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Effect of Fungicides and Application Intervals for the Control of Black Spot of Roses

Christina Jennings, Terri Simmons, Madhav Parajuli, Kumuditha Hikkaduwa Epa Liyanage, and Fulya Baysal-Gurel

Department of Agricultural and Environmental Sciences, College of Agriculture, Tennessee State University, Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110, USA

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Abstract. **The efficacy of the fungicide pydiflumetofen + difenoconazole (Postiva) was evaluated at varying application rates and intervals to control black spot disease (*Diplocarpon rosae*) in rose (*Rosa* spp. 'Coral Drift'). Container-grown roses were arranged in a completely randomized design with five single-plant replications. Experiments were conducted under greenhouse and shade-house conditions (56% shade) in 2021/2022 and 2023. Black spot disease in roses was developed naturally. Pydiflumetofen + difenoconazole at 1.1 , 1.6, and 2.2 mL·L^{-1}, and standard fungicide azoxystrobin + benzovindiflupyr (Mural) at 0.5·g L^{-1} were sprayed on foliage to runoff on a 2- or 4-week interval. Plants that were not treated with fungicide served as the controls. Plants were evaluated weekly for disease severity (0%–100% foliage affected) and defoliation (0%–100% defoliation). The season-long area under the disease progress curve (AUDPC) and area under the defoliation progress curve (AUDFC) were calculated for the evaluation period. Pydiflumetofen + difenoconazole reduced significantly black spot disease severity, AUDPC, defoliation, and AUDFC both in greenhouse and shade-house conditions compared with control plants, and was as effective as azoxystrobin + benzovindiflupyr. All the application rates and intervals of pydiflumetofen + difenoconazole were equally effective in reducing black spot severity and AUDPC. Our findings suggest that pydiflumetofen + difenoconazole at the lowest rate with the longest application interval is the most cost-effective, and has similar efficacy as treatments with higher rates and more frequent intervals.**

The woody ornamental nursery industry generates more than $5.5 billion in annual wholesale values, making it an important agricultural sector in the United States (National Agriculture Statistics Service 2020). Roses are valued for their flowers' color, beauty, fragrance, and symbolism (Asmadi et al. 2020) among woody ornamentals, and are widely grown in residential and commercial plantings, as well as in greenhouses and field nurseries. Roses play an important role in the ornamental industry, being among the top 10 ornamental crops (Bowen et al. 1995; Debener 2019). In 2019, roses contributed $214,614,000 in total sales in the United States (National Agriculture Statistics Service 2020). Several diseases impact the health, aesthetics, and overall value of roses negatively, and render them unmarketable. Black spot disease of roses is a common and destructive foliar disease that causes significant economic losses (Baysal-Gurel and Phillips 2019; Gachomo and Kotchoni 2007).

Black spot is one of the most important diseases of roses (Gachomo et al. 2006; Sinclair and Lyon 1987). Infection not only decreases aesthetic value but weakens the plant as well (Bowen et al. 1995; Neu et al. 2017). In addition to the adaxial leaf surface, infection can occur on buds, petioles, fruit, and young stems (Asmadi et al. 2020; Gachomo and Kotchoni 2007). Susceptible roses grown outdoors are particularly prone to black spot disease during moist, cool weather and high humidity (Ong and Brake 2015). Once established, black spot can be difficult to manage (Gachomo et al. 2009).

Black spot disease of rose is caused by the hemibiotrophic fungal pathogen *Diplocarpon rosae*, the teleomorph stage, and *Marssonina rosae*, the anamorph stage (Bowen et al. 1995; Debener 2019; Gachomo et al. 2006, 2009; Horst 1983). This fungal disease was first reported during the 19th century in Europe and is currently found worldwide as a result of the global trade of roses (Debener 2019). The sexual stage, although previously described, appears much less frequently than the asexual life cycle and is not well explained (Debener 2019; Gachomo and Kotchoni 2007). Two-celled conidium from asexual propagation are the most prevalent propagule of black spot. The life cycle starts when these conidium first develop germination tubes, typically from the larger of the conidial cells, and then form appressoria within the first 12 h of the infection process. Once penetration has occurred, subcuticular hyphae, intercellular hyphae, intracellular hyphae, and haustoria are established and used to siphon nutrients from the host plant (Gachomo et al. 2010). For germination to occur, it is important for conidia to be suspended in water for several hours, with the rate of germination increasing with the amount of time that conidia are immersed in water. High relative humidity is also important for spore germination (Baysal-Gurel and Phillips 2019; Debener 2019; Gachomo and Kotchoni 2007). Germination can occur from 0 to 33 °C, with optimum temperatures for disease development being 18, 21, and 24 °C. However, fungal symptoms do not occur below 10 °C or above 29 °C (Debener 2019; Gachomo and Kotchoni 2007). Symptoms appear as circular brown to black spots or blotches with irregular margins up to 15 mm that appear on the adaxial surface of the leaves, which frequently have a yellow halo (Baysal-Gurel and Phillips 2019; Bowen et al. 1995; Debener 2019; Gachomo et al. 2006; Sinclair and Lyon 1987). In particularly susceptible roses, infection may weaken the plant to the point of additional stressors causing death. Conidia overwinter on plant debris and stems and begin a new cycle in the spring (Debener 2019). Water is the main mode of dissemination for black spot.

Fungal diseases require management practices, such as sanitation and removal of infected material, to prevent the spread of inoculum and overwintering. The use of fungicides is one of the most common and effective methods to protect roses that do not have host plant resistance from black spot disease (Baysal-Gurel and Phillips 2019; Bowen et al. 1995). Fungicides such as thiophanate methyl, propiconazole, fluopyram + trifloxystrobin, trifloxystrobin, propiconazole + chlorothalonil, chlorothalonil, myclobutanil, azoxystrobin, azoxystrobin + benzovindiflupyr, tebuconazole, and metconazole have been used previously to control black spot disease in roses (Baysal-Gurel and Phillips 2019; Bowen and Roark 2001; Gachomo et al. 2009). The fungicides are applied in the growing season in 7- to 14-d intervals. However, there is very little published information on optimal rates and interval of fungicide applications. This understanding can aid in decreasing fungicide use and in reducing production costs. In addition, being able to use less fungicide overall can reduce the risk of fungicide resistance.

The objective of this study was to evaluate the efficacy of application rates and intervals of pydiflumetofen (6.9%) + difenoconazole (11.5%) fungicide (Postiva; Syngenta Crop

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F.B.-G. is the corresponding author. E-mail: fbaysalg@tnstate.edu.

Protection LLC, Greensboro, NC, USA) on the control of black spot of rose, which is a novel, recently developed fungicide. Pydiflumetofen + difenoconazole is considered to provide a broad-spectrum control of diseases, such as Botrytis blight, *Fusarium* crown and root rot, leaf spots, and powdery mildew in ornamentals.

Materials and Methods

Various application rates and intervals of the fungicides pydiflumetofen + difenoconazole and azoxystrobin + benzovindiflupyr (Mural; Syngenta Crop Protection LLC) were evaluated to control black spot disease of rose 'Coral Drift' in greenhouse and shade-house settings. Trials were conducted in 2021/2022 and 2023 at the Tennessee State University Otis L. Floyd Nursery Research Center in McMinnville, TN, USA. Plants were received in 3-gal containers, and nursery mix (processed pine bark, 55%–65%; Canadian sphagnum peat; and sand) (Morton's Horticultural Products, McMinnville, TN, USA) was used as the potting medium for plants. Plants were then fertilized with 399.2 mL liquid fertilizer (24–8–16 Miracle-Gro®; Scotts Miracle-Gro Co., Marysville, OH, USA) and 14.2 g of granular controlled-release fertilizer (18–6–8 Nutricote®; Arysta LifeScience America, New York, NY, USA) preceding their respective trials. The fungicides tested were three rates of pydiflumetofen + difenoconazole (1.1, 1.6, and 2.2 mL·L^{-1}) and a single rate of azoxystrobin + benzovindiflupyr (0.5 g·L^{-1}), with 4% v/v of CapSil spray adjuvant (Aquatrols, Paulsboro, NJ, USA) added to each treatment. Treatments were repeated on a 2- or 4-week interval and were applied to runoff as a foliar spray with a backpack CO_2 pressurized sprayer (Bellspray, Inc., Opelousas, LA, USA) equipped with a TeeJet XR8002VS nozzle at 30 pounds per square inch. Black spot developed naturally in each trial. Every 7 d from the beginning of the trial period until 2 weeks after the last application of fungicides, evaluations for disease severity and defoliation were conducted and expressed as a percentage of the foliage area affected.

Greenhouse trials. In trial 1, plants were in 3-gal containers and were irrigated using overhead irrigation emitters (SpinNet nozzle; Hummert International, Earth City, MO, USA) for 2 min twice a day in Oct, Nov, Dec 2021, and Jan 2022. Plants were arranged in a completely randomized design with five single-plant replications. Fungicides were applied on 10 Nov, 24 Nov, 8 Dec, 22 Dec 2021, and 5 Jan 2022 for the 2-week interval, and on 10 Nov, 8 Dec 2021, and 5 Jan 2022 for the 4-week interval. Five single-plant replications not treated with fungicides served as the controls. Plants were evaluated for disease severity and defoliation, using a scale of 0% to 100% foliage area affected, on 9 Nov, 16 Nov, 23 Nov, 30 Nov, 7 Dec, 14 Dec, 21 Dec, 28 Dec, 4 Jan, 11 Jan, and 28 Jan. Average maximum greenhouse temperatures for 9 to 30 Nov, Dec 2021, and 1 to 18 Jan 2022 were 26.8, 27.4, and 27.2 °C, respectively; average minimum temperatures were 16.1, 15.0, and 15.3 °C, respectively.

In trial 2, plants were irrigated for 2 min twice a day in Jan, Feb, Mar, and Apr 2023. Plants were in 3-gal containers and were arranged in a completely randomized design with five single-plant replications. Fungicides were applied on 30 Jan, 13 Feb, 27 Feb, 13 Mar, and 27 Mar for the 2-week interval, and on 30 Jan, 27 Feb, and 27 Mar for the 4-week interval. Five single-plant replications not treated with fungicides served as the controls. Plants were evaluated for disease severity and defoliation, using a scale of 0% to 100% foliage area affected, on 30 Jan, 6 Feb, 13 Feb, 20 Feb, 27 Feb, 6 Mar, 20 Mar, 27 Mar, 3 Apr, and 10 Apr. Average maximum temperatures for Jan, Feb, Mar, and 1 to 10 Apr were 27.6, 27.8, 27.4, and 26.7 °C, respectively; average minimum temperatures were 17.5, 17.9, 17.7, and 19.0 °C, respectively.

Shade-house trials. In trial 1, plants were irrigated by hand as needed in Jul, Aug, and Sep 2022. Plants were arranged in a completely randomized design in a shade house under 56% shade. Fungicides were applied on 5 Jul, 19 Jul, 2 Aug, 16 Aug, and 30 Aug for the 2-week interval, and on 5 Jul, 2 Aug, and 30 Aug for the 4-week interval. Plants not treated with fungicides served as the controls. Plants were evaluated for black spot disease severity, defoliation, and phytotoxicity on 8 Jul, 15 Jul, 22 Jul, 29 Jul, 5 Aug, 12 Aug, 19 Aug, 26 Aug, 2 Sep, 9 Sep, and 16 Sep using a scale of 0% to 100% foliage area affected. Average maximum temperatures for 5 to 31 Jul, Aug, and 1 to 16 Sep were 32.5, 30.4, and 28.1 °C, respectively; average minimum temperatures were 21.4, 19.6, and 15.5 °C; and total rainfall was 86.9, 63.9, and 67.2 mL, respectively.

In trial 2, plants were irrigated with overhead irrigation (Orbit 55032 1/2" BRS Sprinkler Head; Orbit® Inc., North Salt Lake, UT, USA) for 15 min twice a day in Jul, Aug, and Sep 2023. Plants were arranged in a completely randomized design in a shade house under 56% shade. Fungicides were applied on 13 Jul, 27 Jul, 10 Aug, 24 Aug, and 7 Sep for the 2-week interval, and on 13 Jul, 10 Aug, and 7 Sep for the 4-week interval. Plants not treated with fungicides served as the controls. Plants were evaluated for black spot disease severity, defoliation, and phytotoxicity on 13 Jul, 20 Jul, 27 Jul, 3 Aug, 10 Aug, 17 Aug, 24 Aug, 31 Aug, 7 Sep, 14 Sep, and 21 Sep using a scale of 0% to 100% foliage area affected. Average maximum temperatures for 13 to 31 Jul, Aug, and 1 to 21 Sep were 31.2, 30.3, and 28.2 °C. respectively; average minimum temperatures were 19.9, 19.4, and 16.4 °C, respectively; and total rainfall was 53.6, 118.2, and 10.5 mL, respectively.

Statistical analysis. The season-long area under the disease progress curve (AUDPC) and area under the defoliation progress curve (AUDFC) (Bowen and Roark 2001) were calculated using the formula: $\Sigma\{[(x_i + x_i - 1)/2](t_i - t_{i-1})\}$, where x_i is the rating at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations. Treatment effects on AUDPC and AUDFC were analyzed with one-way analysis of variance (ANOVA) using the PROC generalized linear model in SAS v. 9.4 (SAS Institute, Cary, NC, USA). Means were separated using the Tukey test, with significance at $P < 0.05$. Percent data on severity and defoliation were analyzed using a generalized linear mixed model with a logit link and beta distribution (PROC GLIMMIX) in SAS v. 9.4 and means were separated using least-squares means. Factorial two-way ANOVA was performed to determine the main and interactive effects of application rate and interval. The control treatment was removed to facilitate the two-way factorial analysis. Means were separated using the Tukey test ($P < 0.05$).

Results

Greenhouse trials. In trial 1, the factorial two-way ANOVA showed a significant effect of rate × interval interaction for final disease severity and AUDFC (Table 1). The application rate, application interval, and rate × interval interaction did not have a significant impact on AUDPC or final defoliation. The final black spot disease severity was 24.2% on the untreated controls. Final disease severity and AUDPC were the highest on the untreated controls than on treated plants. All treatments

Table 1. *P* value from two-way analysis of variance testing effects of application rate, application interval, and their interaction on final disease severity, area under the disease progress curve (AUDPC), final defoliation, and area under the defoliation progress curve (AUDFC) in greenhouse and shade-house conditions in 2021/2022, and 2023.

	Trial 1						Trial 2					
	Greenhouse			Shade house			Greenhouse			Shade house		
Variable	Rate	Interval	Rate × interval	Rate	Interval	Rate × interval	Rate	Interval	Rate × interval	Rate	Interval	Rate × interval
Final disease severity	NS[1]	NS	*	NS	NS	NS	**	NS	NS	NS	NS	NS
AUDPC	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS
Final defoliation	NS	NS	NS	NS	*	NS	***	**	***	NS	NS	NS
AUDFC	NS	NS	*	NS	NS	NS	***	*	*	NS	NS	NS

[1] NS, *, **, ***nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Fungicide effects on defoliation (0%–100% defoliation), area under the defoliation progress curve (AUDFC), final black spot (*Diplocarpon rosae*) severity (0%–100% leaf affected), and area under the disease progress curve (AUDPC) of roses (*Rosa* spp. 'Coral Drift') grown in a greenhouse from 10 Nov 2021 to 5 Jan 2022.

Treatment	Application rate	Application interval (weeks)	Final defoliation (%)[i]	AUDFC[ii]	Final severity (%)[i]	AUDPC[ii]
Pydiflumetofen + difenoconazole	1.1 mL·L^{-1}	2	4.3 a[iii]	92.8 a	10.8 b	373.3 b
		4	6.3 a	182.9 a	8.3 b	371.6 b
Pydiflumetofen + difenoconazole	1.6 mL·L^{-1}	2	4.6 a	161.9 a	9.6 b	401.0 b
		4	3.3 a	99.2 a	11.7 b	455.3 b
Pydiflumetofen + difenoconazole	2.2 mL·L^{-1}	2	5.0 a	177.9 a	9.2 b	425.8 b
		4	2.9 a	74.4 a	10.0 b	397.0 b
Azoxystrobin + benzovindiflupyr	0.5 g·L^{-1}	2	4.2 a	129.5 a	11.7 b	430.5 b
		4	4.6 a	180.5 a	12.9 b	504.0 b
Untreated control	—	—	8.3 a	259.6 a	24.2 a	845.8 a
P value	—	—	0.1056	0.6033	<0.000	<0.000
F value	—	—	1.81	0.80	6.51	6.98

[i] Final defoliation and black spot severity evaluation was performed on 28 Jan.

[ii] AUDPC (or AUDFC) = $\Sigma\{[(x_i + x_i - 1)/2](t_i - t_{i-1})\}$, where x_i is the disease severity rating (or defoliation rating) at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations.

[iii] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects on the AUDFC and the AUDPC. Means were compared using Fisher's least significant difference test with $\alpha = 0.05$. Percent data (defoliation and severity) were analyzed according to a generalized linear mixed model with a logit link and beta distribution (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

of pydiflumetofen + difenoconazole and azoxystrobin + benzovindiflupyr reduced significantly the final disease severity and AUDPC (Table 2). Final defoliation and season-long defoliation (AUDFC) were not significantly different between treatments and the untreated controls. The high rate of pydiflumetofen + difenoconazole at the 4-week application interval had a significantly greater width increase compared with the medium rate at the 2-week interval (Table 2).

In trial 2, the two-way ANOVA showed that application rate, application interval, and rate × interval interaction was significant for final defoliation and AUDFC (Table 1). The application rate was significant for final disease severity and AUDPC, although the application interval and rate × interval interaction were not significant (Table 1). Final black spot disease severity for the untreated controls was 40.0%. Final disease severity, AUDPC, final defoliation, and AUDFC were highest in the untreated controls. All treatments of pydiflumetofen + difenoconazole and azoxystrobin + benzovindiflupyr reduced significantly the final disease severity, AUDPC, final defoliation, and AUDFC compared with the untreated controls (Table 3). Both the 2- and 4-week intervals for the low and medium rate of pydiflumetofen + difenoconazole, as well as the 2-week interval of the high rate, had the lowest final black spot disease severity. Azoxystrobin + benzovindiflupyr at the 4-week interval had the highest final disease severity and AUDPC among the treatments, which was significantly greater than the 2-week interval at the low rate, the 4-week interval at the medium rate, and the 2-week interval at the high rate. Azoxystrobin + benzovindiflupyr at the 4-week interval also had the highest AUDFC among treatments, with the rest of the treatments being similar to one another. Treatments with the high rate of pydiflumetofen + difenoconazole at the 4-week interval and azoxystrobin + benzovindiflupyr at the 4-week interval had the highest amount of defoliation among the treatments (Table 3).

Shade-house trials. In trial 1, the two-way ANOVA showed no significant effect of application rate, application interval, or rate × interval on final disease severity, the AUDPC, or the AUDFC (Table 1). The application interval had a significant effect on final defoliation (Table 1). Final black spot disease severity in the untreated controls was 67.0%. The untreated controls had significantly greater final defoliation, AUDFC, final disease severity, and AUDPC. All rates and intervals of pydiflumetofen + difenoconazole and intervals of azoxystrobin + benzovindiflupyr reduced significantly final defoliation, AUDFC, final disease severity, and AUDPC (Table 4). All treatments were equally effective in reducing final defoliation, AUDFC, final disease severity, and AUDPC (Table 4).

In trial 2, The two-way ANOVA showed no significant effects of application rate, application interval, and rate × interval on final disease severity, AUDPC, final defoliation, or AUDFC (Table 1). Final black spot disease severity was 49.0%. The untreated controls had significantly greater final defoliation, final disease severity, and AUDPC. All rates and intervals of pydiflumetofen + difenoconazole and intervals of azoxystrobin + benzovindiflupyr reduced significantly final defoliation, final disease severity, and AUDPC compared with the untreated controls (Table 5). Final defoliation, AUDPC and AUDFC were similar among all treatments. Additionally, the low rate at the 4-week interval, medium rate at the 2-week

Table 3. Fungicide effects on defoliation (0%–100% defoliation), the area under the defoliation progress curve (AUDFC), final black spot (*Diplocarpon rosae*) severity (0%–100% leaf affected), and the area under the disease progress curve (AUDPC) of roses (*Rosa* spp. 'Coral Drift') grown in a greenhouse from Jan to Mar 2023.

Treatment	Application rate	Application interval (weeks)	Final defoliation (%)[i]	AUDFC[i]	Final severity (%)[ii]	AUDPC[ii]
Pydiflumetofen + difenoconazole	1.1 mL·L^{-1}	2	3.0 cd[iii]	35.0 c	3.5 d	86.5 c
		4	2.5 d	40.3 c	5.5 cd	153.7 bc
Pydiflumetofen + difenoconazole	1.6 mL·L^{-1}	2	3.0 cd	52.5 c	5.0 cd	155.4 bc
		4	3.0 cd	38.5 c	3.0 d	70.0 c
Pydiflumetofen + difenoconazole	2.2 mL·L^{-1}	2	4.0 c	84.0 bc	4.5 cd	117.3 c
		4	5.0 b	129.5 bc	6.0 c	200.9 bc
Azoxystrobin + benzovindiflupyr	0.5 g·L^{-1}	2	2.5 d	50.8 c	6.5 bc	298.6 bc
		4	5.0 b	154.0 b	9.5 b	428.1 b
Untreated control	—	—	14.0 a	381.5 a	40.0 a	1070.3 a
P value	—	—	<0.000	<0.000	<0.000	<0.000
F value	—	—	69.14	29.72	73.65	26.00

[i] AUDPC (or AUDFC) = $\Sigma\{[(x_i + x_i - 1)/2](t_i - t_{i-1})\}$, where x_i is the disease severity rating (or defoliation rating) at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations.

[ii] Final defoliation and black spot severity evaluation was performed on 16 Sep.

[iii] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects on the AUDFC and the AUDPC. Means were compared using Fisher's least significant difference test with $\alpha = 0.05$. Percent data (defoliation and severity) were analyzed according to a generalized linear mixed model with a logit link and beta distribution (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

Table 4. Fungicide effects on defoliation (0%–100% defoliation), the area under the defoliation progress curve (AUDFC), final black spot (*Diplocarpon rosae*) severity (0%–100% leaf affected), and the area under the disease progress curve (AUDPC) of roses (*Rosa* spp. 'Coral Drift') grown in a shade house from Jul to Sep 2022.

Treatment	Application rate	Application interval (weeks)	Final defoliation (%)[i]	AUDFC[ii]	Final severity (%)[i]	AUDPC[ii]
Pydiflumetofen + difenoconazole	1.1 mL·L^{-1}	2	1.5 b[iii]	33.3 b	25.0 b	548.8 b
		4	6.0 b	56.0 b	27.0 b	650.0 b
Pydiflumetofen + difenoconazole	1.6 mL·L^{-1}	2	3.0 b	59.5 b	27.0 b	682.9 b
		4	4.5 b	47.3 b	29.0 b	716.1 b
Pydiflumetofen + difenoconazole	2.2 mL·L^{-1}	2	3.0 b	38.5 b	22.0 b	580.0 b
		4	7.0 b	59.5 b	24.0 b	603.4 b
Azoxystrobin + benzovindiflupyr	0.5 g·L^{-1}	2	4.0 b	42.0 b	20.0 b	579.3 b
		4	9.5 b	110.3 b	33.0 b	783.3 b
Untreated control	—	—	39.0 a	553.0 a	67.0 a	1792.7 a
P value	—	—	<0.000	<0.000	<0.000	<0.000
F value	—	—	12.79	10.45	6.42	12.2

[i] Final defoliation and black spot severity evaluation was performed on 16 Sep.

[ii] AUDPC (or AUDFC) = $\Sigma\{[(x_i + x_i - 1)/2](t_i - t_{i-1})\}$, where x_i is the disease severity rating (or defoliation rating) at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations.

[iii] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects on the AUDFC and the AUDPC. Means were compared using Fisher's least significant difference test with $\alpha = 0.05$. Percent data (defoliation and severity) were analyzed according to a generalized linear mixed model with a logit link and beta distribution (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

interval, and high rate at the 2-week interval of pydiflumetofen + difenoconazole were similar in AUDFC to the untreated controls. Treatments at the medium rate of pydiflumetofen + difenoconazole at the 4-week interval and azoxystrobin + benzovindiflupyr at the 4-week interval had the lowest final disease severity; however, these were still similar to all the other treatments besides the high rate of pydiflumetofen + difenoconazole at the 2-week interval (Table 5).

Discussion

Black spot is a major threat in the production of roses. Shade houses and greenhouses are a primary way that roses are produced in Tennessee, USA. Conducting trials in both conditions allowed better insight on black spot infection of roses and ways to combat black spot fungicide resistance using multisite mode-of-action (MOA) fungicides. Black spot disease occurred in both conditions, which indicates that both conditions were able to provide conditions for black spot development. Rose plants can be affected negatively by prolonged black spot disease pressure, with symptoms including defoliation, diminished bloom growth, and overall failure to thrive (Sinclair and Lyon 1987). These results show that being able to treat and control black spot is important.

The fungicide azoxystrobin + benzovindiflupyr (Mural) is commonly used for fungal infection management; pydiflumetofen + difenoconazole (Postiva) is a recently released fungicide being tested for efficacy against black spot disease of roses. Chemicals azoxystrobin (30%), a Fungicide Resistance Action Committee (FRAC) MOA group 11 strobilurin fungicide, and benzovindiflupyr (15%) (also known as SOLATENOL®), a FRAC MOA group 7 succinyl dehydrogenase inhibitor, make up the composition for Mural. Azoxystrobin is a systemic chemical that progresses throughout the plant, offering a prolonged safeguard for new plant growth. In addition, it contributes to plant health by influencing physiological processes. Ethylene production reduction, lower transpiration rates, and heightened efficiency for photosynthesis are some of the physiological benefits that can be provided by azoxystrobin. Benzovindiflupyr is a succinate–dehydrogenase inhibitor (SDHI) that is drawn to the mitochondrial binding site in fungal cells. This binding site is blocked by azoxystrobin + benzovindiflupyr, which kills fungi as a result of the halt of mitochondrial processes (Syngenta 2022). Pydiflumetofen + difenoconazole has components of FRAC MOA groups 7 and 3, respectively (Fungicide Resistance Action Committee 2022). Pydiflumetofen is categorized as an SDHI, which causes inhibition to the growth of fungi via the obstruction of an enzyme involved in fungal cell respiration. Difenoconazole is a demethylation inhibitor fungicide, which impedes fungal growth by repression of ergosterol biosynthesis—an essential part of the plasma membrane in some fungi. Pydiflumetofen + difenoconazole creates a layer of protection by moving into the wax layer of the leaf. Within 24 h of contact with the plant, the fungicide slowly penetrates and spreads throughout the plant tissue. Additional benefits of pydiflumetofen + difenoconazole include inhibition of initial spore germination, fungal penetration, and mycelial growth (Syngenta 2022). Using multiple MOAs defends against multiple pathogens and aids in the prevention of resistance development to fungicides. Both fungicides

Table 5. Fungicide effects on defoliation (0%–100% defoliation), the area under the defoliation progress curve (AUDFC), final black spot (*Diplocarpon rosae*) severity (0%–100% leaf affected), and the area under the disease progress curve (AUDPC) of roses (*Rosa* spp. 'Coral Drift') grown in a shade house from Jul to Sep 2023.

Treatment	Application rate	Application interval (weeks)	Final defoliation (%)[i]	AUDFC[ii]	Final severity (%)[i]	AUDPC[ii]
Pydiflumetofen + difenoconazole	1.1 mL·L^{-1}	2	0.5 b[iii]	6.7 b	17.0 bc	665.7 b
		4	2.2 b	55.3 ab	19.0 bc	779.5 b
Pydiflumetofen + difenoconazole	1.6 mL·L^{-1}	2	2.5 b	48.7 ab	17.5 bc	717.9 b
		4	0.2 b	0.7 b	16.0 c	658.7 b
Pydiflumetofen + difenoconazole	2.2 mL·L^{-1}	2	3.0 b	81.9 ab	23.0 b	926.5 b
		4	0.7 b	15.1 b	19.0 bc	741.7 b
Azoxystrobin + benzovindiflupyr	0.5 g·L^{-1}	2	0.0 b	0.0 b	17.0 bc	693.7 b
		4	0.5 b	6.7 b	15.0 c	590.5 b
Untreated control	—	—	8.5 a	202.0 a	49.0 a	2037.0 a
P value	—	—	0.000	0.004	<0.000	<0.000
F value	—	—	5.15	3.5	19.5	15.07

[i] Final defoliation and black spot severity evaluation was performed on 16 Sep.

[ii] AUDPC (or AUDFC) = $\Sigma\{[(x_i + x_i - 1)/2](t_i - t_{i-1})\}$, where x_i is the disease severity rating (or defoliation rating) at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations.

[iii] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects on the AUDFC and the AUDPC. Means were compared using Fisher's least significant difference test with $\alpha = 0.05$. Percent data (defoliation and severity) were analyzed according to a generalized linear mixed model with a logit link and beta distribution (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute, Cary, NC, USA).

used in our study can be recommended for the control of fungal diseases on ornamental crops.

Practices that reduce the amount of chemical applications should be a priority in current-day fungicide efficacy studies as a result of environmental concerns of overapplication and reductions in costs to growers. Residues resulting from overuse of fungicides can pose an environmental risk by persisting in waterways and soils (Wightwick et al. 2010). Fungicide resistance development is another risk if fungicides are not managed and rotated properly (Corkley et al. 2021). Resistance occurs from the reduced sensitivity to fungicides, which often occurs when fungicides in a group are used too often (Damicone 2017). Fungicides with multiple MOAs are more ideal than single-site MOA fungicides (van den Bosch et al. 2014). Fungicides should be rotated so that chemicals sharing the same FRAC group are not used in conjunction with one another. Reducing application rates or the number of applications per growing season can be tactics to reduce the overall amount of fungicide applied.

In our study, pydiflumetofen + difenoconazole was tested at three rates (low, medium, and high) and two intervals (2 and 4 weeks) for controlling black spot of roses. Black spot disease severity and AUDPC was controlled effectively by all application rates and intervals of pydiflumetofen + difenoconazole compared with the untreated controls. The low rate of pydiflumetofen + difenoconazole provided similar protection from black spot of roses compared with the medium and high rates. Moreover, no significant differences were noted between pydiflumetofen + difenoconazole and azoxystrobin + benzovindiflupyr application intervals in final disease severity and progress. These results show that pydiflumetofen + difenoconazole was as effective as the standard fungicide azoxystrobin + benzovindiflupyr for the control of black spot. This indicates that the low rate can be recommended to growers and landscapers at the 4-week interval tested in our study. Similarly, in a previous study (Jennings et al. 2024) testing pydiflumetofen + difenoconazole at three different application rates (low, medium, and high) and intervals (2, 4, and 6 weeks) on controlling powdery mildew of hydrangea, it was observed that pydiflumetofen + difenoconazole could be used at the 6-week interval at the lowest application rate used for fungal disease control. We did not observe significant differences in plant growth among the treatments, which is likely a result of our short experimental duration. However, plants remain in nurseries for a longer period than those studied herein, and negative impacts on the growth of a plant by disease have more time to develop. This research indicates that both pydiflumetofen + difenoconazole and azoxystrobin + benzovindiflupyr are similarly effective in controlling black spot and can be used when supplementing or developing a fungicide rotation program for black spot of roses.

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