Changes in the Occurrence of Mycoflora on Caraway Seeds after Fungicide Application

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Abstract

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The spectrum of diseases and their harmfulness to caraway has changed within the past few years. The importance of *Mycocentrospora acerina*, recently regarded as the most serious pathogen, has decreased while the severity of other fungi, such as *Erysiphe heraclei* or *Ascochyta carvi*, has increased. This work was undertaken to identify and determine the incidence of fungi which contaminate the seeds and to evaluate the effect of fungicidal treatment. Direct microscopic evaluation confirmed that *Alternaria alternata* and *Cladosporium cladosporiodes* were the most common fungi occurring on caraway seeds. *Cladosporium herbarum*, *Fusarium avenaceum, F. culmorum*, *F. equiseti*, *Fusarium* sp., *Epicoccum nigrum*, *Trichothecium roseum* and *Aureobasidium* sp. were not so frequent. In field trials during 2005 and 2006, fungicide application at flowering of the crop affected mainly the pathogenic fungi *Ascochyta carvi* and *Septoria carvi*, but not the saprophytic fungi *A. alternata* or *Cladosporium* sp. In comparison with the control, in 2005 the yield increased ($*\alpha = 0.05$) only with prothioconazole and dimoxystrobin + boscalid. There was no yield increase at the significance level $\alpha = 0.01$ in either year. The fungicides had only a very small effect on the weight of thousand seeds, the germination rate and the essential oil content. The results of the trials showed that most of the tested preparations would be perspective for use in practice, especially under weather conditions that are more favourable for a higher incidence of severe fungal diseases. Application of fungicides reduced the occurrence of the fungi and had no negative effect on yield parameters and quality.

Keywords: caraway; fungal diseases; fungicides

Caraway (*Carum carvi* L.) is the most important crop in the group of medicinal, aromatic and spice plants. Changes, during the past few years, in the spectrum of caraway diseases and in their harmfulness have become a limiting factor for the farmers. The importance of *Mycocentrospora acerina* (Hartig) Deighton, recently regarded as the most serious pathogen (Evenhuis *et al.* 1995, 1997; Evenhuis 1997), has decreased while the severity of other fungi, such as *Erysiphe heraclei* DC, *Ascochyta carvi* Ondřej or *Septoria carvi* Syd., have increased (Duczek & Slinkard 2003; Bedlan 2005). The fungi *A. carvi* and *S. carvi* are seed-borne. It would be desirable to reduce the incidence of spores of these pathogens. The application of fungicides at the stage of full flowering of a caraway crop can reduce the quantity of spores on the seeds during their maturation. For a long time only a few fungicides had been registered for application against *M. acerina* in caraway, but they are still not efficient enough.

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The aim of this work was to assess the influence of fungicide treatment on differences in the incidence of spores on caraway seeds. It is necessary that the farmers find an adequate substitute for fungicides that contain carbendazim or thiophanate-methyl. Büchler and Volkmann (2003) confirmed the high risk of carbendazim residue in honey.

MATERIAL AND METHODS

Field trials were performed in 2005 and 2006 at Šumperk in the north-eastern part of the Czech Republic, 328 m altitude, in the foothills of the Jeseníky Mountains. The row spacing was 12.5 cm and sowing density for the caraway cultivar Kepron was 12 kg/ha. The small plot trials were designed as complete randomised blocks with three replications. The area of each plot was 10 m^2. Fertilisers, herbicides and insecticides were applied and the crop harvested in accordance with recommendations for caraway cultivation.

Fungicides with different active ingredients or a different combination of active ingredients were used and compared with the untreated variant. The fungicides were applied to caraway at the stage of full flowering of the main umbel. Application rates of the fungicides are shown on Table 1; they were similar to rates registered for other crops like cereals, rapeseed etc.

The effect of fungicide application on yield parameters was evaluated. The quality of the seeds was determined by the essential oil content.

Determination of fungi on the seeds by direct microscopic assessment: 1 g of seeds was immersed in 5 ml of water and stirred for 1 hour. The obtained suspension was centrifuged, the supernatant of the suspension (4 ml) was removed and 1 ml of the precipitate was microscopically assessed. All spores of fungi in the visual field were counted by microscope at a magnification of 450×. The evaluation method was based on the ČSN 46 0610 Czech standard. The following fungi were evaluated: *Alternaria* sp., *Ascochyta carvi*, *Septoria carvi*, *Cladosporium* sp., *Fusarium* sp., and fragments of a dark mycelium with one or more whole cells.

Statistical analysis of the results was carried out by analysis of variance and by multiple comparison testing (Tukey, Schefe).

RESULTS AND DISCUSSION

In comparison with the control, in 2005 the treatments with prothioconazole and dimoxystrobin + boscalid increased yields ($*\alpha = 0.05$), but no treatment increased yields in 2006. No increase in yields was seen at the significance level $*\alpha = 0.01$ in either year (Table 2).

The weight of thousand seeds increased in 2005 only when treated with azoxystrobin at the level $\alpha = 0.05$. There were no significant differences of thousand seeds weight at $\alpha = 0.01$ in 2005, nor at both significance levels in 2006.

No treatment affected the germination rate, with the exception of dimoxystrobin + boscalid which decreased it at the level of $\alpha = 0.05$ in 2006, and azoxystrobin which decreased the germination rate at both significance levels in 2006. The difference in the germination rate between the two years is about 15%, depending on the length of the dormant period (Table 3).

No treatment increased the essential oil content. It was decreased slightly by flusilazole in 2005.

Most fungicide applications influenced, in particular, the pathogenic fungi *Ascochyta carvi* and *Septoria carvi* in both years, whereas the saprophytic species *A. alternata* and *Cladosporium*

Table 1. Fungicides used in fungicide trials

Treatment	Active ingredient(s)	Dose of active ingredient(s) (g/ha)
Untreated	–	–
Pictor 400 SC	dimoxystrobin + boscalid	45.5 + 91
Amistar	azoxystrobin	250
Alert S	flusilazole + carbendazim	125 + 250
Capitan 25 EW	flusilazole	150
Proline 250 EC	prothioconazole	250
Topsin M 70 WP	thiophanate-methyl	560

Table 2. Yield parameters in 2005 and 2006

Treatment	Yield per plot (g)			Weight of 1000 seeds (g)			Germination rate (%)			Essential oil content (%)		
	mean	α = 0.05	α = 0.01	mean	α = 0.05	α = 0.01	mean	α = 0.05	α = 0.01	mean	α = 0.05	α = 0.01
2005												
Untreated	1559.3	ab	ab	2.75	ab	a	66.4	ab	a	3.92	b	b
Pictor 400 SC	1810.6	c	b	2.93	bc	a	64.5	a	a	4.02	b	b
Amistar	1447.0	a	a	2.97	c	a	65.8	ab	a	4.06	b	b
Alert S	1754.6	bc	b	2.91	abc	a	65.7	ab	a	4.25	b	b
Capitan 25 EW	1576.6	ab	ab	2.85	abc	a	69.6	b	a	3.15	a	a
Proline 250 EC	1793.3	c	b	2.76	ab	a	64.3	a	a	4.00	b	b
Topsin M 70 WP	1651.3	abc	ab	2.74	a	a	70.0	b	a	3.87	b	b
2006												
Untreated	1040.0	cd	bc	3.53	a	ab	54.4	c	b	4.31	a	a
Pictor 400 SC	1104.7	d	c	3.81	b	b	46.3	ab	ab	4.41	a	a
Amistar	927.0	ab	ab	3.64	ab	ab	43.6	a	a	4.21	a	a
Alert S	1038.3	cd	bc	3.50	a	ab	52.1	bc	ab	4.31	a	a
Capitan 25 EW	888.7	a	a	3.42	a	a	53.0	c	b	4.30	a	a
Proline 250 EC	1003.5	bc	abc	3.47	a	ab	50.2	abc	ab	4.41	a	a
Topsin M 70 WP	1128.0	d	c	3.50	a	ab	49.1	abc	ab	4.52	a	a

Values with the same letter are not significantly different

sp. were not affected. This may be caused by the fact that the saprophytes grow especially on dead tissues of ripened seeds; fungicides applied during flowering were thus not able to control them. The combination of dimoxystrobin + boscalid was found to be the most effective treatment against both *S. carvi* and *A. carvi*. In comparison with the untreated variant, the occurrence of *A. carvi* was higher in both years on plots treated with prothioconazole. This preparation will not be recommended for the control of *A. carvi*.

To determine the fungal flora on seeds, most authors used the method of isolation on agar medium; they also frequently used special media for certain groups of fungi. Several authors explored the occurrence of mycoflora on the seeds of medicinal, aromatic and spicy plants (Moharram *et al.* 1989; Aziz *et al.* 1998; Srivastava & Jain 1992; Mazur & Nawrocki 2004). In preliminary trials we isolated fungi from caraway seeds on Czapek-Dox agar. The most commonly isolated fungi were *A. alternata* and *C. cladosporioides*. Fungi such as *Fusarium* spp., *Epicoccum nigrum* Link, *Trichothecium roseum* (Pers.) Link, *Mucor* sp. and *Penicillium* spp. were isolated sporadically. *Septoria carvi* and *A. carvi* were not isolated. Therefore, such a method was not suitable for evaluating the incidence of pathogenic fungi on caraway seeds after fungicide application. We instead used direct microscopic search and enumeration of the spores, which appeared to be a more suitable method.

CONCLUSION

Based on our results we can conclude that the microflora of caraway seeds, yield parameters and quality of the harvested seeds are greatly affected by weather conditions during maturation. Yield and weight of thousand seeds are very variable, while the essential oil content and germination rate are independent of fungicide application. In 2005

Table 3. Occurrence of fungi on caraway seeds in 2005 and 2006 – number of spores in visual field of microscope

Treatment	Mean	α = 0.05	α = 0.01	Mean	α = 0.05	α = 0.01	Mean	α = 0.05	α = 0.01	Mean	α = 0.05	α = 0.01	Mean	α = 0.05	α = 0.01	Mean	α = 0.05	α = 0.01
	Alternaria sp.			fragments of dark mycelia			*Ascochyta carvi*			*Septoria carvi*			*Cladosporium* sp.			*Fusarium* sp.		
2005																		
Untreated	1.56	b	bc	2.60	a	a	11.91	de	cd	27.15	d	c	4.41	ab	ab	0.18	cd	abc
Pictor 400 SC	2.05	c	cd	3.84	cd	bc	2.83	a	a	7.80	a	a	5.40	bc	bc	0.01	ab	a
Amistar	0.85	a	a	2.59	a	a	5.93	b	ab	5.70	a	a	4.09	a	ab	0.29	d	c
Alert S	3.65	e	e	5.54	e	d	8.21	bc	bc	5.79	a	a	9.06	e	d	0.24	d	bc
Capitan 25 EW	1.75	bc	bc	2.76	ab	a	8.08	bc	bc	20.38	c	b	3.78	a	a	0.15	bcd	abc
Proline 250 EC	1.40	b	ab	3.34	bc	ab	14.44	e	d	15.05	b	b	6.20	cd	c	0.00	a	a
Topsin M 70 WP	2.59	d	d	4.25	d	c	9.03	cd	bc	19.54	bc	b	6.68	d	c	0.08	abc	ab
2006																		
Untreated	1.15	ab	ab	1.83	ab	a	15.17	bc	bc	5.75	c	c	4.40	a	a	0.10	a	a
Pictor 400 SC	1.13	ab	ab	3.65	c	b	2.85	a	a	0.37	a	a	8.70	c	c	0.02	a	a
Amistar	2.95	c	c	2.13	ab	a	5.40	a	a	2.00	b	ab	4.58	a	a	0.12	a	a
Alert S	0.72	a	a	1.48	a	a	12.48	b	b	2.88	b	b	4.47	a	a	0.95	b	b
Capitan 25 EW	1.72	b	b	2.30	b	a	17.48	cd	c	7.68	d	d	4.90	a	ab	0.13	a	a
Proline 250 EC	1.25	ab	ab	1.65	ab	a	19.00	d	c	10.03	e	e	5.05	a	ab	0.05	a	a
Topsin M 70 WP	1.05	a	ab	4.12	c	b	16.62	cd	bc	2.57	b	b	6.68	b	b	0.12	a	a

Values with the same letter are not significantly different

we achieved an increase in the total yields of the treated variants compared with the untreated control. In 2006, the yield after treatment with Pictor unsignificantly overcome the control, the resting treatments gave unsignificantly lower yield levels as compared to control (Alert S, Proline 250EC, Topsin M70WP), or even significantly lower yield levels (Amistar, Capitan 25EW) (Table 2). The data obtained from 2006 trial were influenced by increased incidence of caraway gall mite (*Aceria carvi*). In 2005 we found that the incidence of the seed-borne pathogenic fungi *Ascochyta carvi* and *Septoria carvi* in most of the treated variants decreased when compared with the untreated control. A similar effect was observed in 2006 when three fungicide variants lowered the occurrence of *A. carvi* and four variants the occurrence of *S. carvi*.

The producers must, therefore, consider carefully what fungicide should be applied, and bear in mind the risk of fungal contamination of the harvested product, favoured by certain weather conditions during ripening.

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