Ohio Vegetable & Small Fruit Research & Development Program

Project Title: Controlling Angular Leaf Spot on pumpkin using seed treatments and foliar applications

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Objective 1: To determine the efficacy of foliar treatments on pumpkin seedlings to reduce the incidence of Angular Leaf Spot on foliage and fruit.

This experiment was designed to compare five compounds to control angular leaf spot in an inoculated field trial using two pumpkin hybrids, susceptible cv. Charmed and a moderately tolerant cv. Solid Gold. All bactericides in this experiment were applied at or near high label field rates. Pumpkins were direct seeded 6 Jun in rolled winter rye in 25 ft long twin row plots at 12 in. spacing at the Ohio Agriculture Research and Development Center research station in South Charleston, OH. The experiment was arranged in a randomized complete block design with four replications per treatment. Prior to seeding, plots were broadcast fertilized with 50 lb/A N, 100 lb/A P_2O_5 and 100 lb/A K_20, then side dressed with 40 lb/A N with 28% liquid N at the 2nd leaf stage on 16 June. Powdery mildew cover sprays were applied to the entire field alternating Quintec 6 oz (quinoxyfen) with Procure 8 oz (triflumizole) and Bravo Weather Stik 2 pt (chlorothalonil) on 2, 11, 22 Aug and 1 Sep. Plots were inoculated on the evening of 29 Jun and 18 Jul with a solution containing ca. $5x10^7$ CFU *Pseudomonas syringae* pv. *lachrymans* in water using a boom sprayer at 35 psi and 30 gal/A at the 5th leaf and pre-vining stages respectively. The first treatment was applied 27 Jun prior to the first inoculation, subsequent treatments were applied 5, 20, 28 Jul; 4, 12, 19, 25 Aug and 2 Sep. Two observers rated five random pumpkin leaves per plot for percent bacterial infections on 6 and 25 Jul and 2 and 17 Aug. On 9 and 12 Sep, all fruit except rotten, cull fruit from each 25 ft long twin row plot were harvested, weighed and graded only for presence or absence of bacterial symptoms. On 19 and 20 Sep, 10 fruit per replicate were randomly chosen and regraded for percent severity of bacterial infections on the rind. Infection of leaves by *P. syringae* pv. *lachrymans* was initially confirmed in July, but only *Xanthomonas* sp. was recovered from lesions on fruit showing symptoms of bacterial disease collected from inoculated plots on 19 Sep. We speculate the hotter temperatures of the summer created environmental conditions not suitable to the *Pseudomonas* bacteria infecting the leaves but favored a local *Xanthomonas* population, hence the lesions on the fruit were not derived from the artificially inoculated leaves. Analysis of bacterial presence on fruit per plot and bacterial fruit infection severity was conducted using SAS 9.1 Proc Mixed routine followed by means separation. All data sets were arcsine transformed for the analysis; original data are shown in table.

Results Objective 1:
Fruit disease presence was significantly reduced in cvs. Charmed and Solid Gold in plots treated with Actigard 50WG at 1 oz/A compared to the non-treated control plots (Table 1). Bacterial disease presence was also lower on Solid Gold fruit in plots treated with Kocide 3000 DF 1 lb/A than in the Solid Gold non-treated control plots, but suppression was significantly less than that provided by the Actigard 50WG 1 0z/A treatment. None of the other treatments significantly suppressed presence of bacterial fruit infection. The incidence of *Xanthomonas* lesions was lower on Charmed fruit from plots treated with Actigard 50WG 1 0z/a and Kasumin 2L 1 qt/A + Activator 90 0.25% than on non-treated control fruit of the same variety. All of the treatments suppressed *Xanthomonas* lesion development on Solid Gold fruit compared to non-treated control fruit. The Actigard 50WG 1 oz/A treatment was more effective in suppressing *Xanthomonas* lesions in Solid Gold fruit than treatment with Kocide 3000 DF 1 lb/A, Serenade Max 3 lb/A or Oxidate 1:100.

Objective 2: Assess the effect of physical and chemical seed treatments on seed germination and seedling vigor.

The effect of seed treatments on germination and seedling vigor was evaluated on non-infested pumpkin seeds (cv. Charmed). Fifty seeds per treatment per replication were treated with 1) chlorine dioxide (3 mg/L) for 1 min; 2) KleenGrow (0.6%) for 15 min; 3) sodium hypochlorite (1:5 (v:v)) for 15 min; 4) Virkon (2.0%) for 15 min; 5) hydrochloric acid (0.6 M) for 15 min, 6) hot water (50˚C) for 20 min, or 7) dry heat (75˚C) for 24 hour. A non-treated control was also included and there were four replications per treatment. The seeds were air dried for 24 hrs before being seeded in 50-cell flats containing Fafard seedling mix. The number of germinated seeds per treatment and replication was counted at 7 and 14 days post-seeding. Seedling height and fresh weight were measured 14 days post-seeding. Data were tested for equal variance and analyzed using ANOVA in Minitab. Means were separated using Fisher's least significance difference test.

Results Objective 2: Amongst all the treatments there were no significant differences observed in the percentage of germinated seed 7 and 14 days post-seeding (Table 2). However, significant differences were observed in seedling vigor (Table 2). Amongst all the treatments, seedling height (cm) was lowest in seedlings produced from non-treated and hydrochloric acid-treated seed. The fresh weight (g) of seedlings produced from sodium hypochlorite treated-seed was significantly lower than the fresh weight of seedlings produced from non-treated-, KleenGrow-, Virkon-, hydrochloric acid- or hot water-treated seed.

Objective 3: Assess the efficacy of physical and chemical seed treatments in eliminating *Pseudomonas syringae* pv. *lachrymans*

Pathogen-free pumpkin seeds (cv. Charmed) were inoculated with a mixture of three *Pseudomonas syringae* pv. *lachrymans* (*Psl*) strains. Fifty seeds per treatment per replication (four replications) were treated with the same materials and using the same rates as described in objective 2. Non-treated, *Psl*-infested seed and non-infested, non-treated seed served as infested and healthy controls respectively. After treatment

application the seeds were air dried for 24 hr and seeded as described in objective 2. After the first true leaves were observed the seedlings were misted for 30 sec/12 min for the remainder of the experiment. The number of germinated seeds was recorded at 7 and 14 days post-seeding. The number of seedlings with ALS symptoms, seedling height and fresh weight were counted 21 days post-seedling. To confirm the presence of *Psl* on pumpkin leaves, five symptomatic and/or asymptomatic leaves were collected from each treatment and replication, surface sterilized using 70% ethanol and ground in 5 ml potassium phosphate buffer (KPB, pH 7.4, 10 mM). Extracts were 10-fold serial diluted and 100 µL of each diluent was plated on Pseudomonas F (PF) medium. The plates were incubated at room temperature and checked daily for the presence of fluorescent, cream colored colonies. Fluorescent colonies were purified on PF and identified using direct colony pick-PCR with *Psl* specific primers (Miller, unpublished). Following incubation at -20 ºC the non-diluted leaf extraction buffer from each diluent was also tested by PCR. Data were tested for equal variance and analyzed using ANOVA in Minitab. Means were separated using Fisher's least significance difference test.

Results Objective 3: After 14 days, the percentage of germinated sodium hypochlorite-, hydrochloric acid- and hot water-treated seed was significantly higher than the percentage of germinated non-treated-infested seed (Table 3). Percent-germinated seed and seedling height was highest when the seed received no treatment (non-treated and non-infested). The fresh weight (g) of seedlings produced from non-treated-infested, chlorine dioxide, hydrochloric acid, and sodium hypochlorite-treated seed was significantly lower than those produced from non-treated, non-infested seed. Overall, disease incidence was low and compared to the non-treated, non-infested control none of the treatments significantly reduced disease incidence. However, except for seedlings grown from chlorine dioxide-treated seed, disease incidence was significantly lower in seedlings produced from treated seed compared to the non-treated-infested control seedlings. Although *Psl* was only isolated from seedlings grown from non-treated and -infested-, chlorine dioxide-, Virkon- and KleenGrow- treated seed (data not shown) it was detected by PCR in leaf extracts from all treatments. *Psl* was not detected in seedlings grown from non-treated and non-infested seed.

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Table 1. Efficacy of bactericides to reduce bacterial leaf spot on pumpkin fruit.

Product and rate/A[X]	Bacterial presence/plot (%)		Bacterial infection/fruit (%)	
	cv. Charmed	cv. Solid Gold	cv. Charmed	cv. Solid Gold
Actigard 50WG 1 oz (1-9)	45.2 b [Z, Y]	18.6 c [Z, Y]	5.8 c [Z, Y]	1.9 c [Z, Y]
Kasumin 2L[W] 1 qt + Activator 90 0.25 % v/v (1-9)	72.0 a	64.8 ab	14.2 bc	4.7 bc
Kocide 3000 DF 1 lb (1-9)	78.0 a	56.5 b	18.9 ab	9.3 b
Serenade Max 3 lb (1-9)	82.7 a	60.7 ab	29.1 ab	7.6 b
OxiDate 1:100 (1-9)	88.0 a	66.5 ab	16.8 abc	8.7 b
Non-treated control (1-9)	83.9 a	71.8 a	29.7 a	16.3 a

[Z] Column numbers followed by the same letter are not significantly different at P≤ 0.05 as determined by SAS Proc Mixed routine.

[Y] Data arcsine transformed for analysis, actual data shown.

[X] Application dates: 1=27 Jun, 2=5 Jul, 3=20 Jul, 4=28 Jul, 5=4 Aug, 6=12 Aug, 7=19 Aug, 8=25 Aug, 9=2 Sep.

[W] Product not labeled for use on pumpkin in OH.

Table 2. Effect of non-infested seed treatments on germination and seedling vigor

Treatment	Application Rate	Germination Rate (%) Day 7	Germination Rate (%) Day 14	Height (cm)	Fresh Weight (g)
KleenGrow™	0.6 %	81.5 a*	84.0 a	6.9 a	4.6 ab
Sodium hypochlorite	1:5 v:v	73.5 a	77.0 a	6.4 bc	4.2 c
Virkon	2 %	78.0 a	81.5 a	6.8 a	4.8 a
Hydrochloric acid	0.6 M	65.5 a	67.0 a	5.8 d	4.6 ab
Hot water	50˚C	74.0 a	80.0 a	6.7 ab	4.6 ab
Dry heat	75˚C	54.5 a	60.0 a	6.2 cd	4.3 bc
Chlorine dioxide	3 mg/L	65.5 a	69.0 a	6.4 bc	4.3 bc
Non-treated	-	58.0 a	64.5 a	5.9 d	4.6 ab
p value		0.535	0.583	≤ 0.001	0.001

* Means followed by the same letter within a column are not significantly different at P≤0.05.

Table 3. Effect of seed treatments on germination, seedling vigor and angular leaf spot disease incidence.

Treatment	Application Rate	Germination Rate (%) Day 7	Germination Rate (%) Day 14	Height (cm)	Fresh Weight (g)	Incidence of symptomatic seedlings (%)	PCR test
KleenGrow™	0.6 %	28.5 cde*	42.0 bc	12.5 c	4.37 abc	4.8 b	+ (4/4)**
Sodium hypochlorite	1:5 v:v	37.0 bcd	57.0 b	13.5 bc	3.97 c	4.4 b	+ (4/4)
Virkon	2 %	37.5 bcd	47.0 bc	15.4 b	4.63 ab	1.9 b	+ (4/4)
Hydrochloric acid	0.6 M	40.0 bc	57.0 b	14.3 bc	4.08 bc	2.2 b	+ (4/4)
Hot water	50˚C	41.0 b	53.5 b	14.9 bc	4.32 abc	3.7 b	+ (4/4)
Dry heat	75˚C	23.5 d	33.5 d	13.8 bc	4.35 abc	1.3 b	+ (4/4)
Chlorine dioxide	3 mg/L	26.0 de	34.0 d	13.4 bc	3.82 c	11.2 a	+ (4/4)
Non-treated, infested	-	27.0 de	36.5 cd	13.8 bc	3.97 c	13.9 a	+ (4/4)
Non-treated, non-infested	-	88.0 a	92.0 a	19.6 a	4.78 a	0 b	- (0/4)
p value		<0.0001	<0.0001	0.038	0.048	<0.001	

* Means followed by the same letter within a column are not significantly different at P≤0.05.

** PCR assay results using leaf extraction buffer as template. The symbol "+"represented for positive result and "-"for negative result. The fraction = number of positive replications/total number of replications.