012 and 2013 field trials of spruce biomass extract were established on commercial strawberry plantations in the territory of the Pūre Horticultural Research Centre (Pūre, Latvia; 57°02' N; 22°52' E) using three-year-old (2012) and two-year-old (2013) plantings of cv. 'Senga Sengana'. Strawberries were grown in 20 wide matted rows with 1 m distance between the rows, without any mulching and artificial irrigation. Plants received no chemical protection against pests and diseases. Hand weeding in rows and mechanical tilling within rows were used. The yield was harvested two or three times per week.

A randomized block design with four replicates per treatment was used in the trials. The size of each plot was 10 m^2. Two application frequencies were used consisting of treatments once or twice a week, from the beginning of flowering until the beginning of fruit harvest. In 2012, the treatment period was from May 25 until June 19 with five and eight individual treatments, respectively. In 2013, the treatment period was from May 31 until June 18 with three and six individual treatments, respectively. The rate of treatment was 500 L ha^{-1} of working solution. In 2012, treatment was performed using 1 and 2% spruce extract, while in 2013 0.5 and 1% treatments were used. Untreated plants were used as a control.

A trial according to the EPPO guidelines

The trial was conducted in 2012 and 2013 in the territory of Ķekavas Dārzs Ltd. (Ķekava, Latvia) on one-year-old strawberry plantings using cv. 'Induka' (2012) and cv. 'Rubin' (2013) according to the European and Mediterranean Plant Protection Organization guidelines (EPPO 1996; EPPO 2002; EPPO 2012). A similar cultivation system as in commercial plantation trials was used.

Spruce biomass extract was applied in three different concentrations, 1, 2 and 4%. Extract was sprayed with an interval of 7 to 10 days, from the beginning of flowering (May 24, 2012 and May 28, 2013) until the maximum of fruit harvesting (June 29, 2012 and July 1, 2013), in total, six times. The rate of treatment was 500 L ha^{-1} of working solution. Untreated plants were used as a control. Fungicide treatment (Signum d.g.) on May 24 and June 27 (2012) and May 28 and June 6 (2013) was used for comparison. A randomized block design with four replicates per treatment was used. The size of each plot was 12 m^2, with 26 healthy plants in each plot.

Measurements and observations

Total yield and average fruit mass were evaluated in each trial. Fruits were picked two to three times per week, separated, weighed and counted. Rotted fruits were counted and weighed separately and the amount of rotted fruits (%) from the total was calculated.

At the beginning of harvesting a sensory evaluation of the fruits was performed to determine a possible influence of spruce biomass extract on fruit overall appearance and flavor in commercial plantation trials. The evaluation was performed by a trained panel test of 10 judges. The evaluation was done for appearance and flavor on a scale of 1 to 9, where 1 corresponds to "unacceptable", 5 to "medium", 9 to "excellent".

In EPPO trials leaf spots caused mostly by *Mycospherella fragariae* and *Diplocarpon earlianum* was evaluated. The incidence (number of damaged plants in plots in %) and severity (damaged leaf surface in %) of leaf spots were estimated at fruit harvest in 2013 and after the fruit harvesting period, both in 2012 and 2013.

Statistical analysis

The statistical evaluation of the results was performed by one- and two-way analysis of variance (ANOVA) and the LSD test using statistical software *R*, version 3.0.2., package "Agricolae" (De Mendinburu 2009) with significance level 5% (*R* Development Core Team 2009).

Table 1. Monthly summary precipitation and average air temperature from April to July in 2012 and 2013 in Pūre and Ķekava

Location	Parameter	Period	April	May	June	July
Pūre	Precipitation (mm)	Long-term average	32.7	37.1	68.7	80.5
		2012	25.5	52.5	38.7	50.8
		2013	1.8	72.2	62.9	6.0
	Temperature (°C)	Long-term average	5.6	11.8	16.1	18.1
		2012	5.9	11.9	14.1	18.4
		2013	4.2	14.2	17.5	18.0
Ķekava	Precipitation (mm)	Long-term average	13.3	16.3	23.3	27.7
		2012	20.3	18.1	21.5	34.8
		2013	12.7	23.3	15.8	22.1
	Temperature (°C)	Long-term average	5.1	10.9	15.1	17.0
		2012	7.4	13.2	15.5	19.5
		2013	4.9	15.7	19.2	19.1

Table 2. Effect of spruce biomass extract treatment on strawberry yield, grey mould damage and fruit quality in a commercial strawberry plantation trial at Pūre in 2012

Treatment	Concentration (%)	Total yield (g m^{-2})	Average fruit mass (g)	Grey mould damaged fruit (%)	Fruit flavor (units)
Control		1155	7.1	8.4	6.8
Once a week	1	1340	6.9	11.9	6.6
	2	1159	6.8	8.3	6.5
Twice a week	1	1152	6.7	7.5	6.8
	2	1213	6.9	8.3	6.6
$LSD_{0.05}$		494	0.74	4.52	0.72

Weather conditions

In both locations where the trials were carried out weather conditions were not favorable for grey mold development in both 2012 and 2013. The season 2012 had a lower amount of precipitation in April, June and July, in comparison to long-term observations (Table 1). In May, the amount of precipitation was higher than the long-term average, but it did not affect grey mold infection level in the field-grown strawberries, as in both locations they started flowering at the middle of the 3rd decade of May. Average air temperature was similar to long-term observations in Pūre, where trials in the commercial fields were carried out. However, the average temperature at Ķekava (EPPO trials) was 2 to 3 °C higher than the long-term average. In 2013, the amount of precipitation and the average air temperature during April and July was lower than in 2012, spring meteorological conditions were reached relatively late (Table 1), whereas in May and July the amount of precipitation and average temperature were higher than in 2012, but relatively typical for this time of the year at the both locations.

Results

In the trial in the commercial strawberry plantation in Pūre in 2012, the highest total yield was obtained in the treatment where spruce biomass extract was applied once a week in 1% concentration (Table 2). However statistically significant differences between treatments and control were not found with respect to yield, average fruit mass, overall fruit quality and flavor. The lowest percentage of rotted fruits was observed in the treatment twice a week with spruce biomass extract at 1% concentration, but the difference with the control was not statistically significant. The effect of application frequency and concentration also was not statistically significant.

In the same trial in 2013, all tested treatments by spruce biomass extract did not significantly affect strawberry yield, average fruit mass and flavor (Table 3). The highest total yield was obtained in the treatment once a week with 1% spruce biomass extract. Lower damage by grey mould was observed in the 0.5% treatment with spruce biomass extract in comparison to 1% treatment. Statistically significant differences between the two application frequencies were not found. The lowest percentage of rotten fruits was observed in the treatment with 0.5% spruce biomass extract once a week.

In the trial following EPPO guidelines, the highest total yield and average fruit mass were observed in the 2% treatment with spruce biomass extract in 2012 and in the 4% treatment in 2013 (Table 4). However, the differences were not statistically significant. It was observed that application of spruce biomass extract in 4% concentration had some negative effect on fruit taste and aroma; berries had specific scent and taste of conifer foliage (data not shown). The lowest percentage of rotted fruits was observed in the treatment with 1% spruce biomass extract but the effect was not significant. However, fungicide treatment significantly diminished fruit damage in 2012 (Table 4).

Spreading of leaf spots caused mostly by *Mycospherella fragariae* (Tul.) Lindau and *Diplocarponn earliana* (Ellis

Table 3. Effect of spruce biomass extract treatment on strawberry yield, grey mould damage and fruit quality in a commercial strawberry plantation trtial at Pūre in 2013. * indicates significant differences in comparison to control ($p < 0.05$)

Treatment	Concentration (%)	Total yield (g m^{-2})	Average fruit mass (g)	Grey mould damaged fruit (%)	Fruit flavor (units)
Control		504	6.6	6.5	6.7
Once a week	0.5	410	5.9	4.5*	6.9
	1	517	6.0	6.1	6.3
Twice a week	0.5	488	5.6	5.5	6.2
	1	500	5.9	6.0	5.9
$LSD_{0.05}$		117.5	0.96	1.70	0.86

Table 4. Effect of spruce biomass extract treatment on strawberry yield, grey mould damage and fruit quality in an EPPO trial at Ķekava in 2012 and 2013. * indicates significant differences in comparison to control ($p < 0.05$)

Treatment	Total yield (g m^{-2})		Average fruit mass (g)		Grey mould damaged fruit (%)	
	2012	2013	2012	2013	2012	2013
Control	725	467	16.15	12.07	4.96	4.78
Signum	767	507	17.46	11.35	2.51*	4.26
Spruce biomass extract (1%)	718	501	16.22	11.24	4.52	4.61
Spruce biomass extract (2%)	741	511	16.57	11.36	5.77	6.03
Spruce biomass extract (4%)	701	532	15.84	11.33	4.79	5.29
$LSD_{0.05}$	247	118	1.89	1.51	2.75	4.65

& Everh) F.A. Wolf were observed in the planting two weeks after start of fruit harvesting. Almost all plants were infected in 2012, with significantly lower level of infection in 2013 (Table 5). Application of spruce biomass extract in concentrations 1% significantly reduced the development of leaf spots in 2012, and in both 1 and 2% concentrations in 2013, compared to the untreated control. However, the effect of spruce biomass extract was significantly less than that of the fungicide treatment.

Discussion

Spruce biomass contains a wide variety of phenolic compounds. The abundant spruce bark constituents stilbene glucosides, astringin, piceid and isorhapontin have been reported to have antifungal and antibacterial activity (Co et al. 2012). Consequently, spruce biomass extract can have some antifungal effect. In a laboratory study, spruce biomass extract had significant inhibitory effect on mycelial growth of *B. cinerea* isolates (Laugale et al. 2013. The efficiency of spruce biomass extract for grey mould control in field trials performed during the present study was not so convincing. Only the treatment with 0.5% spruce biomass extract once a week in 2013 significantly reduced the amount of rotted fruits, compared to untreated control (Table 3). It needs to be taken into account that the overall infection of grey mould on the field was low in both years all trials. This could have significantly influenced the efficiency of the treatments. According to Ceredi et al. (2009), the most important factor that affects severity of grey mould is weather conditions during the flowering phase. Sporulation and subsequent dispersal of *B. cinerea* is significantly enhanced by rainfall (Sutton 1990) and by the total duration of wetness (Sosa-Alvarez 1995). According to Berrie et al. (1998), the factors best related with flower infection are daytime vapor pressure deficit and night-time temperature, with an optimum temperature for grey mould development of about 20 °C. In both years of the trial, the amount of precipitation in May, when flowering of strawberry started, was rather high, whereas later it decreased and was lower than during long-term observations. The average air temperature was lower than 20 °C. It is interesting to note that a higher level of infection was observed in fields where higher total yield was harvested.

In the field trials there was a tendency that treatment with lower spruce biomass extract concentrations showed better results in respect to protection against grey mould. This is in contrast to laboratory tests, where higher concentration of spruce biomass extract in the media (20 g L^{-1}) significantly increased the inhibitory effect on mycelial growth of *B. cinerea* isolates as compared to lower concentration (Laugale et al. 2013). Application frequency had no significant effect on strawberry yield parameters and grey mould intensity.

Treatments with spruce biomass extract had no significant effect on strawberry yield and fruit size. Only the highest concentration (4%) of the extract had some negative influence on fruit taste and aroma, and it slightly reduced average size of berries, although the reduction

Table 4. Effect of spruce biomass extract treatment on strawberry leaf spot incidence and severity in an EPPO trial at Ķekava in 2012 and 2013. * indicates significant differences in comparison to control ($p < 0.05$). a, evaluated at harvest time; b, evaluated after harvest

	Leaf spot incidence (%)			Leaf spot severity (%)		
	2012a	2013a	2013b	2012a	2013a	2013b
Control	100.00	34.00	76.50	16.35	1.41	13.30
Signum	75.00	11.50*	10.50*	3.13*	0.20*	0.53*
Spruce biomass extract (1%)	97.00	27.50	48.50*	6.90*	0.56*	2.77*
Spruce biomass extract (2%)	99.00	30.00	57.50*	13.66	0.86*	6.02*
Spruce biomass extract (4%)	99.00	33.00	51.50*	13.83	0.78*	7.47*
$LSD_{0.05}$	17.12	10.39	10.25	6.00	0.47	2.24

was not statistically significant. The most pronounced positive effect was significant reduction of leaf spot diseases by spruce biomass extract applications in 1 and 2% concentrations. The effect was seen during both years in the trial conducted at the farm Ķekavas Dārzs.

In conclusion, the efficiency of spruce biomass extract for grey mould control on strawberries in the field conditions was not convincing. Better level of control was observed when lower concentrations of spruce biomass extract were used. The increase of application frequency did not result in any significant effect on grey mould development. More investigations on spruce biomass extract effectiveness are necessary with additional testing with lower concentrations of extract and with different application techniques. The influence of the extract on development of other diseases needs to be investigated.

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