A Natural Lipid, Lysophosphatidylethanolamine (LPE), can Mitigate Adverse Effect of Fungicide, Chlorothalonil, on Fruit Set and Yield in Cranberries

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Abstract

Fruit rot is a major problem for cranberries. Several fungicide applications are necessary during the growing season to prevent significant losses, especially for cranberries cultivated in Massachusetts and New Jersey where field rot is a major concern. Cholorothalonil (Bravo) is one of the widely used fungicides to control cranberry fruit rot. However, application of cholorothalonil has been reported to cause damage on the flowers, reduction in fruit set and yield. Recently a natural lipid, lysophosphatidylethanolamine, has been shown to improve fruit ripening, enhance storage life and protect membrane degradation. We investigated the potential use of LPE to reduce undesirable effects of cholorothalonil on cranberry (*Vaccinium macrocarpon* 'Stevens') fruit. For this purpose 1 m x 2 m plots were established in cranberry beds with five replications of each treatment at four separate locations. Plots were sprayed at the rate of 6 L·ha^{-1} of Bravo and LPE (100 and 200 mg·L^{-1}) combinations at 20 and 80% bloom. Cranberry fruits and flowers on the upright shoots were counted from an area in the plot to determine the fruit set. Flooded plots were harvested with a hand rake to determine total yield and other fruit quality parameters. Bravo applications resulted in reduction in fruit set and yield, while adding LPE prevented fruit set and yield decline by Bravo. Applications of LPE alone showed 20% and about 7% greater fruit set compared with Bravo alone and the untreated control, respectively. The results of the present study show that: (1) applications of LPE can improve fruit set, and yield when applied at the time of flowering; (2) application of LPE together with Bravo can mitigate injury by Bravo.

INTRODUCTION

Cranberry fruit rot, which is caused by fungi, is considered the most yield-limiting disease problem in cranberry production, especially in northeastern United States (Eck, 1990; Caruso, 1998; Oudemans et al., 1998). Fruit-rotting fungi cause cranberries to rot, either before harvest (field rot) or after harvest (storage rot). Fruit rot is caused by as many as fifteen different fungal species (Oudemans et al., 1998). Cranberry fruit-rotting fungi appear to be indigenous to cranberry beds, overwintering each year in older woody plant tissues or as latent infections in living leaves. The most effective control measures rely on broad-spectrum, protectant fungicides such as chlorothalonil (Bravo). In cranberry beds usually three fungicide applications are necessary to reduce field rot (Oudemans et al., 1998). Fruit rot fungi appear to infect the fruit during late bloom and early fruit set (Oudemans et al., 1998). Usually, fungicide applications begin during early bloom and are repeated every ten to fifteen days. However, recent studies have shown that chlorothalonil may have phytotoxic effects on cranberry fruit. This phototoxicity can appear as flower and fruit damage, as well as reduction of fruit set and yield (Caruso, 1998; Cox, 1997; Jeffers, 1991a, and 1991b; Oudemans et al., 1998). In Wisconsin, chlorothalonil applied during bloom has been shown to reduce fruit set up to 60% depending upon the environmental conditions (Jeffers, 1991a, and 1991b).

Recent studies from our laboratory suggest a specific role of lysophosphatidyl-ethanolamine (LPE) on membrane degradation during senescence. LPE is a phospholipid,

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which is rich in egg yolk and soy lecithin (Ryu et al., 1997). LPE can retard senescence of attached and detached tomato leaves, retard tomato fruit senescence and improve storage life (Farag and Palta, 1993a and 1993b). In another study, the vase-life of LPE-treated cut flowers was prolonged to six days, compared with three days for the control (Kaur and Palta, 1997). Reduced leakage of electrolytes in LPE treated leaves, flowers and postharvest fruit suggests that LPE may protect membrane integrity. Also, LPE inhibited the activity of phospholipase D (PLD), a membrane-degrading enzyme, whose activity is increased during plant senescence (Ryu et al., 1997). Furthermore, LPE has been found to mitigate injury by ethephon, an ethylene-releasing compound, on tomato plants (Farag and Palta, 1993a). The purpose of the present study was to investigate use of LPE, a natural phospholipid, in mitigating phytotoxic effects of chlorothalonil on cranberries. Specifically, we measured fruit set, fruit rot incidence, and overall yield.

MATERIALS AND METHODS

Experimental Design and Treatments

Experimental plots were established in commercial 'Stevens' cranberry beds at Yellow River and Crawford Creek located in central Wisconsin. Experiments were conducted in two beds at each location in 2000. Plots, 2 m x 1 m, were sprayed with a volume equivalent to 1000 $L \cdot ha^{-1}$ with a hand held sprayer. Treatments included (i) untreated control; (ii) chlorothalonil (Bravo WeatherStik) at the rate of 6 $L \cdot ha^{-1}$; (iii) and (iv) LPE 100 and 200 $mg \cdot L^{-1}$ alone; (v) and (vi) LPE 100 and 200 $mg \cdot L^{-1}$ applied 3 hours prior to chlorothalonil; (vii) and (viii) LPE 100 and 200 $mg \cdot L^{-1}$ applied mixed with chlorothalonil. Temperature at the time of applications was 28 and 30 °C at Yellow River and Crawford Creek locations, respectively. LPE used in our experiments was obtained from Doosan Serdary Research Lab. (Englewood N.J.) and was purified from egg yolk. LPE was suspended in water by sonication (Sonic Dismembrator model 550; Fisher Scientific, Pittsburgh Pennsylvania.). Applications were made at 20% (22 June) and 80% bloom (6 July). Plots were harvested from flooded beds using a hand rake 2 and 6 Oct. at Yellow River and Crawford Creek locations, respectively. Experimental plots were laid out using a randomized block design with five replications at four different locations.

Data Collection (Total Yield, Fruit Set and Fruit Rot)

After fruit set 50 individual uprights were randomly selected from each plot. Fruit set was determined by dividing the number of fruits present by the number of pedicels on each upright. At harvest, sound marketable fruit from each plot was weighed to determine total yield.

Data were analyzed using a general linear mixed model (MIXED) procedure of SAS Statistical Software (SAS Institute, Inc., Cary, N.C.). Blocks and locations were modeled as random effects, and treatments were modeled as fixed effects. Blocks were nested within locations. Treatments were also compared with pair-wise comparison at $p<0.05$ and $p<0.01$ levels.

RESULTS AND DISCUSSION

Yield

There were significant differences among the treatments in terms of total yield (Table 1). LPE 100 $mg \cdot L^{-1}$ combined with Bravo significantly increased yield by 7% compared with Bravo alone (Tables 1 and 2). The difference between LPE alone and Bravo alone was about 15% (Tables 1). There were no significant differences between LPE alone when applied at 100 $mg \cdot L^{-1}$ and 200 $mg \cdot L^{-1}$. We found fruit scarring in mid-season in plots treated with Bravo alone (data not shown).

Fruit Set

Fruit set was significantly influenced by the treatments (Table 1). Bravo treatments resulted in 10% reduction of fruit set compared to control plants. However, LPE

application either prior to Bravo or mixed with Bravo applications maintained fruit set similar to control (Table 1). LPE alone (both concentrations) resulted in a 7-8% increase in fruit set compared with untreated control and a 21-23% increase in fruit set compared with the Bravo alone treatment. Fruit rot level was less than 5% in all the locations. Differences among all the treatments were not significant, suggesting that this year fruit rot was quite low.

Overall, LPE-Bravo mixtures increased fruit set significantly compared with Bravo alone. Also, LPE-Bravo treated plants maintained total yield and fruit set as compared with control. LPE alone resulted in significantly higher yield, and more fruit set compared with Bravo alone.

Result of the present study suggests that LPE has the potential to mitigate phytotoxic effects of chlorothalonil (Bravo) on cranberries. Total yield in cranberry is dependent on number of uprights, proportion of fruiting uprights, number of flowers per upright, fruit set and berry weight (Eaton and Kyte, 1978; Eaton and MacPherson, 1978). It has been reported that fungicides can reduce pollen germination and consequently reduce fruit set in cranberries (Bristow and Shawa, 1981; Shawa et al., 1966), which in turn could lead to yield reduction.

Mode of action for chlorothalonil involves its combination with glutathione inside fungus cells (Tillman et al., 1973). As these glutathione-chlorothalonil derivatives form, the cells' available glutathione becomes bound, leaving glutathione-dependent enzymes unable to function. Several enzymes that are important in cellular respiration are glutathione dependent. Inhibition of respiration can lead to chlorothalonil's toxic effects (Tillman et al., 1973).

We do not know the exact mechanism by which LPE mitigates phytotoxic effects of Bravo. However, we have demonstrated that LPE can mitigate ethylene-promoted senescence in potato leaves (Park and Palta, 2001) and mitigate damage by ethephon, an ethylene releasing compound, on tomato leaves (Farag and Palta, 1993b). Furthermore, reduced leakage of electrolytes was found in LPE-treated leaves (Farag and Palta, 1993b); flowers (Kaur and Palta, 1997) and fruits (Farag and Palta 1993a) suggesting that LPE can protect membrane integrity. LPE has been found to be involved in the regulation of membrane lipid degradation by inhibiting Phospholipase D (PLD) activity during senescence (Ryu et al., 1997). Taken together these studies suggest that LPE may be mitigating Bravo injury to cranberry flowers by protecting cell membranes from phytotoxic injury. Results of the present study suggest that LPE can be applied as a mixture with Bravo to mitigate injury by this fungicide to plants.

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Tables

Table 1. Means of treatments from all the locations combined on yield and fruit set of 'Stevens' cultivar cranberry fruit in 2000 season. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS Statistical Software. Blocks and locations were modeled as random effect and treatments were modeled as fixed effects. Blocks were nested within locations. Mean of each treatment over all locations and blocks. Overall analysis showed that treatment effect on yield ($p = 0.0014$) and fruit set ($p < 0.0001$) were highly significant.

Treatments[1]	Yield ($g \cdot m^{-2}$)	Fruit set (%)
C	1401.9	32.90
B	1328.0	28.84
L100	1478.7	34.93
L200	1467.0	35.50
BL100	1350.0	32.23
BL200	1344.4	33.30
MIX100	1425.0	32.06
MIX200	1359.0	32.65
Std Error	129.6	0.60

[1]C = control;
B = Bravo;
L100 = LPE (100 $mg \cdot L^{-1}$);
L200 = LPE (200 $mg \cdot L^{-1}$);
BL100 = LPE (100 $mg \cdot L^{-1}$) applied three hours prior to Bravo;
BL200 = LPE (200 $mg \cdot L^{-1}$) applied three hours prior to Bravo;
MIX100 = LPE (100 $mg \cdot L^{-1}$) applied mixed with Bravo;
MIX200 = LPE (200 $mg \cdot L^{-1}$) applied mixed with Bravo.

Table 2. Pair wise comparison of treatments using yield, and fruit set of 'Stevens' cultivar cranberry fruit in 2000 season at two different locations. P-values shaded are statistically significant. Pair-wise comparisons of the treatments from all locations combined. Experiment plots were laid out using a randomized block design with five replications in four different locations. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS Statistical Software. Blocks and locations were modeled as random effect and treatments were modeled as fixed effect. Blocks were nested within locations.

YIELD

Treatments[1]	C	B	L100	L200	BL100	BL200	MIX100
B	.0897						
L100	.0780	.0007**[2]					
L200	.1347	.0016*	.7866				
BL100	.2306	.6142	.0034**	.0076**			
BL200	.1861	.7042	.0023**	.0053**	.9009		
MIX100	.5930	.0264*	.2170	.3341	.0842	.0644	
MIX200	.3130	.4871	.0061**	.0130*	.8484	.7522	.1239

FRUIT SET

Treatments	C	B	L100	L200	BL100	BL200	MIX100
B	.0001**						
L100	.0002**	.0001**					
L200	.0001**	.0001**	.2783				
BL100	.2011	.0001**	.0001**	.0001**			
BL200	.4389	.0001**	.0022**	.0001**	.0412*		
MIX100	.1097	.0001**	.0001**	.0001**	.7451	.0184*	
MIX200	.6324	.0001**	.0001**	.0001**	.4221	.2114	.2601

[1]C = Control;
B = Bravo;
L100 = LPE (100 mg·L^{-1});
L200 = LPE (200 mg·L^{-1});
BL100 = LPE (100 mg·L^{-1}) applied three hours prior to Bravo;
BL200 = LPE (200 mg·L^{-1}) applied three hours prior to Bravo;
MIX100 = LPE (100 mg·L^{-1}) applied mixed with Bravo;
MIX200 = LPE (200 mg·L^{-1}) applied mixed with Bravo;

[2]*, ** Significant at $P < 0.05$ or 0.01, respectively.