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Efficacy of *Reynoutria sachalinensis* for Control of Bacterial Leaf Spot and Powdery Mildew on Tomato in Florida

Jason Osborne, Timothy Johnson, David Warman, Hai Su*, Marja E. Koivunen, and Pam G. Marrone

Marrone Bio Innovations, Inc., 2121 Second Street, Suite B-107, Davis, CA 95618

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The extract of *Reynoutria sachalinensis* F. Schmidt (formulated as Regalia®), controls powdery mildew and other diseases on many crops by induction of plant systemic resistance and by inhibition of conidia germination. A field study was conducted near Hobe Sound, FL, by Glades Crop Care on tomato (*Lycopersicon esculentum* Mill.) to test the efficacy of the extract of *Reynoutria sachalinensis* against tomato bacterial leaf spot [*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye] and powdery mildew (*Oidium neolycopersici* L. Kiss sp. nov.). Formulated *Reynoutria sachalinensis* extract was tested as a stand-alone treatment as well as in a tank mix or in rotation with maneb, copper or chlorothalonil. All *Reynoutria sachalinensis* treatments resulted in a significant reduction in the severity of bacterial leaf spot, and the efficacy was not significantly different to that obtained with maneb (Maneb® 75DF) and copper (Kocide® 2000) rotated with chlorothalonil (Bravo Weather Stick®). For powdery mildew control, *Reynoutria sachalinensis* as a stand-alone treatment significantly reduced disease incidence and performed better at 1.0% than at 0.5% (v/v, formulated product) concentration. There were no significant differences between tank-mix and rotation treatments in the control of both diseases.

Bacterial leaf spot, caused by the bacterium *Xanthomonas campestris* pv. *vesicatoria*, is a common disease on tomato in Florida. Frequent epidemics of this disease cause tremendous yield loss (Pohronezny and Volin, 1983) and control is limited to bactericide applications. Currently, bactericides containing copper, mancozeb or maneb are commonly used in commercial production. However, due to the limited efficacy and choices, and frequent application, resistance to these chemicals has long been a problem (Stall and Thayer, 1962; Marco and Stall, 1983; Ritchie and Dittapongpitch, 1991).

Tomato powdery mildew (*Oidium neolycopersici*) was first detected and became epidemic in Florida in 1996 (Pernezny and Sonoda, 1998), and since then it has also posed a regular threat to tomato production in Florida. Myclobutanil and strobilurin fungicides are normally used to control powdery mildew on tomato (Mickler and Keathley, 2006). Resistance to these fungicides is commonly found in powdery mildew as well as other disease pathogens. Thus, pesticides with novel modes of action are needed to combat fungicide resistance.

Reynoutria sachalinensis was recently formulated as Regalia® by Marrone Bio Innovations, Inc., Davis, CA (Su et al., 2009). Its mode of action is based on induced systemic resistance (ISR) which involves synthesis of phytoalexins or other phenolic compounds that are toxic to pathogens. *Reynoutria sachalinensis* extract increases the activity of flavonoid biosynthesis enzymes (chalcone synthase and chalcone isomerase) which lead to the accumulation of flavonoid compounds (Fofana et al. 2005; McNally et al., 2003). Simple phenolics, such as ρ-coumaric acid, caffeic acid, ferulic acid, ρ-coumaric acid methyl ester, which are all fungitoxic, are accumulated in plants after exposure to *R. sachalinensis* extract (Daayf et al., 1997, 2000).

Other phenomena involved in plant immune system are also present after plants are treated with *R. sachalinensis*, such as increased activity of peroxidase, chitinase, and β-1,3-glucanase (Schneider and Ullrich, 1994) as well as papillae formation and lignification in cell walls (Fofana et al., 2005; Wurms et al., 1999).

Direct effect of *R. sachalinensis* on plant pathogens has also been studied. It has been shown that *R. sachalinensis* inhibits the germination of *Leveillulia taurica* (tomato powdery mildew) (Konstantinidou-Doltsinis et al., 2006) and *Blumeria graminis* f.sp. *tritici* (DC) E.O. Speer f. sp. *tritici* Em (wheat powdery mildew) (Randoux et al., 2006).

The unique mode of action of *R. sachalinensis* extract and its effect on powdery mildew of different crops makes it a great candidate in controlling tomato diseases and for managing fungicide resistance. This experiment was designed to evaluate the efficacy of formulated *R. sachalinensis* extract in controlling tomato leaf spot and powdery mildew when used alone or in combination with other currently used fungicides.

Materials and Methods

The experiment was conducted near Hobe Sound, FL from 19 Dec. 2008 to 4 Mar. 2009. The field with fine clay-loam soil and pH 7 was conventionally tilled. Tomato cv. " FL 47" was planted on 11 Nov. 2008. Plants were drip and sprinkler irrigated and managed following commercial practices.

*Corresponding author; email: hsu@marronebio.com; phone: (530) 750-2800

The following treatments were applied during the experiment:

1. Untreated control;
2. *R. sachalinensis* at 0.5% (v/v) formulated product (FP);
3. *R. sachalinensis* at 1.0% (v/v) FP;
4. *R. sachalinensis* at 1.0% FP in a tank mix with maneb and copper each at 1.5 lb/acre FP;
5. *R. sachalinensis* at 1.0% FP in a tank mix with chlorothalonil at 1.5 pt/acre FP;
6. *R. sachalinensis* at 1.0% FP in rotation with a tank mix of maneb and copper each at 1.5 lb/acre FP;
7. *R. sachalinensis* at 1.0% FP in rotation with chlorothalonil at 1.5 pt/acre FP;
8. Tank mix of maneb and copper each at 1.5 lb/acre FP in rotation with chlorothalonil at 1.5 pt/acre FP.

The treatments were arranged in a randomized complete-block design with four replications. The plot size of each treatment was 5 inches × 30 inches. The treatments were applied with a compressed CO_2 backpack sprayer in 42- to 56-gal total spray volume per acre. The first application was done on 19 Dec. 2008 and the spray interval was 6–8 d, depending on weather conditions. There were eight applications during the experiment.

Diseases developed naturally. After disease was first detected on 7 Jan. in the field, 10 plants were chosen from each plot to rate the severity of tomato leaf spot and continuously every 14 d after that. Disease severity was evaluated according to Horsfall–Barrett 1–10 scale.

Powdery mildew was also assessed at the same time with leaf spot. The number of plants infected with the disease, the number of fruit, and weight of fruit per plot were recorded in the end of the study on 4 Mar. 2009.

Data of disease severity of bacterial leaf spot, incidence of powdery mildew, number of fruit, and total fruit weight were analyzed by ANOVA with SAS program ver. 9.1 (SAS Institute, Cary, NC). Means were compared with Tukey test at $\alpha = 0.05$ level.

Result and Discussion

The results show that formulated *R. sachalinensis* extract either used alone, in rotation or in combination with chlorothalonil, copper, or maneb, provided effective control of tomato bacterial leaf spot and tomato powdery mildew (Tables 1–2).

For controlling bacterial leaf spot, both rates of formulated *R. sachalinensis* provided as good control as maneb tank-mixed with copper in rotation with chlorothalonil, which is a common control program adopted by growers. There were no differences between the treatments of formulated *R. sachalinensis* at 0.5% and 1.0%, which both were as effective as the common fungicide program. Disease severity was significantly reduced by either *R. sachalinensis* tank-mixed with maneb and copper, or in rotation with mixture of maneb and copper compared to the untreated control. This indicates that application of *R. sachalinensis* can reduce the use of copper and maneb to obtain good control and reduce the risk of fungicide resistance (Ritchie and Dittapongpitch, 1991).

For powdery mildew control, *Reynoutria sachalinensis* extract significantly reduced disease incidence and the efficacy increased with increased rate of the formulated product (from 0.5% to 1.0%). At the higher rate, efficacy of *R. sachalinensis* alone was the same as that provided by the rotation of chlorothalonil and maneb tanked-mixed with copper. Due to low disease pressure, the advantage of *R. sachalinensis* mixed or rotated with maneb and copper or chlorothalonil was not obvious. Effect of treatments on fruit counts and weight was also not significant and may have been impacted by two late season frosts that damaged uncovered portions of the trial plot. However, integration of *R. sachalinensis* with a different mode of action to the pesticide program is beneficial for managing resistance in powdery mildew pathogen.

This study demonstrates that formulated *R. sachalinensis* extract can effectively control tomato bacterial leaf spot and powdery mildew. In addition, tank mix of *R. sachalinensis* with

Table 1. Effect of formulated *Reynoutria sachalinensis* extract applied alone, tank-mixed, or rotated with chlorothalonil, copper, or maneb in controlling tomato leaf spot near Hobe Sound, FL, from 19 Dec. 2008 to 4 Mar. 2009.

	Disease severity				
Treatment	7 Jan.	21 Jan.	6 Feb.	18 Feb.	4 Mar.
Untreated control	2.3 a[z]	2.8 a	3.4 a	3.9 a	5.6 a
R. sachalinensis, 0. 5% (v/v) FP[y]	2.2 a	2.3 a	2.4 b	2.6 b	3.5 b
R. sachalinensis, 1.0% (v/v) FP	2.3 a	2.3 a	2.6 b	2.8 b	3.7 b
R. sachalinensis, 1.0% FP +[x] maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP	1.9 a	1.8 a	2.0 b	2.4 b	3.2 b
R. sachalinensis, 1.0% FP + chlorothalonil, 1.5 pt/acre FP	2.2 a	2.2 a	2.5 b	2.4 b	3.3 b
R. sachalinensis, 1.0% FP/ (maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP)	1.6 a	2.0 a	2.4 b	2.5 b	3.7 b
R. sachalinensis, 1.0% FP/ chlorothalonil, 1.5 pt/acre FP	2.1 a	2.3 a	2.4 b	2.5 b	3.5 b
(maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP)/ chlorothalonil, 1.5 pt/acre FP	2.2 a	2.1 a	1.9 b	2.3 b	3.5 b
	NS[w]	NS	$P<0.05$	$P<0.001$	$P<0.0001$

[z]Means separation (in columns) by Tukey test, 5% level.
[y]Formulated product.
[x]+ == tank mix, / = alternate with.
[w]Nonsignificant.

Table 2. Effect of formulated *Reynoutria sachalinensis* extract applied alone or in combination with chlorothalonil, copper, maneb on tomato powdery mildew incidence (%), number of fruit, and fruit weight (lb) near Hobe Sound, FL, on 4 Mar. 2009.

Treatment	Incidence	Fruit count	Fruit wt
Untreated control	3.1 a[z]	123 a	57.4 a
R. sachalinensis, 0. 5% (v/v) FP[y]	0.7 b	113 a	49.9 a
R. sachalinensis, 1.0% (v/v) FP	0.2 c	87 a	39.1 a
R. sachalinensis, 1.0% FP +[x] maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP	0.1 c	119 a	51.2 a
R. sachalinensis, 1.0% FP + chlorothalonil, 1.5 pt/acre FP	0.1 c	90 a	44.4 a
R. sachalinensis, 1.0% FP/ (maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP)	0.1 c	111 a	49.2 a
R. sachalinensis, 1.0% FP/ chlorothalonil, 1.5 pt/acre FP	0.1 c	101 a	41.4 a
(maneb, 1.5 lb/acre FP + copper, 1.5 lb/acre FP)/ chlorothalonil, 1.5 pt/acre FP	0.2 c	106 a	45.4a
	$P<0.0001$	NS[w]	NS

[z]Means separation (in columns) by Tukey test, 5% level.
[y]Formulated product.
[x]+ = tank mix, / = alternate with.
[w]Nonsignificant.

other pesticides or incorporation of *R. sachalinensis* in disease control programs will greatly reduce risk of pesticide resistance and increase marketable yield.

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