

## FINAL PROJECT REPORT

**Project Title:** Systemic acquired resistance to bacterial canker of sweet cherry

**PI:** Ken Johnson

**Organization:** Oregon State University

**Telephone/email:** 541-737-5249 johnsonk@science.oregonstate.edu

**Address:** Dept. Botany and Plant Pathology

**Address 2:** 2082 Cordley Hall

**City:** Corvallis

**State/Zip:** OR 97331-2902

**Cooperators:** Bob Spotts, MAREC, Oregon State University, Hood River

**Other funding sources:** None

**Total Project Funding:** \$39,200

### Budget History:

Item	Year 1 of 2	Year 2 of 2	(type additional year if relevant)
<b>Salaries</b> FRA 3mo	10,000	10,300	
<b>Benefits</b> OPE 63%	6,300	6,489	
<b>Wages</b>			
<b>Benefits</b>			
<b>Equipment</b>			
<b>Supplies</b>	2,000	2,111	
<b>Travel</b> local	500	500	
<b>Miscellaneous</b> plot fee	500	500	
<b>Total</b>	19,300	19,900	

**Footnotes:** Annually: FRA 3 mo plus fringe, 2K M&S, 1K plot fee, 3% inflation

## **OBJECTIVES**

- 1) Evaluate use of root drenches and trunk paints of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), for protection of young cherry trees from bacterial canker.**
- 2) In conjunction with SAR treatment, evaluate treatments for protection of heading cuts on young sweet cherry trees from bacterial canker.**

### **MODIFIED OBJECTIVE 2:**

- 2) Examine the relationship between length of the orphan stub\* and initiation of bacterial canker in the trunk tissue below the heading cut.**

\* The 'orphan stub' is the unsupported trunk tissue below the heading cut but above the topmost lateral (unsupported because there is no potential for further vascular flow through it).

## **SIGNIFICANT FINDINGS**

- Based on June 2011 observations of 2010 plantings, a second heading cut made in May on April-planted cherry trees reduced the amount of dead, shrunken trunk tissue (canker) that developed below the heading cut. Larger cankers developed on those trees headed only once compared to those headed a second time. Moreover, canker lengths were positively correlated with lengths of orphan stubs.
- Spring trunk paint and root dip treatments with the SAR inducer, acibenzolar-*S* methyl (ASM), onto trees plants in 2010 did not suppress bacterial canker. In fact, trunk paint treatments were phytotoxic to cherry and exacerbated canker symptoms. Additional fall treatments of ASM to 2010 trees did not provide a response a response in 2011.
- In a 2011 planting, long orphan stubs increased the incidence of gummosis associated with the heading cut by three fold compared to short orphan stubs. Heading the trees a second time, however, did not reduce gummosis or initial canker development.
- Drench and spray treatments of ASM were applied to the 2011 planting in September and October. These trees will be evaluated in summer 2012.

## **METHODS**

**2010.** Parallel 200-tree plantings of sweet cherry cv. 'Bing' on 'Mazzard' rootstock were established in Hood River and Corvallis. Trees with 3/4" trunk diameters were planted in Corvallis; trees with 5/8" trunk diameters were planted in Hood River.

The experimental design is shown in Table 2 on the next page. The timetable of plot treatment activity was as follows:

**Table 1. 2010 plantings.**

<b>Experimental Activity</b>	<b>Corvallis</b>	<b>Hood River</b>
Planting & ASM root dip	7 Apr	13 Apr
ASM trunk paint	15 Apr	26 Apr
First heading & pathogen inoculation	19 Apr	26 Apr
Fertilize (50 g (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> per tree)	19 Apr	11 May
Second heading & wound sealant	28 May	11 May
Drench ASM	12 Aug & 14 Sept	13 Sept
Spray ASM	13 Oct	
Fall pathogen inoculation	29 Oct	
Tree Measurements	5 Sept 27 June 2011	14 Sept 27 June 2011

**Table 2. 2010 treatments in randomized completed block with 4 replications.**

<b>Primary Spring Treatment</b>	<b>Heading cut treatment applied to each primary treatment</b>
1) ASM Hi Root Dip 10 g/L in 1% Pentrabark	<b>a)</b> Heading near planting - inoculated cut with <i>Pseudomonas syringae</i> (sprayed 10 <sup>6</sup> cfu/ml immediately after heading)  <b>b)</b> Second heading 10-12 cm below the first - cut treated with a mix of kasugamycin (100 ppm) and oxytetracycline (200 ppm), then sealed with TreeKote® tree wound dressing. This cut was made at the beginning of a warm, dry period of weather (3 days at Corvallis, 4 days at Hood River)
2) ASM Lo Root Dip 5 g/L in 1% Pentrabark	
3) ASM Hi Trunk Paint 30 g/L in 2% Pentrabark	
4) ASM Lo Trunk Paint 15 g/L in 2% Pentrabark	
5) Untreated control	

**Table 3. Additional late summer/fall ASM treatments**

<b>Primary Spring Treatment</b>	<b>Additional late summer/fall ASM treatments</b>
1) ASM Hi Root Spray 10 g/L in 1% Pentrabark	Per tree: 300 mg ASM in 250 ml crown drench; 13 Sept Hood River, 14 Sept Corvallis
2) ASM Lo Root Spray 5 g/L in 1% Pentrabark	Per tree: 200 mg ASM in 250 ml crown drench; 13 Sept Hood River, 14 Sept Corvallis. Additionally, in Corvallis only, trees were sprayed with ASM (1 g/L) in 0.5% PentraBark on 13 Oct. <sup>#</sup>
4) ASM Lo Trunk Paint 15 g/L in 2% Pentrabark	Per tree: In Corvallis only, 200 mg ASM in 250 ml drench on both Aug. 12 and Sept 14.

<sup>#</sup> This treatment is based similar spray we applied to lilac in fall 2009 that significantly reduced *Pseudomonas blight* in spring of 2010.

2011. A third 240-tree planting of sweet cherry cv. ‘Bing’ (3/4” caliper) on ‘Mazzard’ rootstock was established at Corvallis. The timetable of plot treatment activity design were as follows:

**Table 4. 2011 planting.**

<b>Experimental Activity</b>	<b>Corvallis</b>
Planting	28 Apr
First heading& pathogen inoculation	7 May
Second heading (70% EtOH on cuts)	19 May
Fertilize (50 g (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> per tree)	6 June
Drench ASM	19 September
Spray ASM	19 Oct
Fall pathogen inoculation	21 Oct
Tree Measurements	15 Sept

**Table 5. 2011 treatments in randomized completed block with 10 replications**

<b>Primary Spring Treatment Secondary fall treatment</b>	<b>Heading cut treatment applied to each primary treatment</b>
1) Long orphan stub Half the trees received ASM as drench (Actigard 1 g/tree) in September followed by ASM spray (Actigard 0.3 g /L to runoff) in October	<b>a)</b> Headed near planting - inoculated cut with <i>Pseudomonas syringae</i> (sprayed 10 <sup>6</sup> cfu/ml immediately after heading)  <b>b)</b> Second heading 10-12 cm below the first - cut treated with 70% ethanol. This cut was made at the beginning of a warm, dry period of weather (6 days in length).
2) Short orphan stub Half the trees received ASM as above.	

## **RESULTS -- 2010 plantings.**

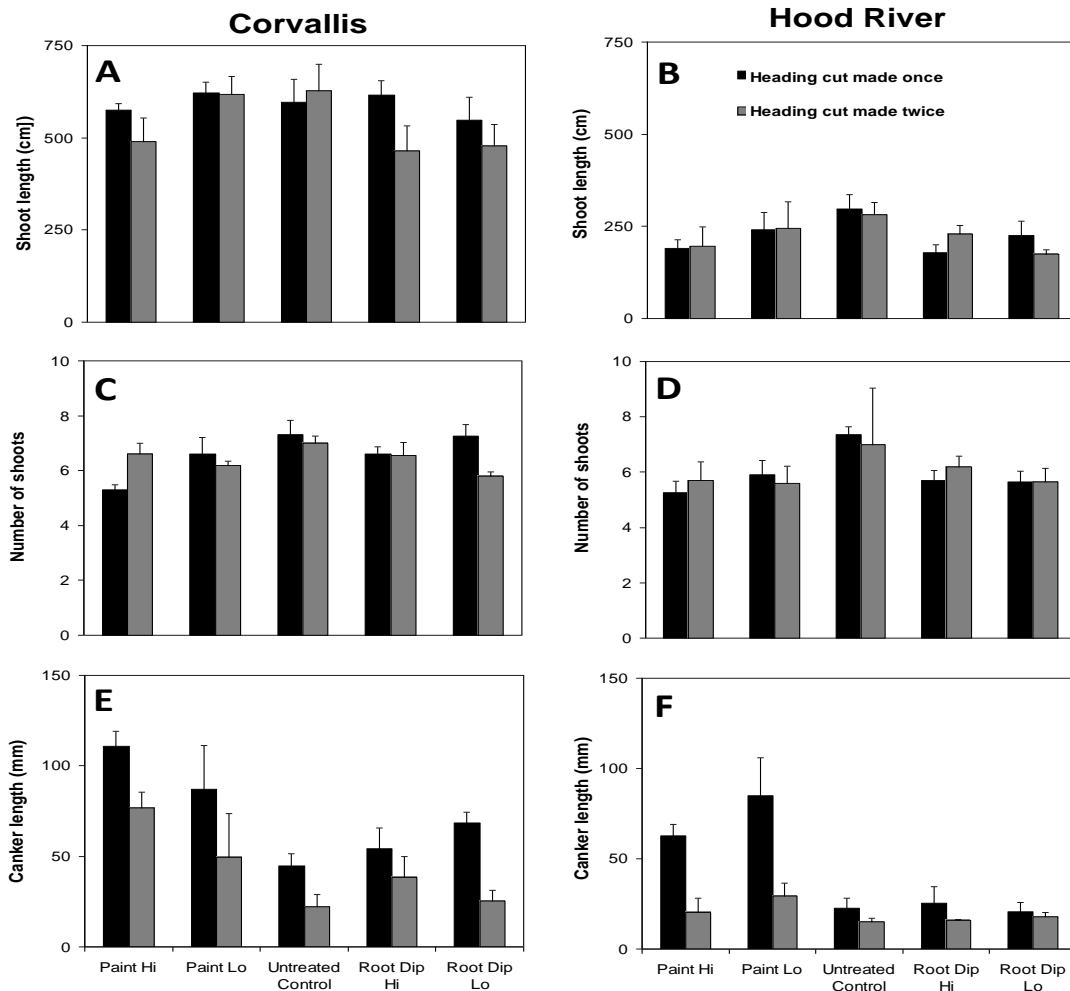
**Tree establishment and growth.** All trees received from the nursery established and developed healthy shoots. Although the trees at Hood River were smaller caliper than at Corvallis, initial budbreak and shoot growth was faster at the Hood River site. However, as the seasons progressed, total new shoot growth at Corvallis (6 meters per tree) was superior to Hood River (2.5 meters per tree) (Fig 1. A, B, C, D). Initial tree caliper was probably a reason for this difference, but at Hood River, moderate deer damage in outer rows of the plot area and planting the trees into soil that had hosted cherry previously also may have contributed to poorer growth at this site.

Overall, applying a second heading cut to the trees had no significant effect on number of shoots per tree and total shoot length (Fig 1. A, B, C, D).

**Effect of spring ASM treatments.** Spring-applied ASM treatments were not beneficial to tree growth (Fig 1. A, B, C, D), nor did they suppress the initial development of bacterial canker (Fig 1. E, F). In fact, at both Corvallis and Hood River, painting the trunk with ASM in 2% PentraBark was apparently phytotoxic, resulting in larger cankers associated with the heading cut compared to trees that received the other treatments (Fig 1. E, F). At Hood River, the largest cankers developed on the trees painted with ASM and headed only once (Fig. 1F). At both sites, the distribution of lateral shoots on ASM-painted trees were generally skewed lower on the trunk compared to both untreated and root dip treated trees (data not shown).

The ASM root dip treatments reduced total shoot length and number of shoots per tree by 5-15%, compared to the untreated control (Fig 1. A, B, C, D) but the trees showed no apparent symptoms of phytotoxicity. At Hood River, canker development on ASM root-dipped trees was similar to that observed on the untreated controls (Fig 1. E, F), but at Corvallis, root-dipped trees headed only once developed larger cankers relative to the untreated controls.

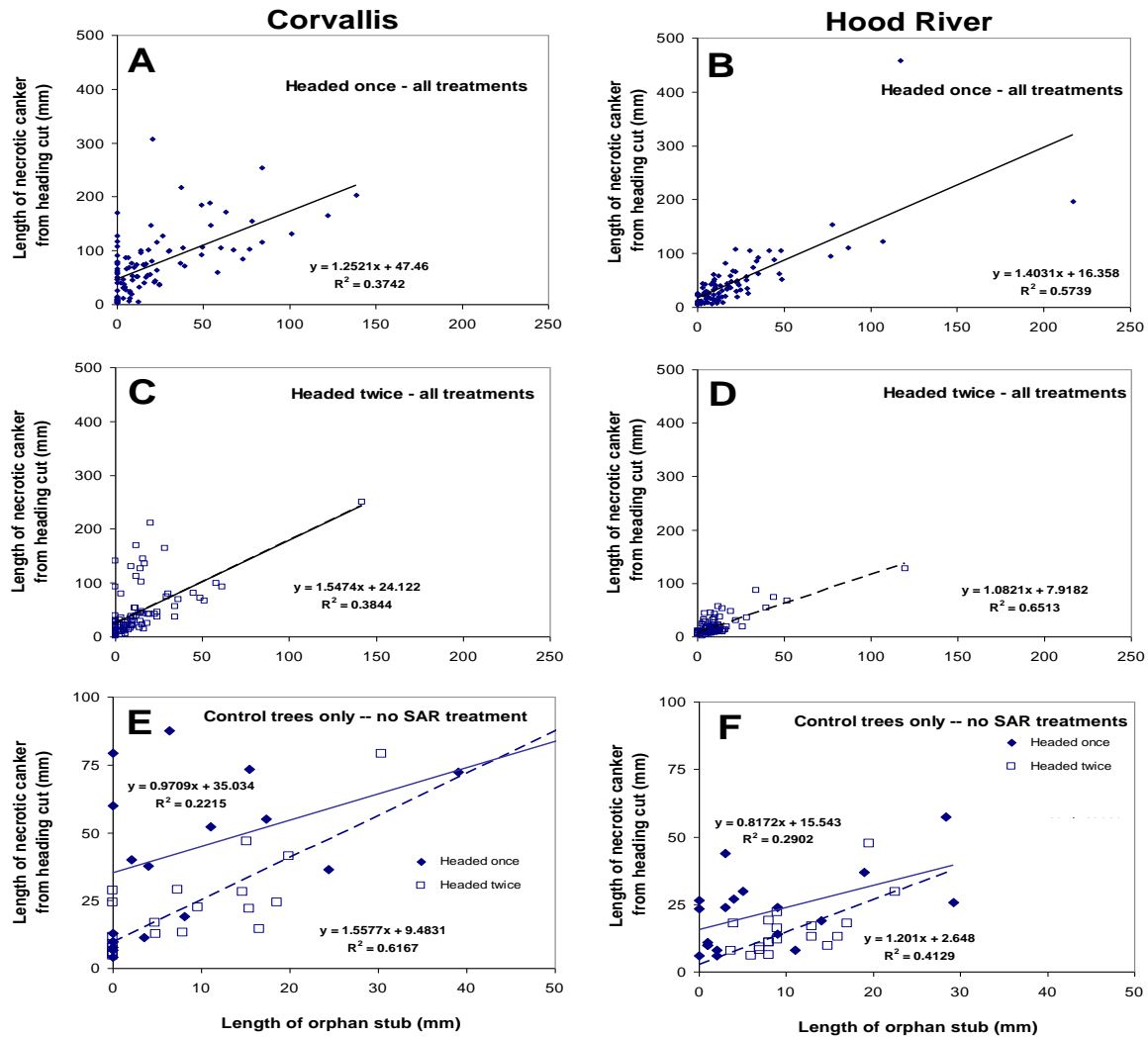
**Effect of the second heading cut on canker development.** In general, application of the second heading cut reduced the amount of dead, shrunken trunk tissue that developed immediately adjacent to the cut (what we are calling the ‘initial canker’). The treatment effect was most dramatic for the trees the received the ASM-paint treatment at Hood River, but consistent reductions in the initial canker size also were observed for double-headed trees of the other treatments (Fig 1. E, F).



**Fig. 1. Effects of spring- and fall applied root dips, paints, drenches and sprays of the systemic acquired resistance inducer (SAR), acibenzolar-S methyl (ASM), on total shoot length, number of shoots, and length of the dead, shrunken trunk tissue (canker) that developed immediately below the heading cut on trees of sweet cherry cv. ‘Bing’ (Mazzard rootstock) planted at Corvallis and Hood River, OR. After planting in April 2010, trees were headed once near the day of planting (black bar), or headed a second time two (Hood River) to five (Corvallis) weeks after the first heading (hatched bar). The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated with antibiotics and sealed with TreeKote® tree wound dressing. Measurements were made mid- to late June 2011. Lines at tops of bars are  $\pm$  one standard error of the mean; experimental design was RCB with 5-tree plots replicated four times.**

**Effect of the orphan stub length on canker development.** The amount of dead, shrunken trunk tissue that developed immediately adjacent to the heading cut (the ‘initial canker’) was positively correlated with the length of the orphan stub (Fig. 2 A-F). Longer stubs occurred on trees that received ASM, which resulted in longer lengths of necrotic cankers. In Fig. 2 A, B, C, D, nearly all of the points above the regression lines represent trees that received a paint treatment of ASM.

For control trees that did not receive an ASM treatment (Fig. 2 E, F), trees headed a second time had smaller cankers than those head once. Furthermore, trees headed a second time with a short orphan stub generally developed a small cankers; this relationship was less consistent for trees headed only once.

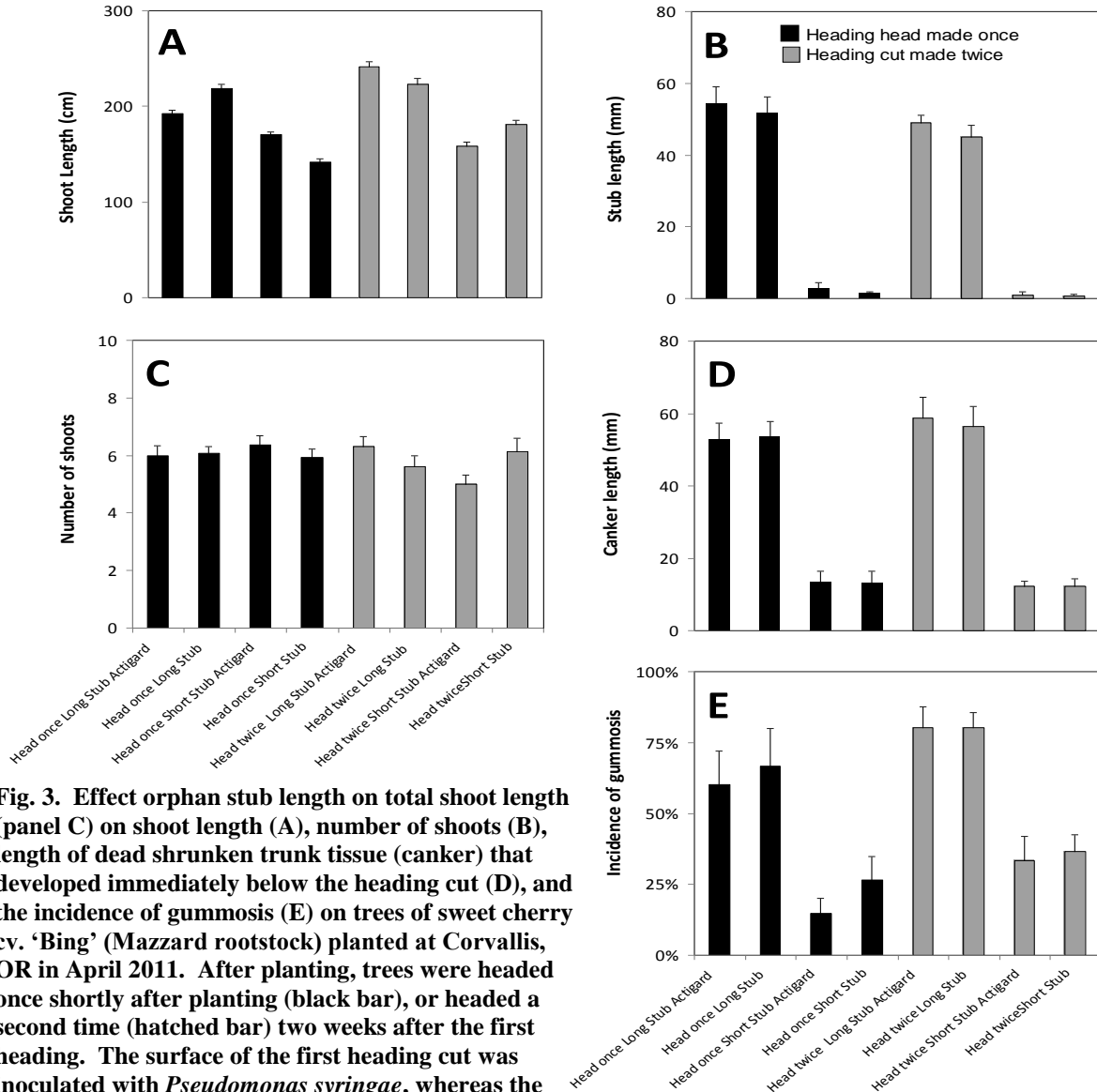


**Fig. 2.** Regression of ‘length of necrotic canker from heading cut’ on ‘length of the orphan stub’. Panels A-D includes all trees in the experiment including those that received spring and fall treatments of the systemic acquired resistance inducer, acibenzolar-*S* methyl (ASM); panels E and F are trees in the plots of the untreated control. After planting in April 2010, trees were either headed once near the day of planting (solid diamond in panels A, B, E, F), or headed a second time two (Hood River) to five (Corvallis) weeks after the first heading (open square in panels C, D, E, F). The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated with antibiotics and sealed with TreeKote® tree wound dressing. Measurements were made mid- to late June 2011.

## 2011 planting.

**Tree establishment and growth.** All trees received from the nursery established and developed healthy shoots. Total new shoot growth was intermediate (Fig. 2 A) to that observed in the first year of the 2010 Corvallis (highest) and Hood River (lowest) plantings (see last year's report). Initial tree caliper, although purchased as  $\frac{3}{4}$ ", also was intermediate to the  $\frac{3}{4}$  (Corvallis) and  $\frac{5}{8}$  caliper (Hood River) trees planted in 2010.

Long or short orphan stubs, and heading once or twice had no consistent effect on number of new shoots per tree (Fig. 3 B), but total shoot length on trees with short orphan stubs was reduced ~15-20% compared to trees with long stubs regardless if the tree was headed once or twice (Fig. 3 A).



**Fig. 3. Effect orphan stub length on total shoot length (panel C) on shoot length (A), number of shoots (B), length of dead shrunken trunk tissue (canker) that developed immediately below the heading cut (D), and the incidence of gummosis (E) on trees of sweet cherry cv. 'Bing' (Mazzard rootstock) planted at Corvallis, OR in April 2011. After planting, trees were headed once shortly after planting (black bar), or headed a second time (hatched bar) two weeks after the first heading. The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated 70% ethanol. Measurements were made mid-September 2011. Lines at tops of bars are  $\pm$  one standard error of the mean; experimental design was an RCB with 3-tree plots replicated 10 times. Labels below the panels that include 'Actigard' had not yet received this treatment at the time of measurement (it was applied late-Sept and Oct 2011).**

**Effect of orphan stubs on necrotic canker development.** As the season progressed, trunk tissue associated with the orphan stub became necrotic with canker lengths and stub lengths having similar values at the time of measurement in September (Fig. 3 D). Unlike 2010, the length of necrotic tissue was not influenced by the number of times the trees were headed. Similarly, the incidence of gummosis associated with the orphan stub was not affected by the number of times the trees were headed (Fig. 3 E), but long stubs showed an incidence of gummosis that was 2.5 times greater than observed on trees with short stubs (long stubs, 70% incidence of gummosis, versus short stubs, 30%).

## DISCUSSION

**Systemic acquired resistance.** The rationale for this project was based on research in citrus that has shown systemic acquired resistance (SAR) inducers applied to the root zone protects trees from canker caused by *Xanthomonas citri* spp. *citri*. The citrus industry in Florida has begun to utilize this technology for canker control in commercial orchards. Moreover, in our pear and apple research, we are continuing to observe significant effects of ASM on fire blight canker expansion in pear and apple rootstock protection from the fire blight pathogen.

In cherry, however, we did not see a benefit from spring application of ASM, and in fact, some treatments were phytotoxic. Moreover, fall ASM treatments in 2010 to onto trees that received the spring treatments provided no apparent response. Fall treatments also were applied to the 2011 planting, and these will be evaluated in summer 2012; at this point in time (October 2011), we have no expectation that these treatments will show an effect.

**Orphan stubs.** The rationale for the study of orphan stubs in association with the heading cut was based on results of Spotts et al. (2010) who found that *P. syringae* infections initiated in the heading cut resulted in large cankers and frequent death of the newly planted sweet cherry trees. In our plots, we had only a few trees die, of which all received a phytotoxic paint treatment of ASM. One difference in our methods relative to Spotts et al. (2010) was inoculum dose sprayed onto heading cut wounds. Spotts et al. (2010) inoculated heading cuts with suspensions of *P. syringae* prepared at  $10^8$  CFU/ml whereas we used a concentration that was 100-fold smaller ( $10^6$  CFU/ml). Our reason for the smaller dose was that we believed it would more realistically represent what the typical tree in a commercial orchard was likely to experience. In hindsight, a higher inoculum dose may have helped to clarify our data.

Bacterial canker of cherry is most severe in trees/tissues that received some kind of stress (Kennelly & Sundin 2009). The tissues associated with the orphan stub are stressed because they are unsupported by the vascular system of the tree. Orphan stubs typically dieback to the point of the topmost lateral regardless of whether *P. syringae* is present in the necrotic tissue. Nonetheless, a stress pathogen like *P. syringae* readily colonizes the unsupported stub tissues, which results in an exacerbated rate of necrosis and canker expansion. The pathogen also can utilize the colonized stubs an energy source to further invade healthier portions of the trunk. Our results indicate that newly planted cherry will remain healthier if the length of orphan stubs is minimized. The best results (i. e. smallest cankers) were observed when a heading cut was made ~5 mm above the emerging bud of (what will become) the topmost lateral. Our heading cuts were angled downward 45° from just above the emerging topmost lateral toward the distal side of the trunk.

**Double heading.** The rationale for double heading is that cherry trees are planted in March or April when weather is showery, and mature cherry, pear and apple are flowering. With warmer



temperatures and the abundance of flowers and new leaves, epiphytic *P. syringae* populations in the surrounding environment increase very rapidly. Just after planting, the orchardist heads the newly planted tree, and the large, perhaps horizontally-oriented, slowly-healing wound becomes contaminated with *P. syringae* being dispersed in showery spring weather (the *P. syringae* also could come from the tree itself). This initiates bacterial canker at the heading cut. The second heading cut 2 to 5 weeks after the first is made going into a period of warmer and dry weather, such that the wound has a chance to heal before it gets wet again.

Results from 2010 plantings indicated that a second heading cut in dry weather 2 to 5 weeks after the heading cut made near planting reduced the size of the canker associated with the orphan stub, but the results from the 2011 plantings showed no benefit from this second heading. Based on these conflicting results, we recommended that growers consider the second heading (especially if the first was made in wet weather) or alternatively, delay the first heading until it can be made going into a period of warm dry weather. Based on the data, producing a short orphan stub and avoiding wet weather when making heading cuts are likely more important than whether the tree is headed a second time.

**Clarification on what is a ‘stub’.** Researchers at Cornell University (Carroll et al. 2011) are recommending leaving a ‘stub’ when pruning laterals on sweet cherry. The objective in their research was to determine if by leaving pruning stubs, trunk and scaffold branch cankers caused by *P. syringae* could be reduced. Their data demonstrated that leaving lateral stubs protected against bacterial canker by distancing the main trunk and scaffolds from the pruning wounds where invasion by *P. syringae* occurred; lateral stubs also tended to increase the formation of new laterals by leaving existing buds (on the stubbed branch) on the tree. Compared to this study, their use of the term stub means ‘it is the length of branch left on the tree, but potential new laterals are (ideally) distributed along this length’. What we are calling the ‘orphan stub’ is the ‘unsupported trunk tissue above the topmost lateral on the trunk of the newly planted tree’; unsupported because there is no potential for further (future) vascular flow through this tissue.

**Existing plots.** This 640 trees planted in is this study are still in the ground. The experimental trees will be measured again in summer 2012; 2010 plantings will be removed at that time. The 2011 will be maintained and measured again in summer 2013.

## LITERATURE CITED

- Carroll, J. E., Burr, T. J., Robinson, T. L., Hoying, S. A., and Cox, K. D., 2011. Evaluation of pruning techniques and bactericides for managing bacterial canker of sweet cherry. (Abstract) *Phytopathology* 101:S27
- Kennelly, M. M., Cazorla, F. M., de Vicente, A., Ramos, C., Sundin, G. W. 2007. *Pseudomonas syringae* diseases of fruit trees: Progress toward understanding and control. *Plant Dis.* 91:4-17
- Spotts, R. A., Wallis, K. M., Serdani, M., and Azarenko, A. N. 2010. Bacterial canker of sweet cherry in Oregon: Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. *Plant Dis.* 94:345-350.

## **EXECUTIVE SUMMARY**

The purpose of the study was to evaluate practices with potential to reduce the development of bacterial canker associated with heading cuts on newly-planted sweet cherry.

### **Specific objectives evaluated:**

Use of root drenches and trunk paints of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), for protection of young cherry trees from bacterial canker.

and, the relationship between single or double heading and the resulting length of the orphan stub\* on initiation of bacterial canker in the trunk tissue below the heading cut.

\*The 'orphan stub' is the unsupported trunk tissue below the heading cut but above the topmost lateral (unsupported because there is no potential for further vascular flow through it).

### **Results:**

- Spring trunk paint and root dip treatments with ASM in 2010 did not suppress bacterial canker. In fact, trunk paint treatments were phytotoxic to cherry and exacerbated canker symptoms.
- A second heading cut made in May on April-planted cherry trees reduced the amount of dead, shrunken trunk tissue (canker) that developed below the heading cut. Larger cankers developed on those trees headed only once compared to those headed a second time.
- Canker lengths were positively correlated with lengths of orphan stubs.
- Long orphan stubs increased the incidence of gummosis associated with the heading cut compared to short orphan stubs.

### **Recommendation to orchardists:**

Growers should consider a second heading cut in dry weather 2 to 5 weeks after the first heading cut, or alternatively, delay the first heading until it can be made going into a period of dry weather. Whichever the case, the final heading should strive to achieve a short orphan stub by cutting ~5 mm above the emerging bud of (what will become) the topmost lateral angled downward 45° toward the distal side of the trunk. Based on the data, producing a short orphan stub and avoiding wet weather when making heading cuts are likely more important than whether or not the tree is headed a second time.

### **Future directions:**

The current consensus among researchers is management/prevention of bacterial canker of sweet cherry is best approached through horticultural practices and/or host resistance. Resistance in the pathogen to copper is widespread, rendering copper fungicides ineffective for suppression of this disease. Sprays of other chemicals (e.g., antibiotics) are cost prohibitive because they are not persistent enough to suppress *P. syringae* for long periods of time. Further understanding of bacterial canker and its suppression needs to continue to focus on how and when wounds are made and the causes of stress in host tissues.