2004-2005 NURSERY RESEARCH FINAL REPORT

TO
Oregon Department of Agriculture
Oregon Association of Nurseries

TITLE: **Use of biocontrol and proprietary chemicals for controlling postharvest diseases of barerooted deciduous nursery plants.**

PROJECT LEADER: Leslie H. Fuchigami
Department of Horticulture
Oregon State University
Corvallis, OR 97331-7304
Ph. (541) 737-5445

PROJECT COOPERATORS: Srisangwan Laywisadkul
Department of Horticulture
Oregon State University
Corvallis, OR 97331-7304

Guihong Bi
Department of Horticulture
Oregon State University
Corvallis, OR 97331-7304

Robert Lindermann
USDA ARS HCRL
3420 NW Orchard Ave.
Ph. (541) 752-9455

Mark Mazzola
USDA-ARS tree fruit research lab
1104 N. Western Avenue
Wenatchee, WA 98801

Walt Mahaffe
USDA ARS HCRL
3420 NW Orchard Ave.
Ph. (541) 752-9455

Michael Wisniewski
USDA ARS, 45 Wiltshire Rd.
Kearneysville, WV 25430
Ph. (304) 725-3451

Objectives

1. Determine whether fall urea/defoliant treatment predisposes deciduous nursery stock to *Phytophthora syringae*
2. Determine the best timing of applying urea and Cu-EDTA to control *Phytophthora syringae* in relation to pathogen inoculation time.
3. Determine the best timing of applying PhytoFOS to control *Phytophthora syringae* in relation to pathogen inoculation time.
4. Determine whether the degree of plant dormancy influences infection of *Phytophthora syringae*.
5. Determine whether wounding predisposes deciduous nursery stock to *Phytophthora syringae*

Experimental Procedure

Plants material

Pear (*Pyrus communis*) OHF-97 rootstocks were planted into containers containing a mixture of firbark, peat moss, and pumice (1:1:1) in 1 June 2004. The plants were grown in a lathhouse at OSU and trained to a single stem. During this period, the plants were fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients (Plantex Corp., Ontario, Canada) once a week, from 14 June to August, 18 2004.

Phytophthora syringae isolation

Stock cultures of *P. syringae* were maintained on V8 juice agar medium at 20° C in the dark. For preparation of V8 juice agar, calcium carbonate (4 g) was added to 340 ml of V8 juice and filtered through cheese cloth. One hundred ml of V8 juice, 17g of agar and 900ml of distilled water were mixed and autoclaved (121° C, 20 min). Fresh cultures were prepared by transferring agar plugs to plates containing V8 agar which were then incubated at 20 ^{o} C.

Wound inoculation test

Phytophthora syringae (isolated from kalmia by USDA) grown on V-8 agar medium was used to inoculate all trees. Four-mm-diameter disks taken from the actively growing margin of 7-to10-day-old colonies on V-8 juice agar were placed into wounds (removed bark to expose wood) made with cork borer on the stem of each tree to the depth of the cambium. Wound was wrapped with paraffin. After all applications, disease development as the length of lesion was measured eight weeks after inoculation.

Experiment 1: (Objective 1, 2, and 3) Effect of urea, Cu-EDTA, and PhytoFOS on *P syringae* infection

Plants were divided into two sets. Each of four plants were sprayed with either 1) urea (3%) +Cu-EDTA (1%) (UD), 2) PhytoFOS (1%) (P), 3) urea (3%) +Cu-EDTA (1%) + PhytoFOS (1%) (UDP), or 4) water (control) on October 22, 2004 and November 24, 2004. Inoculation with *P. syringae* was made either 1) 1 week before chemical application, or 2) 2 days, 3) 1 week, and 4) 3 week after chemical application in each set. Three wounds per plant were made: two wounds were inoculated with the pathogen and the last wound was inoculation with V8 agar to serve as control. Three inoculations without artificial wounding were also made on the same plant: 2 positions inoculated with the pathogen and the last position was inoculation with V8 agar to serve as control.

Results

All plants inoculated with V-8 agar showed no infection (data not shown). In both sets of treatments, no infection occurred on the no wounded treatments. Plants treated with water and UD in October showed smaller lesions than the treatments in November. In November, plants treated with UD showed smaller lesion than the control, suggesting UD did not predispose plants to infection (figure 1). This data also suggested that environmental conditions may be involved in disease development because the colder and wetter weather during late fall appeared to favor *P. syringae* infection. Lesion extension (or infection) from inoculation was prevented by applying PhytoFOS 1% when application was made before inoculation.

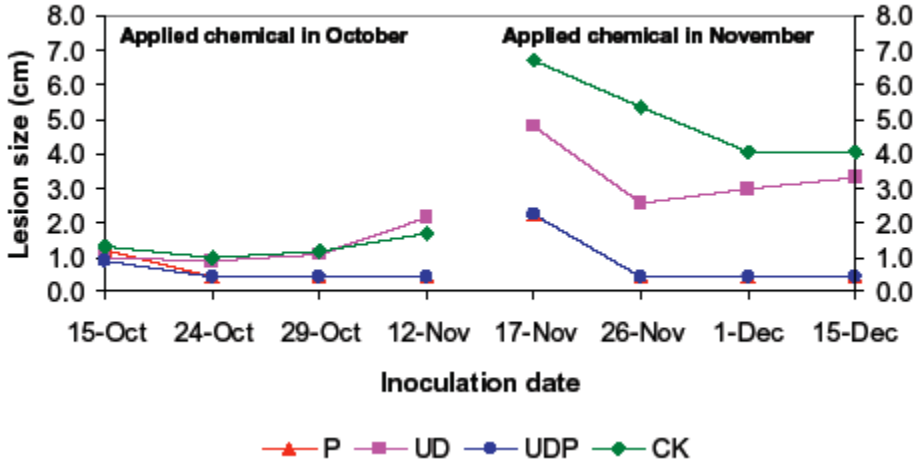

Figure 1. Effect of chemicals on preventing the extended lesion on OHF 97 pear caused by direct inoculation of *P. syringae* to the cambium made either 1 week before chemical application, or 2 days, 1 week, or 3 week after chemical application in each set.

Experiment 2: (Objective 4) Stage of plant growth and *Phytophthora syringae* infection

Plants were grown under natural condition (lathhouse, LH) and divided into two sets. Before start of the experiment, one set was moved to a greenhouse (GH, 16h/8h light/dark) and the other set kept in the LH condition. Plants in LH underwent natural defoliation while plants in GH were still growing. For the dormancy test, each of five natural defoliated plants from LH and hand defoliated plants from GH was kept in GH for monitoring the new bud break. During the same time, each of 15 plants from GH and LH were inoculated with *P. syringae* and V8 agar. After inoculation, five of each plant in each group was incubated in LH, GH, and cold room (CR, 4 ^{o}C) until the end of experiment.

Result

For dormancy test, plants from the lathhouse and greenhouse have a terminal bud break two weeks after placement in the greenhouse condition. Plants grown under greenhouse condition prior to inoculation showed smaller lesions than plant grown under lathhouse condition prior to inoculation in all incubation conditions (figure 2). Disease expression was greatest under cold room condition and decreased when incubated in lathhouse and greenhouse conditions, respectively, suggesting that the cause of the disease progression was due to the differences in the environment. However, under the same incubation condition, plants exposed to different growing environment prior to inoculation showed different disease expression, suggesting growth status of the plants may be involved in disease expression other than environment condition. All plants inoculated with V-8 agar showed no infection (data not shown).

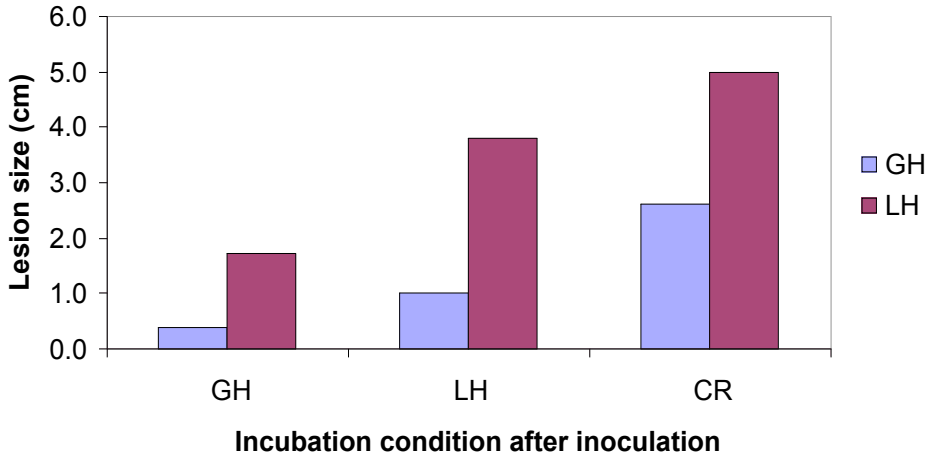

Figure 2 Effect of incubation condition and stage of plant growth on disease expression of *P. syringae*. Growing environment of plants prior to inoculation (greenhouse, lathhouse

Experiment 3: (Objective 3) Effect of pre-harvest application of urea, Cu-EDTA, and PhytoFOS on post-harvest infection by *P syringae*

Plants were divided into two sets. Each of five plants sprayed with either 1) urea (3%) +Cu-EDTA (1%) (UD), 2) PhytoFOS (1%) (P), 3) urea (3%) +Cu-EDTA (1%) + PhytoFOS (1%) (UDP), or 4) water (control) in October 22, 2004 and November 24, 2004. After natural defoliation, all plants were harvested and bare-rooted for cold storage on December 22, 2004. The same stem of each tree was inoculated with pathogen at 0, 2, and 4 weeks after cold storage. Each stem was wounded and inoculated with V8 agar as control.

Result

All plants inoculated with V-8 agar showed no infection (data not shown). The size of the lesions decreased with increasing length of cold storage. Plants treated with UD showed the same trend as the control except the response for decreasing lesion size was later. Plants treated with UD in October showed greater lesion than in November in all inoculation time after cold storage, suggesting that UD application timing in the field might influence infection in cold storage. The growth of the lesion from plants that were inoculated and placed in cold storage was prevented by pre-harvest application of PhytoFOS 1%.

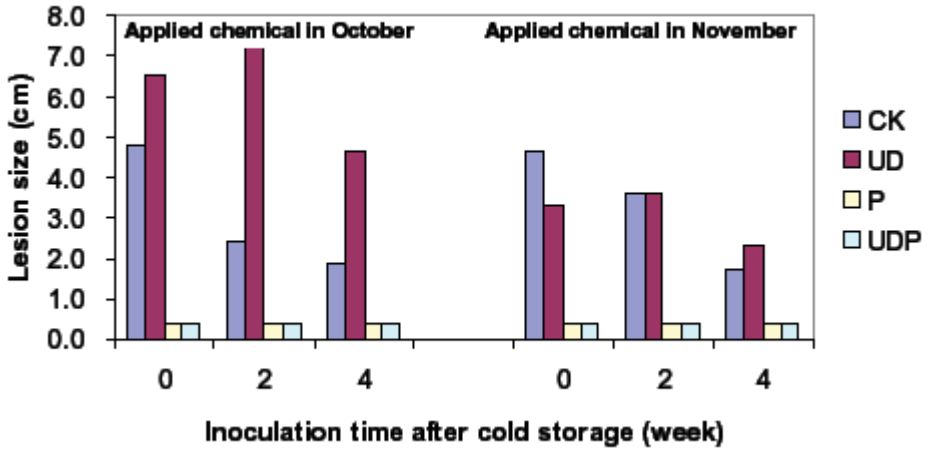

Figure 3 Effect of pre-harvest chemical application timing on post-harvest disease severity.

Experiment 4: (Objective 5)

Each of eight plants was divided into two sets. One set was kept in the lathhouse and the other set was kept in the cold room. Each plant had been wounded at different times and inoculated at the same time. The stem of the same plant was wounded 4, 3, 2, 1, and 0 week before inoculation with pathogen occurred. In addition, each tree was wounded and inoculated with V8 agar to serve as control.

Results

Table1. Effect of wound age on *P syringae* infection

Wound age (weeks)	Percentage of infected wound		Average lesion size ** (cm)	
	Lathhouse	Cold room	Lathhouse	Cold room
4	0.0	0.0	0.4*	0.4
3	0.0	0.0	0.4	0.4
2	0.0	0.0	0.4	0.4
1	25.0	50.0	0.6	1.4
0	100.0	100.0	6.6	6.6

* 0.4 cm is uninfected because the pathogen does not extend from the inoculation site.
** Average from only symptomatic wound

Pathogen caused extended lesion when inoculated to fresh wound under both environmental conditions and the size of the lesions decreased as the wound age increased. There are no extended lesions when inoculation occurred 2 weeks after wounding. The data suggested that healing of the wound within two weeks prevented pathogen infection.

Conclusions

1. Disease infection increased with later treatment dates under natural conditions in the fall while disease infection with early treatment was similar at all inoculation date.
2. November application of Urea and/or CuEDTA caused greater lesions than the October treatments.
3. PhytoFOS application before inoculation was effective in controlling *P. syringae* infection but not after inoculation.
4. Healing of wound reduced infection