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Phytoprotection

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Article abstract

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Evaluation of phytosanitary products for the management of raspberry late leaf rust [*Pucciniastrum americanum* (Farl.) Arthur]

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Different phytosanitary products (Actinovate SP, Cabrio EG, Double Nickel 55, Fullback 125 SC, Kumulus DF, Nova, Phostrol, Pristine WG, Serenade Opti, Sirocco, StorOx) registered in Canada for the management of various diseases of horticultural crops but not for the management of late leaf rust (LLR) of raspberry were tested against the latter. Efficacy of each product was first determined in vitro on raspberry leaf discs. Based on in vitro efficacy, tested products can be ranked (from the most to the least effective) as follows: Nova, Sirocco, Fullback 125 SC, Pristine WG, Phostrol, Kumulus DF, StorOx, Cabrio EG, Serenade Opti, and Actinovate SP/Double Nickel 55. Five products (Fullback 125 SC, Kumulus DF, Nova, Phostrol, Sirocco) were further tested in the field. Foliar applications of Fullback 125 SC and Nova significantly reduced disease severity as compared with the control. This study shows that the triazole fungicides Fullback 125 SC and Nova were effective against LLR of raspberry as determined using in vitro and field assays and proposes a fast, inexpensive, and easy in vitro method to be used to select phytosanitary products to be tested for field assays.

Keywords: phytosanitary products, late leaf rust, *Pucciniastrum americanum*, raspberry, in vitro assay, field assay.

[Évaluation de différents produits phytosanitaires pour lutter contre la rouille jaune tardive du framboisier [*Pucciniastrum americanum* (Farl.) Arthur]]

Différents produits phytosanitaires (Actinovate SP, Cabrio EG, Double Nickel 55, Fullback 125 SC, Kumulus DF, Nova, Phostrol, Pristine WG, Serenade Opti, Sirocco, StorOx) homologués au Canada pour lutter contre diverses maladies affectant les plantes horticoles, mais non homologués pour lutter contre la rouille jaune tardive (RJT) du framboisier ont été testés pour leur efficacité à réduire le développement de cette maladie. L'efficacité de chaque produit a d'abord été évaluée in vitro sur des disques foliaires de framboisier. Selon l'efficacité observée in vitro, les produits à l'étude se classent (du plus efficace au moins efficace) comme suit : Nova, Sirocco, Fullback 125 SC, Pristine WG, Phostrol, Kumulus DF, StorOx, Cabrio EG, Serenade Opti et Actinovate SP/Double Nickel 55. Cinq produits (Fullback 125 SC, Kumulus DF, Nova, Phostrol, Sirocco) ont par la suite été testés au champ. Le Fullback 125 SC et le Nova, appliqués sur le feuillage, ont réduit significativement la sévérité de la maladie comparativement au traitement témoin. Cette étude montre que le Fullback 125 SC et le Nova, fongicides de la famille des triazoles, se sont avérés efficaces contre la RJT du framboisier lors d'essais in vitro et au champ et propose une méthode in vitro, rapide, peu coûteuse et facile d'utilisation afin de sélectionner pour les essais au champ les produits phytosanitaires davantage efficaces.

Mots clés : produits phytosanitaires, rouille jaune tardive, *Pucciniastrum americanum*, framboise, essai in vitro, essai au champ.

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INTRODUCTION

Late leaf rust (LLR) of raspberry (*Rubus idaeus* L.) is caused by the fungus *Pucciniastrum americanum* (Farl.) Arthur, an obligate parasite for which white spruce [*Picea glauca* (Moench) Voss] is known as alternate host (Luffman and Buszard 1988; Nelson 2011; Nickerson 1991). Symptoms of LLR are observed on leaves (small yellow spots turning brown and yellow-orange powdery pustules on adaxial and abaxial leaf surfaces) and fruits (light yellow/orange pustules); affected fruits being inadequate for the fresh market. In severe cases, the disease causes a decrease in plant vigour, early defoliation, and increased sensitivity to winter frost (Carrier 2007; Lambert *et al.* 2013; Luffman and Buszard 1988; Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec [MAPAQ] 2020; Nickerson 1991).

In the Québec province, LLR causes important economic loss in the production of fall-fruiting raspberries, where the cultivar "Pathfinder" is widely used (Landry *et al.* 2017). The disease was also reported in the Atlantic Canadian provinces (Luffman and Buszard 1988, 1989; Nickerson 1991) and in different countries including Argentina (Lucero *et al.* 2008), Mexico (Rebollar-Alviter *et al.* 2003), and the USA (Nelson 2011; Nickerson 1991).

Management of LLR relies on chemical control and different cultural practices such as avoiding sprinkler irrigation, allowing good air circulation between plants, and avoiding planting raspberries near white spruce trees (Carrier 2007; Luffman and Buszard 1988; MAPAQ 2020; Nickerson 1991). In regard to chemical control, few active ingredients [ferbam (Ferbam 76 WDG), calcium polysulfide (Lime Sulphur), penthiopyrad (Fontelis)] are currently registered in Canada for the management of raspberry LLR; calcium polysulfide being the only one registered in organic production (Health Canada 2020; MAPAQ 2019a).

In this study, different phytosanitary products were tested against LLR using in vitro and field assays.

Table 1. Phytosanitary products tested

Product	Manufacturer	Active ingredient(s)	RIH/RIE[a]	Spray mixture recommended by the manufacturer
Actinovate SP	Monsanto Canada Inc. (Winnipeg, MB, Canada)	*Streptomyces lydicus* (strain WYEC 108)	5/1	425 g in 1100 L[b]
Cabrio EG	BASF Canada Inc. (Mississauga, ON, Canada)	Pyraclostrobin	35/75	0.56 kg - 1 kg in 1000 L[c]
Double Nickel 55	Certis USA L.L.C. (Columbia, MD, USA)	*Bacillus amyloliquefaciens* (strain D747)	5/1	NA
Ferbam 76 WDG	Loveland Products Canada Inc. (Dorchester, ON, Canada)	Ferbam	260/32	2 kg - 3.75 kg in 1000 L[d]
Fullback 125 SC	FMC Corporation (Philadelphia, PA, USA)	Flutriafol	87/123	NA
Kumulus DF	BASF Canada Inc.	Sulphur	12/5	120 g in 100 L[e]
Lime Sulphur	Loveland Products Canada Inc.	Calcium polysulfide	180/100	513 mL in 20 L, 2.6 L in 100 L or 5.1 L in 200 L[d]
Nova	Dow AgroSciences Canada Inc. (Calgary, AB, Canada)	Myclobutanil	42/57	175 g in 250 L[f]
Phostrol	Engage Agro Corporation (Guelph, ON, Canada)	Phosphites of Na, K, and NH_4^+	5/1	5.2 L in 400 L[g]
Pristine WG	BASF Canada Inc.	Boscalid and pyraclostrobin	96/136	NA
Serenade Opti	Bayer CropScience Inc. (Calgary, AB, Canada)	*Bacillus subtilis* (strain QST 713)	-/1	NA
Sirocco	AEF Global Inc. (Lévis, QC, Canada)	Potassium bicarbonate	5/1	2.8 kg - 5.6 kg in 1000 L[b]
StorOx	BioSafe Systems, LLC. (East Hartford, CT, USA)	Hydrogen peroxide	500/1	100 mL in 10 L[h]

[a] Risk index for health (RIH) and environment (RIE) (Cécyre and Lambert 2019; MAPAQ 2019a, 2019b).
[b, c, d, e, f, g, h] Spray mixture recommended for the management of powdery mildew in strawberry, anthracnose in strawberry, rusts in raspberry, powdery mildew in greenhouse cucumber, yellow rust in raspberry, *Phytophthora* root rot in raspberry, and grey mold in greenhouse tomato, respectively.
NA (Not applicable) No mixture concentrations are provided by the manufacturers for Double Nickel 55, Fullback 125 SC, Pristine WG, and Seranade Opti but rather a quantity to apply per ha: 2.5 kg, 1000 mL, 1.6 kg, and 3.3 kg, respectively.

MATERIALS AND METHODS

Phytosanitary products

Actinovate SP, Cabrio EG, Double Nickel 55, Ferbam 76 WDG, Fullback 125 SC, Kumulus DF, Lime Sulphur, Nova, Phostrol, Pristine WG, Serenade Opti, Sirocco, and StorOx were tested (Table 1).

Effect of phytosanitary products on LLR incidence (in vitro assay)

Each phytosanitary product was dissolved/suspended in sterile distilled water at the maximal concentration recommended by the manufacturer (Table 1). Double Nickel 55, Fullback 125 SC, Pristine WG, and Serenade Opti were dissolved/suspended in sterile distilled water at concentrations of 5 g L^{-1}, 2 mL L^{-1}, 3.2 g L^{-1}, and 6.6 g L^{-1}, respectively. Leaf discs (10 mm diam) were cut randomly from healthy raspberry plants (cultivar "Pathfinder"). They were soaked in sterile distilled water (control) or phytosanitary product solutions/suspensions for 30 s and subsequently placed on humidified Whatman filter papers (grade 1) in Petri dishes. Each product was applied on 50 leaf discs. After a drying period of 30 min, a suspension (10 µL) of *P. americanum* uredospores [1×10^6 uredo-spores mL^{-1}; uredospores were dislodged with an inoculating loop from naturally infected raspberry leaves collected at Fraisière Garneau (Lévis, QC, Canada) and suspended in water] was then deposited on each leaf disc. After an incubation period of seven days at room temperature, discs were rated for the presence or absence of LLR symptoms by visual inspection using a magnifying glass 30×; disease incidence (number of leaf discs showing presence of symptoms) was determined for each product. Efficacy of tested products was calculated as follows: {[Incidence (control) - Incidence (phyto-sanitary products)] / Incidence (control)} × 100. Relative efficacy as compared to Lime Sulphur and Ferbam 76 WDG (currently registered for the management of raspberry rusts) was calculated as follows: [Efficacy (phytosanitary products) / Efficacy (Lime Sulphur or Ferbam 76 WDG)] × 100. The experiment was repeated three times.

Effect of phytosanitary products on LLR severity (field assay)

Field assay was conducted in summer 2019 at Fraisière Garneau as a randomized complete block design with three replicates. Experimental plots were 4 m × 1.5 m and consisted of one row of raspberry plants ("Pathfinder") every 18 inches. Experimental plots were sprayed [400 L ha^{-1} except for Sirocco (1000 L ha^{-1})] with Kumulus DF (120 g 100 L^{-1}; 8 sprays), Fullback 125 SC (512 mL ha^{-1}; 3 sprays), Nova (175 g ha^{-1}; 3 sprays), Phostrol (5.2 L ha^{-1}; 4 sprays) or Sirocco (2.8 kg ha^{-1}; 9 sprays). Control plots were sprayed with water. Raspberry plants were sprayed with a portable sprayer from July 17 to September 22 when weather conditions (light winds, weak morning dew, and no precipitation in the coming days) allowed a good coverage of plants. The spray schedule is presented in Table 2.

Disease severity was evaluated on August 29, September 15, and September 28, on three raspberry canes randomly selected in each experimental plot, according to a visual scale of 0-5, where 0 = no symptoms, 1 = disease symptoms on ≤ 10% of foliar surface, 2 = disease symptoms on 10-30% of foliar surface, 3 = disease symptoms on 31-50% of foliar surface, 4 = disease symptoms on 51-70% of foliar surface, and 5 = disease symptoms on > 70% of foliar surface. Disease severity represents the mean of disease symptoms rated on the leaves of three canes.

Analysis of variance

Analyses of variance (ANOVAs) were performed on the data of LLR severity (field assay) using PROC MIXED in SAS system 9.4 (SAS Institute Inc., Cary, NC, USA). When significant ($P \leq 0.05$), treatment means were compared using Tukey's test. Data of LLR severity were also analysed using Kruskal-Wallis ($P \leq 0.05$), a non-parametric test, using PROC NPAR1WAY in SAS as previously reported by Delisle-Houde and Tweddell (2020).

Table 2. Phytosanitary products spray schedule (field assay)

Product	Date								
	Spray 1	Spray 2	Spray 3	Spray 4	Spray 5	Spray 6	Spray 7	Spray 8	Spray 9
Fullback 125 SC[a]	July 17	July 24	August 2	-	-	-	-	-	-
Kumulus DF[b]	July 17	July 24	August 2	August 11	August 20	August 27	September 5	September 15	-
Nova[c]	July 17	July 24	August 2	-	-	-	-	-	-
Phostrol[d]	July 17	July 24	August 2	August 11	-	-	-	-	-
Sirocco[e]	July 17	July 24	August 2	August 11	August 20	August 27	September 5	September 15	September 22

Recommendations of manufacturers:
[a] Do not apply more than 2048 mL per hectare per season; do not apply within 8 days of harvest.
[b] Use a maximum of 8 applications per growing season; do not apply within 1 day of harvest.
[c] Use a maximum of 3 applications per growing season; do not apply within 6 days of harvest.
[d] Use a maximum of 4 applications per growing season; can be applied until harvest day.
[e] Use a maximum of 10 applications per growing season; can be applied until harvest day.

Table 3. Efficacy and relative efficacy of phytosanitary products to reduce late leaf rust incidence on raspberry leaf discs

Product	Efficacy[a] (%)	Relative efficacy[b] (%)	
		Lime Sulphur	Ferbam 76 WDG
Nova [0.7 g L^{-1}][c]	95.7 ± 6	109.9	202.3
Sirocco [5.6 g L^{-1}]	78.5 ± 7	90.1	166
Fullback 125 SC [2 mL L^{-1}]	76.8 ± 10	88.2	162.4
Pristine WG [3.2 g L^{-1}]	65.6 ± 4	75.3	138.7
Phostrol [13 mL L^{-1}]	58.1 ± 16	66.7	122.8
Kumulus DF [1.2 g L^{-1}]	51.8 ± 7	59.5	109.5
StorOx [10 mL L^{-1}]	38.7 ± 3	44.4	81.8
Cabrio EG [1 g L^{-1}]	27.9 ± 16	32	59
Serenade Opti [6.6 g L^{-1}]	2.1 ± 7	2.4	4.4
Actinovate SP [0.39 g L^{-1}]	0 ± 2	0	0
Double Nickel 55 [5 g L^{-1}]	0 ± 4	0	0

[a] Efficacy was calculated as follows: {[Incidence (control) - Incidence (phytosanitary products)] / Incidence (control)} × 100. Each value represents the mean of 3 replicates ± standard deviation.
[b] Relative efficacy was calculated as follows: [Efficacy (phytosanitary products) / Efficacy (Lime Sulphur or Ferbam 76 WDG)] × 100. Each value represents the mean of 3 replicates. Efficacy of Lime Sulphur [26 mL L^{-1}] and Ferbam 76 WDG [3.75 g L^{-1}] were 87.1% and 47.3%, respectively.
[c] Concentration applied on leaf discs between brackets.

RESULTS

In vitro assay

Phostrol, Sirocco, Kumulus DF, Pristine WG, Fullback 125 SC, and Nova showed efficacy values higher than 50% (Table 3); Nova showing the highest efficacy with a value of 95.7%. Efficacy values between 25% and 40% (Cabrio EG and StorOx) or lower than 2.5% (Actinovate SP, Double Nickel 55, and Serenade Opti) were obtained with the other tested products. Sirocco, Fullback 125 SC, Pristine WG, Phostrol, and Kumulus DF were shown more effective than Ferbam 76 WDG to reduce disease incidence on leaf discs with values of relative efficacy of 166%, 162.4%, 138.7%, 122.8%, and 109.5%, respectively (Table 3). Nova was more effective than Lime Sulphur (relative efficacy of 109.9%) and Ferbam 76 WDG (relative efficacy of 202.3%) (Table 3).

Field assay

Results of the field assay showed significant effect of tested phytosanitary products on LLR severity evaluated on August 29 (Fig. 1a), September 15 (Fig. 1b), and September 28 (Fig. 1c) according to ANOVA and the Kruskal-Wallis test. Application of Nova and Fullback 125 SC significantly reduced disease severity rated on August 29, September 15, and September 28 as compared with the control.

DISCUSSION

In the Québec province, LLR is problematic for fall-fruiting raspberry producers especially when growing seasons are wet and rainy, environmental conditions that are conducive to disease development. Under these conditions, management of LLR relies more on chemical control. In the present study, eleven phytosanitary products, not currently registered in Canada for the management of raspberry LLR but registered for the management of either powdery mildew (*Podosphaera aphanis*, syns. *Sphaerotheca aphanis, Sphaerotheca macularis* f. *fragariae*) in strawberry, anthracnose (*Colletotrichum* spp.) in strawberry or raspberry, yellow rust (*Phragmidium rubi-idaei*) in raspberry, *Phytophthora* root rot in raspberry (*Phytophthora* spp.), powdery mildew (*Podosphaera fusca*, syn. *Podosphaera xanthii*) in greenhouse cucumber or grey mold (*Botrytis cinerea*) in raspberry, strawberry or greenhouse tomato, were tested for their efficacy to control LLR. Ferbam 76 WDG and Lime Sulphur, currently registered for the management of raspberry rusts, were also tested.

The efficacy of the phytosanitary products was first tested in vitro on raspberry leaf discs. Products were tested at the highest concentrations recommended by the manufacturers (Table 1). For Double Nickel 55 and Fullback 125 SC (registered against powdery mildew in strawberry) and Pristine WG and Seranade Opti (registered against grey mold in raspberry), no mixture concentrations are provided by the manufacturers, but rather a quantity to apply per ha: 2.5 kg, 1000 mL, 1.6 kg, and 3.3 kg, respectively. In these cases, a water volume of 500 L per ha, commonly recommended for fungicides application, was used to calculate the concentrations to apply on leaf discs. In terms of efficacy, tested products can be ranked (from the most to the least effective) as follows: Nova, Sirocco, Fullback 125 SC, Pristine WG, Phostrol, Kumulus DF, StorOx, Cabrio EG, Serenade Opti, and Actinovate SP/Double Nickel 55 (Table 3). Based on relative efficacy values, Sirocco, Fullback 125 SC, Pristine WG, Phostrol, and Kumulus DF were shown more effective than Ferbam 76 WDG while Nova was shown more effective than Ferbam 76 WDG and Lime Sulphur. Among these products, the five products with the lowest risk index for health and environment were selected for field assay.

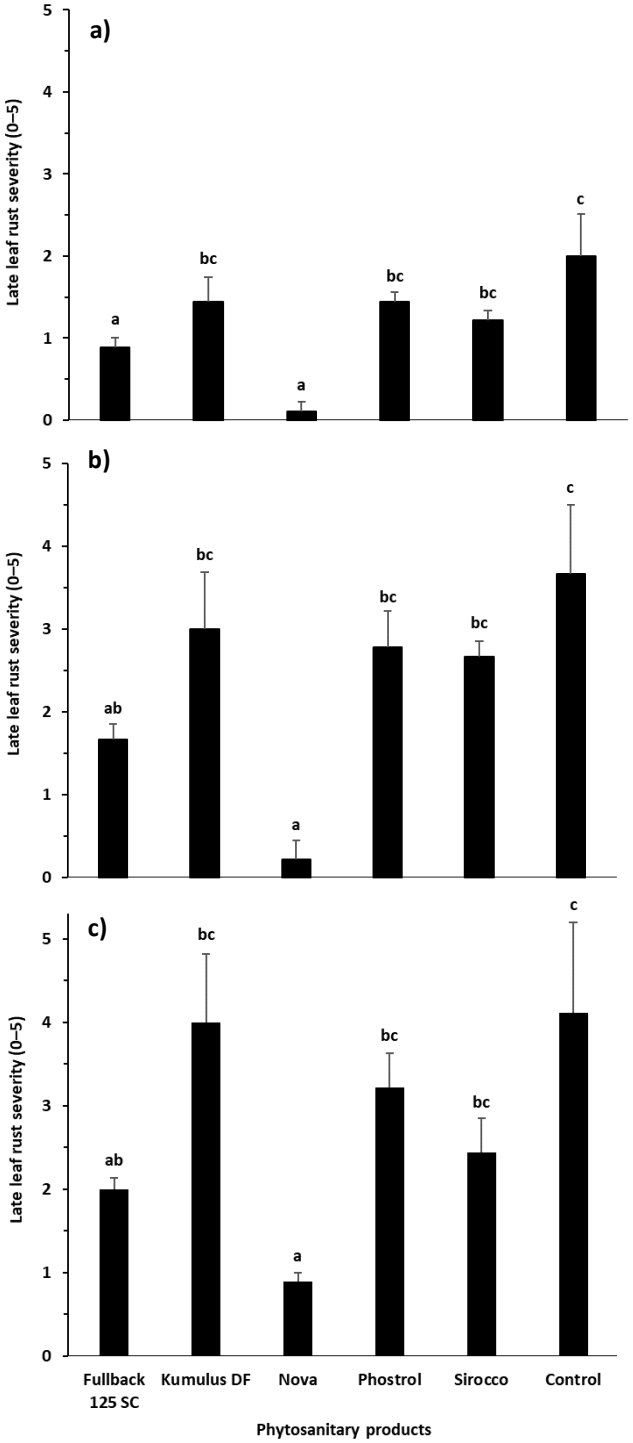

Figure 1. Effect of Fullback 125 SC (512 mL ha^{-1}), Kumulus DF (480 g ha^{-1}), Nova (175 g ha^{-1}), Phostrol (5.2 L ha^{-1}), and Sirocco (2.8 kg ha^{-1}) on raspberry late leaf rust severity. Disease severity was evaluated on August 29 (**a**), September 15 (**b**), and September 28 (**c**) 2019 according to a visual scale of 0-5. Each value represents the mean of three replicates ± standard error. Means with a similar letter are not significantly different according to Tukey's test ($P \leq 0.05$).

Sirocco, Fullback 125 SC, Phostrol, Kumulus DF, and Nova were sprayed on raspberry plants according to the recommendations of the manufacturers from July 17 to September 22 according to the spray schedule presented in Table 2. Application of Nova and, to a lesser extent, Fullback 125 SC was shown to reduce the development of LLR as evidenced by the reduced symptom severity observed on plants treated by these fungicides belonging to the triazole family. Previous studies reported the efficacy of triazole fungicides to control rusts such as faba bean rust (*Uromyces viciae-fabae*) (Emeran *et al.* 2011), wheat brown leaf rust (*Puccinia recondita*) (Tyagi *et al.* 1973), wheat yellow rust (*Puccinia striiformis*), and wheat leaf rust (*Puccinia recondita*) (Basandrai *et al.* 2013). Nova and Fullback 125 SC are currently registered in Canada against yellow rust of raspberry and powdery mildew of strawberry for an application up to 6 days and 8 days before harvest, respectively (Health Canada 2020). While Lime Sulphur and Ferbam 76 WDG can be applied during delayed dormant period and just before bloom, respectively. Registration of Nova and/or Fullback 125 SC against LLR could give additional alternatives to producers, particularly in regard to late sprays. Considering triazole fungicides are prone to resistance development (Ribas e Ribas *et al.* 2016), the eventual use of Nova or Fullback 125 SC for the management of LLR should be done according to the recommended strategies for preventing the emergence of fungicide resistance.

It is important to indicate that Fullback 125 SC and Sirocco were respectively applied on raspberry plants at a rate of 512 mL ha^{-1} and 2.8 kg ha^{-1} while manufacturers recommend an application range of 512-1024 mL ha^{-1} (Fullback 125 SC) and 2.8-5.6 kg ha^{-1} (Sirocco) for the control of strawberry powdery mildew. It would be interesting in future work to test the efficacy of these fungicides against LLR when applied at higher rates and under different levels of disease pressure, especially for Sirocco which is currently registered in organic production of strawberry with pre-harvest interval of 0 days.

Based on LLR severity rated on September 28, the ranking of the products tested in the field from the most effective (lowest LLR severity) to the least effective (highest LLR severity) is as follows: Nova, Fullback 125 SC, Sirocco, Phostrol, Kumulus DF (Fig. 1c). It is interesting to note that the in vitro assay with raspberry leaf discs allowed an interesting approximation of the ranking obtained in the field assay. In this regard, the in vitro assay conducted in the present study could be a fast, inexpensive, and easy method to be used to select fungicides to be tested against *P. americanum* for field assays.

The results reported herein show that the triazole fungicides Fullback 125 SC and Nova were effective in reducing raspberry LLR incidence or severity as determined using in vitro and field assays.

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