FINAL REPORT
WTFRC Project #AH-01-86 **WSU Project #13C-3661-4367**

Project Title: Epidemiology and control of gray mold of apples

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Objectives:
1. Determine effects of preharvest applications of fungicides on postharvest decay including gray mold and blue mold.
2. Determine effects of preharvest applications of biocontrol agents for control of postharvest decay including gray mold and blue mold.
3. Determine preharvest colonization of floral parts of fruit by *B. cinerea* and its relationship to postharvest decay.
4. Determine inoculum sources of *Botrytis cinerea* in the orchard.
5. Test sensitivity to thiabendazole (TBZ) among *B. cinerea* isolates from apples collected from various apple production areas in eastern Washington.
6. Study the populations of *Botrytis* isolates from apples in eastern Washington.

Significant findings:
- In the experiments conducted in 2001 to 2003, the fungicide fenhexamid (Elevate) was consistently effective in controlling gray mold originating from infection of wounds by *Botrytis cinerea*. Fenhexamid applied one day prior to harvest reduced the incidence and severity of postharvest gray mold originating from wound infection by 75-86 and 88-92%, respectively, in comparison with the non-fungicide treated control. *With the increasing commercial use of SmartFresh (1-MCP) on fresh apples, fewer apples will be drenched with DPA and thiabendazole (TBZ, Mertect). Gray mold is one of the major decay problems, particularly on fruits that are not treated with fungicide before storage. Alternatives to TBZ drench for decay control are needed. Preharvest use of effective fungicides such as fenhexamid would meet this need for decay control under the new SmartFresh-based fruit handling system.*
  Fenhexamid is considered a low-risk fungicide. Based on the data generated in this research project, an IR4 request has been submitted for use of fenhexamid as preharvest treatments for control of postharvest gray mold on apples and pears in Washington State. We are planning to continue to work with related organizations and the chemical company to make this fungicide available to the fruit industry.
- Most of *B. cinerea* (gray mold fungus) isolates tested in this study were sensitive to TBZ (Mertect), the fungicide that is commonly used as postharvest treatments to control decay. A strain that is able to grow at 10 ppm is considered to be resistant to TBZ. Approximately 70% of the isolates had EC50 values (the concentrations that result in reduction of fungal growth by 50%) between 0.41 and 0.60 ppm. Only 1.5% of the isolates tested were highly resistant to TBZ.
- No TBZ-resistant isolates of *B. cinerea* were observed among the 70 isolates that were recovered from Mertect-drenched apple fruit. The EC50 values of TBZ-sensitive isolates were very similar between isolates from non-drenched sources and those from Mertect-drenched, indicating that the current postharvest decay control recommendations, including avoiding benzimidazole fungicides in the orchards and using thiabendazole only after harvest in the packing facility, are an effective measure for fungicide resistance management.

- Fludioxonil has recently been registered for commercial use as postharvest treatments on stone fruits for decay control and will likely be available soon for commercial use on pome fruits. We have established the baseline sensitivity of *B. cinerea* from apple to this fungicide. The *B. cinerea* population from apple in Washington State is sensitive to this fungicide. All isolates tested have not been exposed to this fungicide. Thus, the baseline sensitivity data will be useful in monitoring the development of resistance in the *B. cinerea* populations to this fungicide.
- There was apparently no cross-resistance in *B. cinerea* isolates to the two postharvest fungicides, thiabendazole and fludioxonil.
- Effectiveness of preharvest applications of biocontrol agents in controlling postharvest gray mold and blue mold varied in the two-year trials. Overall, they were not effective to reduce incidence of decay originating from wound infection by *B. cinerea* and *P. expansum*, though they might reduce severity of decay. The relatively low level of the yeast or bacteria cells introduced at the wound might be responsible for the unsatisfactory control.
- *B. cinerea* colonized floral parts of fruit during bloom and survived in these tissues throughout the growing season, but no correlation was observed between colonization of floral parts and calyx-end decay in storage. Colonization of floral parts by *Botrytis* can be used as an indicator of inoculum potential of the pathogen in the orchard.
- *B. cinerea* formed sclerotia on decayed fruit in the spring. *B. cinerea* was also recovered from previous season's thinned fruits on the orchard floor. This indicated that removal of decayed fruit from the orchard floor would be beneficial in reducing inoculum level in the orchard.

Methods:
Experiments were conducted on Fuji in 2001, 2002 and 2003 and on Gala in 2002 and 2003 to evaluate the effectiveness of preharvest applications of fungicides for control of postharvest gray mold. Fungicides, including fenhexamid (Elevate), captan, and thiabendazole, thiram and ziram, were tested. Fungicides were applied onto the fruit at two weeks or one day before harvest. Fruit was harvested from each replicate tree. Thirty or 50 fruits from each replicate were immediately wounded with a finish nail head and inoculated with spore suspensions of *B. cinerea* or *P. expansum*. Fruit was packed on sterilized trays with poly liners and stored in RA at 32ºF for postharvest decay evaluation.

In 2002 and 2003 we also evaluated preharvest applications of biocontrol agents for control of gray mold caused by *B. cinerea* and blue mold caused by *P. expansum*. Three biocontrol agents, the yeast *Cryptococcus laurentii* 87-108, Aspire, and Bio-Save, were tested on Gala and Fuji. Biocontrol agents were applied onto fruits at nine days or one day before harvest. The populations of introduced yeasts or bacteria on the fruit surface were monitored. The procedure for fruit inoculation after harvest was the same as described above.

To determine formation of *B. cinerea* sclerotia on decayed fruit on the orchard floor, in the fall Fuji apples were inoculated with *B. cinerea* isolates in the lab. Botrytis-decayed fruit was then placed in mesh bags and laid on the orchard floor. Botrytis-infected fruit was examined for formation of sclerotia in spring.

During the bloom period in May, 40 flowers from each of five commercial orchards in different locations were collected for fungal isolation. Plates were examined for presence of *Botrytis* and *Penicillium* colonies. To determine preharvest colonization of calyx tissues by *B. cinerea* and its relationship to postharvest decay, 30 blossom clusters on each of four Gala and four Braeburn trees were tagged and inoculated with conidia suspensions of *B. cinerea*. Fruit was harvested and stored in RA for further decay evaluation. At harvest, 20 boxes of fruit (one box per tree) were also harvested from each of four commercial orchards and stored in air at 32ºF for decay evaluation.

During 2001 to 2003, 300 isolates of *B. cinerea* were recovered from floral parts of fruit collected from apple orchards or decayed apple fruit sampled from commercial packinghouses across the state of Washington. All 300 isolates were single-spored and stored at -80ºC. Sensitivity of mycelial growth of 139 isolates were determined on 1/2-strength potato dextrose agar (PDA) amended with thiabendazole or fludioxonil. Concentrations in the media were 0, 0.1, 0.5, 0.75, 1, 10 and 100 ppm for thiabendazole and 0, 0.0001, 0.001, 0.01, 0.1 and 1 ppm for fludioxonil. The fungicide concentration that inhibited mycelial growth by 50% (EC50) in comparison with the non-amended control was calculated for each isolate.

Results and discussion:

Preharvest fungicides for control of postharvest gray mold and blue mold. Experiments were conducted on Fuji in 2001, 2002 and 2003 and on Gala in 2002 and 2003. At the time of writing this report, the 2003 Fuji experiment is still in progress (in storage). It will be completed in December, and the data will be presented at the research review meeting in January 2004.

(1) 2001 Fuji experiment: All fungicides applied one day prior to harvest significantly reduced gray mold originating from wound infections by *B. cinerea* compared to the non-fungicide treated control. The fungicide fenhexamid (Elevate) provided the best control for postharvest gray mold. Fenhexamid, captan and thiabendazole reduced the incidence of gray mold by 86, 51, and 37%, respectively, in comparison with the non-fungicide treated control. The data had been reported.

(2) 2002 experiments: Preharvest applications of captan and fenhexamid significantly reduced the severity (decayed area) of gray mold rot on fruit inoculated with *B. cinerea* but had no effect on blue mold rot on fruit inoculated with *P. expansum*. The data had been reported. On Fuji, fungicides captan, fenhexamid and thiabendazole were applied one day before harvest. Fruit was wounded and inoculated with *B. cinerea* and *P. expansum*. A very low level of decay developed in inoculated fruit. The data were not reliable to separate treatment differences.

(3) 2003 Gala experiment: Treatments including fenhexamid (Elevate) applied at two weeks or one day before harvest, thiram and ziram applied at two weeks before harvest, and a non-treated control were tested in 2003. The fungicide fenhexamid (Elevate) was very effective in controlling gray mold on apples (Fig. 1). Elevate was significantly more effective when applied at one day before harvest than when applied at 14 days before harvest.

    Thiram and ziram applied at 14 days before harvest did not significantly reduce incidence and severity of both gray mold (Fig. 1) and blue mold (Table 1) in comparison with the non-treated control. We have seen a great variation in amount of decay between replicates of thiram or ziram treatments. Variation in micro-environmental conditions between replicates might affect residues of these two fungicides on apple fruit. Thiram and ziram are contact fungicides. In this study, lack of satisfactory control provided by these two fungicides might indicate that they were not effective to prevent infection of wounds by *B. cinerea*. The benefit of preharvest application of thiram and ziram in decay control may be largely attributed to the reduction of spore levels of decay pathogens on the fruit surfaces by these fungicides.

In 2002, 1-MCP (SmartFresh) received registration for use on fresh apples. With the increasing commercial use of SmartFresh on apples, fewer apples will be drenched with DPA and TBZ. Gray mold rot is one of the major diseases on non-drenched apples. Alternatives to TBZ drench for decay control are needed. Preharvest use of effective fungicides such as fenhexamid would meet this need. Fenhexamid is considered a low-risk fungicide. Based on the data generated in this research project, an IR4 request has been submitted for use of fenhexamid as preharvest treatments for control of postharvest gray mold on apples and pears in Washington State. We are planning to continue to work with relevant organizations and the chemical company to make this fungicide available to the fruit industry.

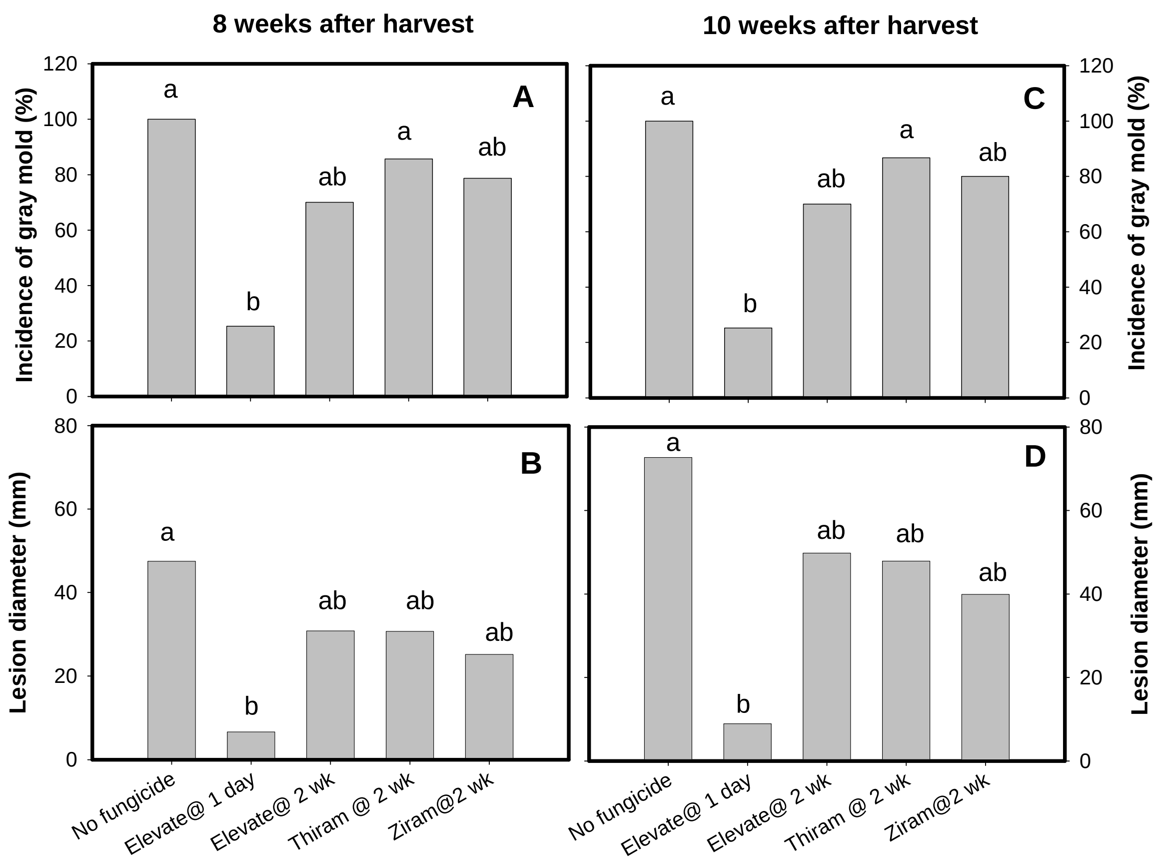

Fig. 1. Effects of pre-harvest fungicides on gray mold on Gala apples after 8 weeks (**A**: incidence; **B**: lesion size) and 10 weeks (**C**: incidence; **D**: lesion size) of storage in air at 32ºF. The fungicide Elevate was applied at 2 weeks or 1 day before harvest; and Thiram and Ziram were applied at 2 weeks before harvest. Fruit was wound-inoculated at harvest with spores of *Botrytis cinerea*.

Table 1. Effects of preharvest fungicides on postharvest blue mold on Gala apples[1].

Treatment	6 weeks after harvest		8 weeks after harvest	
	Decay incidence (%)	Lesion diameter (mm)	Decay incidence (%)	Lesion diameter (mm)
Nontreated	100	24	100	30.1
Thiram applied at 2 weeks before harvest	100	22.6	100	31.5
Ziram applied at 2 weeks before harvest	100	23.3	100	30

[1] There was no significant difference in incidence and severity of blue mold between fungicide-treated and non-treated fruit.

Preharvest applications of biocontrol agents for control of postharvest gray mold and blue mold.

(1) 2002 Gala experiment: The yeast *Cryptococcus laurentii* 87-108 was applied one day before harvest. It significantly reduced the severity of gray mold originating from wound infection by *B. cinerea* but not incidence of the disease. It had no effect on blue mold. The data had been reported.

(2) 2002 Fuji experiment: Three biocontrol agents, Aspire, Bio-Save, and *C. laurentii* 87-108, were applied one day before harvest. After eight weeks of storage in air at 32ºF, variation in amount of decay among replicates within the same treatment, except Aspire, was observed. Overall, the biocontrol agent *Cryptococcus laurentii* 87-108 significantly reduced the number of Botrytis-inoculated fruit that developed decay in storage. All three agents did not provide satisfactory control for blue mold, although *C. laurentii* 87-108 significantly reduced the size of decay compared with the non-treated control (Table 2).

(3) 2003 experiments: On Gala, the biocontrol agent *Cryptococcus laurentii* 87-108 was applied at nine days or one day before harvest. Overall, this biocontrol agent did not provide satisfactory control for gray mold that originated from infection of wounds by *B. cinerea* (Table 3). Three biocontrol agents were also tested on Fuji. At the time of writing this report, the experiment is still in progress (in storage). The data will be presented at the research review.

Table 2. Effects of preharvest biocontrol agents on postharvest gray mold and blue mold on Fuji apples in 2002.

Treatment[1]	Gray mold		Blue mold	
	Decay incidence (%)	Lesion diameter (mm)	Decay incidence (%)	Lesion diameter (mm)
Nontreated	67.5a[2]	29.2a	100a	29.9ab
Aspire	65.8ab	23.2a	100a	31.5a
BioSave	60.8ab	24.8a	96.6b	33.2a
Cryptococcus laurentii 87-108	34.2b	11.7a	100a	25.7b

[1] All biocontrol agents were applied at one day before harvest.
[2] Values with the same letter in the same column are not significantly different based on the LSD test (P = 0.05). Decay was evaluated after 8 weeks of storage in air at 32ºF.

Table 3. Effects of preharvest biocontrol agents on postharvest gray mold and blue mold on Gala apples in 2003.

Treatment	Gray mold		Blue mold	
	Decay incidence (%)	Lesion diameter (mm)	Decay incidence (%)	Lesion diameter (mm)
Nontreated	98.4a	26.1a[1]	100a	21.1a
Cryptococcus laurentii 87-108 applied at 9 days before harvest	97.5a	22.4b	100a	17.2a
Cryptococcus laurentii 87-108 applied at 1 day before harvest	93.4a	24.4ab	100a	15.7a

[1] Values with the same letter in the same column are not significantly different based on the LSD test (P = 0.05). Decay was evaluated after six weeks of storage in air at 32ºF.

Preharvest colonization by Botrytis *in the orchard.*

(1) In the spring of 2001 and 2002, *Botrytis* spp. were isolated from the floral parts of fruit in all five commercial orchards sampled during bloom period. The data had been reported. The percentage of flowers from which *Botrytis* spp. were recovered ranged from 10 to 48% in 2002. In

comparison with the 2001 data, the percentage of flowers colonized by *Botrytis* varied from season to season and from orchard to orchard.

(2) At harvest in 2001 and 2002, fruit were sampled from three commercial orchards. *Botrytis* spp. were isolated from calyx tissues, with isolation frequency ranging from 18 to 58% (Table 4).

(3) Fruit harvested from the previous year's experiment was evaluated for decay in the spring of 2001 and 2002. Fruit harvested from the same commercial orchards developed a very low level of calyx-end rot caused by *B. cinerea*. No correlation between calyx colonization and postharvest calyx-end decay was observed.

Table 4. Isolation frequency of *Botrytis* spp. from calyx tissues of fruit at harvest.

Variety	% fruit from which *Botrytis* recovered[1]		% fruit with *Botrytis* recovered from sepals		% fruit with *Botrytis* recovered from stamens or pistils	
	2001	2002	2001	2002	2001	2002
Gala	20	20	10	15	13	13
Gala	58	18	23	10	43	18
Braeburn	48	30	10	8	40	30

[1] Forty fruits were sampled from commercial orchards at harvest.

Lack of correlation between calyx colonization and calyx-end decay may be due to unfavorable climatic conditions for disease development in eastern Washington. It is uncertain whether over-tree irrigation would result in a higher level of the disease. However, colonization of floral parts by *Botrytis* can be used as an indicator of inoculum potential of the pathogen in the orchard.

<u>*Inoculum source of* Botrytis cinerea</u>. In 2001 and 2002, we determined potential sources of *Botrytis* inoculum in apple orchards. We had reported that *Botrytis* was recovered from the previous season's thinned fruit on the orchard floor. In the fall of 2001, fruit inoculated with *B. cinerea* was laid on the orchard floor. The pathogen formed sclerotia on decayed fruit in the spring of 2002. This indicates that removal of decayed fruit from the orchard floor would be beneficial in reducing inoculum level in the orchard.

<u>*Resistance of* Botrytis cinerea *isolates to TBZ*</u>. In the pathology literature, an isolate that is able to grow at 10 ppm is considered to be resistant to TBZ (Mertect). Of 139 isolates of *B. cinerea* tested, two were highly resistant to TBZ, with EC50 values of 74 and 83 ppm, respectively (Fig. 2). Interestingly, the two highly resistant isolates were recovered from decayed apple fruit that had not been drenched after harvest with Mertect (thiabendazole). This indicates that TBZ-resistant strains of *B. cinerea* are still present in these apple orchards, even though benzimidazole fungicides such as benomyl have not been used in the orchards for many years. Approximately 70% of the isolates had EC50 values between 0.41 and 0.60. Sensitivity distribution indicates that only less than 2% of *B. cinerea* isolates were highly resistant to TBZ and that most of the *B. cinerea* isolates from apples were sensitive to TBZ (Fig. 2). This indicates that TBZ is still effective to control gray mold.

No TBZ-resistant isolates of *B. cinerea* were observed among the 70 isolates that were recovered from Mertect-drenched apple fruit. The EC50 values of TBZ-sensitive isolates were very similar between isolates from non-drenched sources and those from Mertect-drenched (Table 5), indicating that the current postharvest decay control recommendations, including avoiding benzimidazole fungicides in the orchards and using thiabendazole only after harvest in the packing facility, are an effective measure for fungicide resistance management.

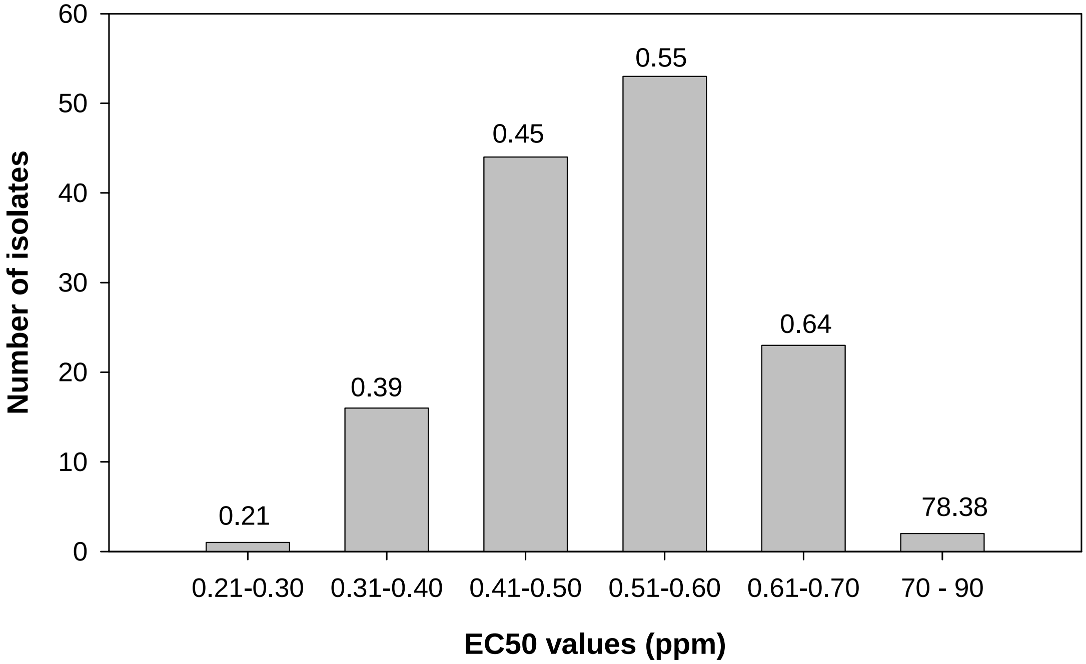

Fig. 2. Frequency distribution of EC50 value (the concentration that results in 50% reduction in fungal mycelial growth compared with the non-treated) to thiabendazole determined for *Botrytis cinerea* isolates recovered from apple in Washington State. Numbers above bars are mean EC50 values of isolates within corresponding ranges. A total of 139 isolates was tested.

Table 5. Sensitivity to thiabendazole (Mertect) of *Botrytis cinerea* isolates recovered from apple from different sources in Washington State.

Sources of isolates[1]	Number of isolates	Percentage of resistant isolates (grow at 10 ppm)	EC50 values of resistant isolates (ppm)		EC50 values of sensitive isolates (ppm)	
			Range	mean	Range	mean
Non-drenched	69	2.9	74.0 - 82.7	78.4	0.21 - 0.69	0.49
Mertect-drenched	70	0	NA[2]	NA	0.38 - 0.69	0.52

[1] Isolates were recovered from floral parts of fruit collected from apple orchards or decayed apple fruit sampled from commercial packinghouses across the fruit production region in Washington State. Isolates recovered from orchards are considered to be from non-drenched sources.

[2] No resistant isolates were observed among the 70 isolates that were recovered from the Mertect-drenched apples.

<u>*Baseline sensitivity of* B. cinerea *from apples to the new postharvest fungicide fludioxonil.*</u>
Fludioxonil has recently been registered for commercial use as postharvest treatments on stone fruits for decay control and will likely be available soon for commercial use on pome fruits. Distribution of baseline sensitivity of *B. cinerea* to this fungicide indicates that the fungal population from apple was sensitive to this fungicide (Fig. 3). All isolates tested have not been exposed to this fungicide. Thus,

the baseline sensitivity data will be useful in monitoring the development of resistance in the *B. cinerea* populations to this fungicide.

Thiabendazole belongs to benzimendazole fungicide and fludioxonil belongs to a different class of fungicide, phenylpyrroles. The two TBZ-resistant isolates had EC50 values to fludioxonil at 0.003 and 0.005, respectively. The baseline sensitivity data indicate that there was apparently no cross-resistance to these two fungicides.

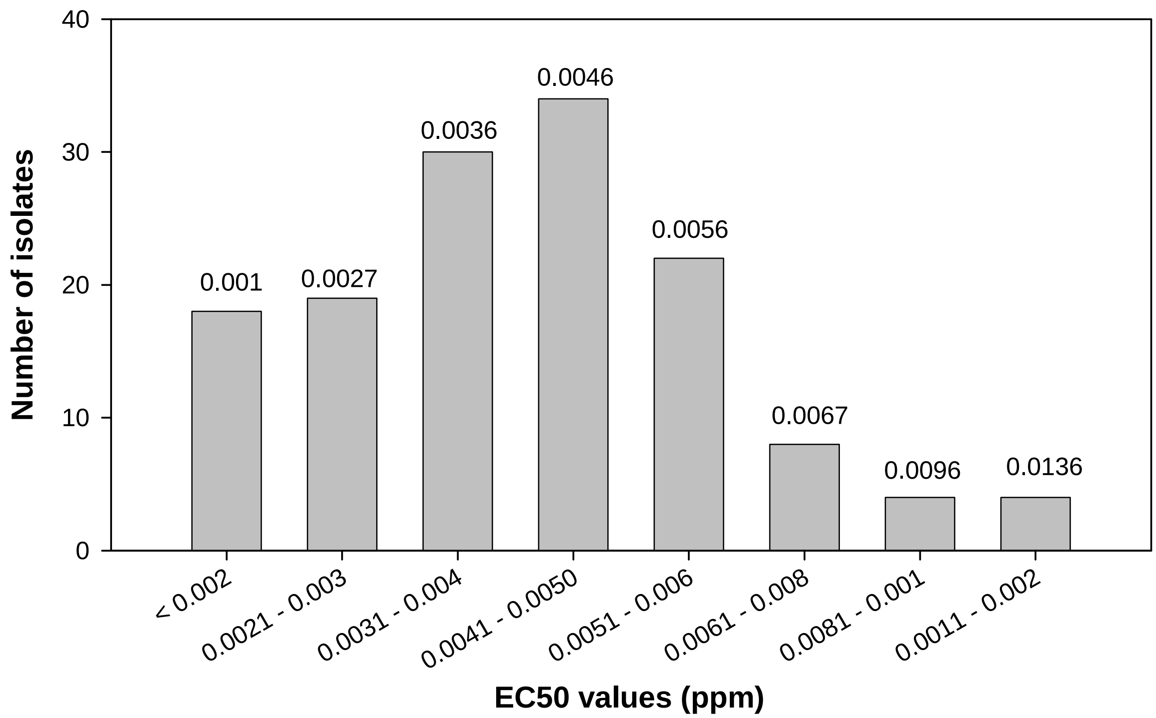

Fig. 3. Frequency distribution of EC50 value (the concentration that results in 50% reduction in fungal mycelial growth compared with the non-treated) to fludioxonil determined for *Botrytis cinerea* isolates recovered from apple in Washington State. Numbers above bars are mean EC50 values of isolates within corresponding ranges. A total of 139 isolates was tested.

Budget:

Project title: Epidemiology and control of gray mold of apples
PI: Chang-Lin Xiao
Project duration: 2001-2003
Project total (3 years): $124,544

Item	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Salaries[1]		30,000	31,200
Benefits (37%)		10,800	11,544
Wages	12,000	--	
Benefits	2,000	--	
Equipment	4,500	--	
Supplies[2]	4,500	5,500	4,500
Travel[3]	3,000	3,000	2,000
Miscellaneous		--	
Total	26,000	49,300	49,244

[1] Salary for a Postdoctoral Research Associate.

[2] Lab and field supplies including isolation media, chemicals, Petri dish plates, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates, and fungicides. Cost of fruit bought from commercial orchards.

[3] Travel to selected commercial orchards and packinghouses in various locations are required for sampling. We used a leased vehicle.