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Original scientific paper

Efficacy of fungicides with different modes of action in raspberry spur blight (*Didymella applanata*) control

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SUMMARY

Efficacy trials of four multi-site fungicides (copper hydroxide, mancozeb, chlorothalonil and dithianon), as well as six fungicides with specific modes of action (fluopyram, boscalid, fluazinam, tebuconazole, azoxystrobin and pyraclostrobin) in raspberry spur blight (*Didymella applanata)* control were carried out in the seasons 2014 and 2016. The experiments were conducted as a randomized block design with four replicates in a commercial raspberry orchard in the locality Trešnjevica (Arilje) in western Serbia. All fungicides were applied preventively, four times until the beginning of harvest and once after harvest. The effects of the products tested were assessed three weeks after the last fungicide application according to the intensity of cane infection. Disease severity in control (untreated) plots were 53.7 (2014) and 76.3% (2016). In both years, the highest efficacy was achieved by tebuconazole (96.3 and 99.6%), followed by fluopyram (95.7 and 99.3%) and boscalid (94.7 and 95.9%). The broad-spectrum multi-site fungicides mancozeb, chlorothalonil, copper hydroxide and dithianon were effective against *D. applanata* and they reduced disease severity significantly, in comparison with the untreated plots. The efficacy of these compounds was between 64.4 and 81.7%. Conversely, azoxystrobin, pyraclostrobin, and fluazinam showed very low efficacy (13.7-37.8%) in control of raspberry spur blight.

Keywords: *Didymella applanata*; Fungicides; Chemical control; Raspberries

INTRODUCTION

Raspberry (*Rubus idaeus L.)* is the most important berry-like fruit in Serbia. With a total production of 65,000 tons per year, Serbia is one of the leading global producers and exporters of raspberries (Nikolić et al., 2008). Spur blight, caused by the phytopathogenic fungus *Didymella applanata* (Niessl.) Sacc. (anamorph *Phoma argillacea*), is one of the most detrimental and widespread cane diseases of red raspberry which limits the production of this berry fruit (Williamson & Hargreaves, 1981). The disease is common in all raspberry growing regions across Europe, America and Australia (Josifović, 1941; Lousberg et al. 1973; Dimitrijević & Petronijević, 1976; Ranković & Garić, 1996). Spur blight incidence leads to economically notable losses that are able to reach 60% in humid years (Williamson & Hargreaves, 1981). The pathogen reduces yields in several ways: by blighting the fruit-bearing spurs, inducing premature leaf drop, and killing buds on canes that later develop into fruit-bearing side branches (Lousberg et al., 1973; Fox, 2006).

Disease control is based on an integrated approach involving some significant non-chemical methods, such as the removal and destruction of all old fruited floricanes after harvest, elimination of the first flush of young canes and selection of resistant cultivars. However, to achieve superior disease control, multiple applications of fungicides are unavoidable (Williamson, 1991; Fox, 2006). Unfortunately, a range of constraints could be a hindrance to spur blight control with fungicides. Raspberry, as a minor crop on a world scale, does not receive adequate consideration by chemical companies developing new active ingredients and formulations as the market is very small (Williamson, 2003). Similarly, older chemicals are under sustained review by registration authorities, so that modern toxicological standards are applied and the environmental issues re-examined minutely (Williamson, 2003). Consequently, many of the chemicals which have been documented as effective in spur blight control are not approved for further use in the EU, such as benomyl, captafol, dichlofluanid, ferbam and zineb (EU Pesticides Database, 2014).

The problem of raspberry spur and cane blight has become more prominent over the past years as the use of some chemicals has been restricted in integrated production (Mikulic-Petkovsek et al., 2014). At present, copper compounds, azoxystrobin, and tebuconazole are the only fungicides registered in the Serbian pesticide market for control of *Didymella applanata* (Anonymous, 2015). Furthermore, fungicide application is limited in practice by the long-lasting harvest season, and is usually performed only after harvest. Considering the biology of the pathogen, fungicide application is therefore not efficient enough since the pathogen is able to infect raspberry crops far earlier under favorable weather conditions. The basic assumption is that fungicides should be applied much earlier before harvest, taking into account the pre-harvest interval (PHI), in order to achieve maximum efficiency of fungicides. It would provide not only higher yields in the next season, but also the longevity of plantations, which progressively wilt over the years due to inappropriate disease control. Finally, it is important to highlight that control of cane diseases is usually less effective than the control of fruit or foliar diseases, since it is difficult to cover canes equally by pesticides.

According to Faby (2008), spraying is only efficient if the solution entirely covers the primocanes from the ground to the tip. Considering the significance of appropriate fungicide treatment on the one side, and the very few available products for alternative use on the other, an extensive knowledge about the efficacy of different fungicides against *D. applanata* could be very useful. Therefore, the aim of this study was to widen the spectrum of prospective fungicides for spur blight control by screening the efficacy of several multi-site (copper hydroxide, mancozeb, chlorothalonil, and dithianon), as well as site-specific fungicides (fluopyram, boscalid, fluazinam, tebuconazole, azoxystrobin, and pyraclostrobin) under field condition.

MATERIALS AND METHODS

Fungicide treatments

The experiments were carried out in a commercial raspberry field at the location Trešnjevica (Arilje) (GPS: N 43 68. 826'; E 20 132. 45') during the growing seasons of 2014, 2015 and 2016. Ten commercial products of fungicides with different modes of action were tested for their ability to reduce the severity of raspberry spur blight (Table 1). In 2015, disease incidence in untreated plots was very low due to unfavorable weather conditions, and the efficacy assessment was not considered as reliable (data not shown).

The experiment examined 10 different fungicides in treated plots, as compared to the untreated (control) plots, and the design was a complete randomized block system with four replicates. Each plot was a 25 m long row. The fungicides were applied preventively, five times in total (four times before and once after harvest). The sprays were applied by a knapsack sprayer (Solo 423, Germany). The timing of application was generally adjusted to local practice in Serbia. Fungicide application dates and growth stages of raspberry are shown in Tables 2 and 3. The most common cultivar Willamette (19 years old planting), which is highly susceptible to spur blight, was used in this experiment.

Table 1. Fungicides used in the experiment

Active ingredient (a.i.)	Trade name	Manufacturer	Content of a.i. (g L^{-1} $(kg)^{-1}$)
Copper hydroxide	Kocide-2000	Du Pont	538
Mancozeb	Mankogal-80	Galenika -Fitofarmacija	800
Pyraclostrobin	Retengo	BASF	200
Dithianon	Delan 700-WG	BASF	700
Fluazinam	Zignal 500 SC	Cheminova	500
Boscalid	Cantus	BASF	500
Azoxystrobin	Quadris	Syngenta	250
Fluopyram	Luna	Bayer Crop Science	500
Chlorothalonil	Dakoflo 720-SC	Galenika-Fitofarmacija	720
Tebuconazole	Akord WG	Galenika -Fitofarmacija	250

Table 2. Raspberry growth stages and fungicide application dates in 2014

Treatment	Growth stage	Date
1.	5-10 cm length of primocanes	Apr, 12
2.	15-20 cm length of primocanes	Apr, 30
3.	25-35 cm length of primocanes	May, 11
4.	Beginning of flowering	May, 24
5.	After harvest	Aug, 05
Date of assessment	–	Aug, 26

Table 3. Raspberry growth stages and fungicide application dates in 2016

Treatment	Growth stage	Date
1.	5-10 cm length of primocanes	Mar, 29
2.	15-20 cm length of primocanes	Apr, 14
3.	25-35 cm length of primocanes	May, 05
4.	Beginning of flowering	May, 23
5.	After harvest	Aug, 05
Date of assessment	–	Aug, 27

Type of assessment

Disease severity was assessed three weeks after the last fungicide application, when disease symptoms were clearly detected in untreated plots. The assessment was performed on 50 randomly selected primocanes per plot, counting the number of spur blight lesions ranked according to the scale: 0 = without symptoms; 1 = one lesion per primocane; 2 = two to three lesion per primocane; 3 = more than three lesions per primocane.

Disease severity (DS) was calculated using Townsend-Heuberger's formula:

$$DS\ (\%) = \Sigma(nV) / NV \times 100$$

where n – degree of infection according to the scale; v – number of primocanes per category; V – total number of primocanes screened; N – highest degree of category

The efficacy of fungicides was calculated using Abbott's formula:

$$Efficacy\ (\%) = (X - Y) / X \times 100$$

where X – disease severity in the control; Y – disease severity in the treatment

Statistical Analysis

The data were subjected to standard statistical processing, using the analysis of variance (One Way ANOVA) and Statistica for Windows (2001) software. The significance of differences between different treatments was determined by Duncan's multiple range test at the significance level of 5% (P<0.05).

RESULTS

Weather conditions over the growing season 2014 were highly favorable for spur blight development, resulting in a high level of disease incidence in untreated plots (53.7%). Fungicides used in that experiment showed very different degrees of efficacy (Table 4).

The DMI fungicide tebuconazole showed the highest efficacy against *D. applanata* (96.3%), followed by the newly developed succinate dehydrogenase inhibitors (SDHI) fluopyram (95.7%) and boscalid (94.7%). The efficacy of the broad-spectrum fungicides dithianon and copper hydroxide was 78.9 and 78.0 % respectively, followed by two other 'multi-site' inhibitors, chlorothalonil and mancozeb, which showed a bit lower efficacy (70.5 and 67.1 %, respectively). The lowest efficacy was achieved by two QoI fungicides, azoxystrobin and pyraclostrobin, as well as an oxidative phosphorylation inhibitor, fluazinam, which showed poor effectiveness (13.7, 32.9 and 20.2 %, respectively) in spur blight control.

In experiments carried out during the season 2016, disease severity in untreated plots (76.3%) was very high (Table 5). Under the conditions, the highest efficacy was achieved by fluopyram (99.6%), followed by tebuconazole and boscalid (99.3 and 95.9%, respectively). The preventive fungicides copper hydroxide and dithianon showed good efficacy of 80.8 and 81.7 % respectively, followed by chlorothalonil and mancozeb (71.7 and 64.4 %, respectively). The efficacy of azoxystrobin and pyraclostrobin (16.2 and 37.8 %, respectively), as well as fluazinam (27.1%), was unsatisfactory.

Table 4. Disease severity and fungicide efficacy in 2014

Fungicide	Concentration rate (%)	Disease severity[1] (%)	Efficacy (%)
Kocide-2000	0.2	11.8 b	78.0
Mankogal-80	0.25	17.7 b	67.1
Retengo	0.1	36.0 c	32.9
Delan 700-WG	0.1	11.3 b	78.9
Zignal 500 SC	0.1	42.9 c	20.2
Cantus	0.12	2.8 a	94.7
Quadris	0.075	46.3 d	13.7
Luna	0.075	2.3 a	95.7
Dakoflo 720-SC	0.2	15.8 b	70.5
Akord WG	0.075	2.0 a	96.3
Control (untreated)	–	53.7 d	–

[1]Mean values in columns marked by different letters are significantly (p<0.05) different according to Duncan's test

Table 5. Disease severity and fungicide efficacy in 2016

Fungicide	Concentration rate (%)	Disease severity[1] (%)	Efficacy (%)
Kocide-2000	0.2	14.0 b	81.7
Mankogal-80	0.25	27.2 b	64.4
Retengo	0.1	47.5 c	37.8
Delan 700-WG	0.1	14.7 b	80.8
Zignal 500 SC	0.1	55.7 c	27.1
Cantus	0.12	3.2 a	95.9
Quadris	0.075	64.0 d	16.2
Luna	0.075	0.3 a	99.6
Dakoflo 720-SC	0.2	21.6 c	71.7
Akord WG	0.075	0.5 a	99.3
Control (untreated)	–	76.3 d	–

[1]Mean values in columns marked by different letters are significantly (p<0.05) different according to Duncan's test

Figure 1. Raspberry primocanes with typical symptoms of spur blight in untreated plot

Figure 2. Plot treated with tebuconazole (Akord WG 0.075%)

DISCUSSION

Spur blight, caused by the phytopathogenic fungus *D. applanata*, is one of the most important diseases of raspberry in Serbia. The level of infection by this disease varies widely from year to year depending mainly on weather conditions. In our presented trials, among the products tested, the demethylation inhibiting (DMI) fungicide tebuconazole showed the highest efficacy against *D. applanata*, followed by the newly developed succinate dehydrogenase inhibitors (SDHI) fluopyram and boscalid. Despite the fact that resistance of different phytopathogenic fungi *(Venturia inaequalis*, *Monilinia fructicola*, *Podosphaera xanthii*, *Uncinula necator*, *Sclerotinia homoeocarpa*, *Cercospora beticola,* etc.) to DMIs have been reported (Golembiewski et al., 1995; Gubler & Ypema, 1996; Köller et al., 1997; Zehr et al., 1999; Karaoglanidis et al., 2000; McGrath & Shishkoff, 2001; Stević et al, 2010), they still provide excellent control of raspberry spur blight. However, some recorded cases of DMI resistance development imply a necessity of permanent monitoring program for early detection of resistant strains in pathogen population.

A newly developed class of fungicides, succinate dehydrogenase inhibitors (SDHI), are characterized as broad-spectrum antifungal compounds that may be used on various crops (Stammler et al., 2007; Yanase et al., 2007). SDHIs as fungicides with a specific mode of action based on inhibition of mitochondrial electron transport have been classified as medium to high risk of resistance development (FRAC, 2016). Considering the fact that the fungicide resistance phenomenon is broadly accepted as a crucial factor in both limiting the efficacy and lifetime of fungicides, anti-resistance strategy relies on well-timed information about the origin, development and spread of resistant strains. Therefore, regular monitoring of *D. applanata* isolates' sensitivity to this group of fungicides, as well as assessment of their efficacy under field condition, should be conducted despite the fact that they are highly effective in spur blight control.

The results obtained in this study showed that a slightly lower efficacy in spur blight control was achieved by the broad-spectrum multi-site fungicides mancozeb, chlorothalonil copper hydroxide and dithianon. Considering the high disease severity, and only a preventive action of these fungicides, the achieved effectiveness can be considered as satisfactory. Data showed that the use of broad-spectrum fungicides for *D. applanata* control is desirable not only because of their high efficacy, but also for reducing the risk of resistance development in *D. applanata* populations to some effective fungicides with specific mode of action, such as quinone outside inhibitors (QoI).

QoI fungicides are one of the most significant classes of fungicides due to their broad-spectrum activity against the main groups of plant pathogenic fungi, low application rates and some yield benefits (Bartlett et al., 2002). However, our present study showed that the QoI fungicides azoxystrobin and pyraclostrobin did not provide effective control of *D. applanata* in both experimetal years. This result was not surprising since two isolates, Da4E and Da17B, originating from the planting where the experiment was carried out, were highly resistant to pyraclostrobin with RF values 290.8 and 325.5 (Mirković et al., 2015). In contrast to our results, Stevanović et al. (2014) found azoxystrobin to be very effective in controlling spur blight (95.5-96.5%) in a field with moderate disease pressure (33.1-34.5%). Such conflicting results might be attributed to resistance developed to QoI fungicides in a pathogen population. The highly unique mode of action (inhibition of mitochondrial respiration in fungi by binding to the Qo site of the cytochrome bc1 complex, blocking electron transfer and halting ATP synthesis) of fungicides belonging to this group has proven to be a serious weakness, since a significant number of target pathogens have already developed resistance to QoIs (Bartlett et al., 2002; Fernández-Ortuno et al., 2008). An additional study is expected to reveal if the low efficacy of these fungicides in our experiment is the consequence of intensive production technology and frequent use of QoI fungicides in this area.

A diversity in fungicides with respect to their chemistry and mode of action is essential for ensuring continued and increased raspberry production, and for managing fungicide resistance. As mentioned before, the development of resistance to fungicides with a specific mode of action is a major threat to effective chemical control. This threat may also refer to fluazinam, a new protective fungicide belonging to the chemical group of phenylpyridinamines with a highly unique mode of action (acts by interrupting the fungal cell's energy production process by an uncoupling effect on oxidative phosphorylation) (Guo et al., 1991). Our study revealed that this fungicide exhibited very low efficacy in the control of *D. applanata* in both trials. Considering the fact that both isolates (Da4E and Da17B) derived from the planting where the experiments were carried out, were marked as sensitive to fluazinam, it is unlikely that low efficacy of this fungicide is a consequence of fungicide resistance. Certainly, extensive field studies complemented by parallel laboratory experiments have to be carried out in the future in order to determine whether fluazinam is an appropriate fungicide for *D. applanata* control.

CONCLUSION

Weather conditions during the growing seasons of 2014 and 2016 were favorable and led to high levels of raspberry spur blight severity (53.7 and 76.3%, respectively). The results of this study revealed that of all products tested in both years, tebuconazole, fluopiram and boscalid showed the highest efficacy against *D. applanata* (94.7-99.6%) in the field with high disease pressure. The high levels of disease control provided by the SDHI fungicides fluopyram and boscalid in our study indicate that they are a welcome addition to the group of fungicides for the control of spur blight disease. The broad-spectrum multi-site fungicides mancozeb, chlorothalonil, copper hydroxide and dithianon were found to be effective against *D. applanata*, and the efficacy of these fungicides ranged from 64.4% to 81.7%. However, the site-specific fungicides including azoxystrobin, pyraclostrobin and fluazinam provided very poor suppression of *D. applanata* (13.7-37.8%). Considering the fact that fungicide use is indispensable to successful control of spur blight, repeated evaluation of their efficacy is recommended.

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Efikasnost fungicida različitog mehanizma delovanja u suzbijanju kestenjaste pegavosti izdanaka maline (*Didymella applanata*)

REZIME

U toku 2014. i 2016. godine, ispitivana je efikasnost četiri fungicida sa nespecifičnim (bakar-hidroksid, makozeb, hlorotalonil i ditianon) i šest sa specifičnim (fluopiram, boskalid, fluazinam, tebukonazol, azoksistrobin i piraklostrobin) mehanizmom delovanja, u suzbijanju prouzrokovača kestenjaste pegavosti izdanaka maline (*Didymella applanta*). Ogledi su izvedeni u komercijalnom zasadu maline na lokalitetu Trešnjevica (Arilje), u zapadnoj Srbiji, po tipu potpunog slučajnog blok sistema sa četiri ponavljanja. Svi fungicidi su primenjeni preventivno, a obavljeno je ukupno pet tretiranja, četiri pre berbe i jedno nakon završene berbe. Efikasnost testiranih preparata ocenjena je tri nedelje nakon poslednje primene fungicida. Intenzitet oboljenja u kontrolnim (netretiranim) parcelama iznosio je 53,7% (2014) i 76,3% (2016). Tokom obe godine ispitivanja, najviša efikasnost postignuta je primenom tebukonazola (96,3% i 99,6%), fluopirama (95,7% i 99.3%) i boskalida (94,7% i 95,9%). Fungicidi nespecifičnog mehanizma delovanja mankozeb, hlorotalonil, bakar-hiroksid i ditianon bili su efikasni u suzbijanju *D. applanata* i značajno su smanjili intenzitet oboljenja u poređenju sa netretiranim parcelama. Efikasnost ovih preparata bila je između 64,4% i 81,7%, dok su azoksistrobin, piraklostrobin i fluazinam pokazali veoma nisku efikasnost (13,7-37,8%) u subijanju prouzrokovača kestenjaste pegavosti izdanaka maline.

Ključne reči: *Didymella applanata*; Fungicidi; Hemijsko suzbijanje; Malina