Harvested Grape Clusters as Inoculum for Pierce's Disease

ALEXANDER H. PURCELL and STUART SAUNDERS, Department of Environmental Science, Policy and Management, University of California, Berkeley 94720-3112

ABSTRACT

Purcell, A. H. and Saunders, S. 1995. Harvested grape clusters as inoculum for Pierce's Disease. Plant Dis. 79:190-192.

Harvested fruit clusters from grapevines with Pierce's disease (PD) did not serve as sources from which an efficient insect vector (the blue-green sharpshooter, *Graphocephala atropunctata*) acquired the causal bacterium *Xylella fastidiosa*. Sharpshooters fed for 6 hr on fruit clusters harvested from PD-infected vines, then were tested twice for *X. fastidiosa* by exposure to healthy grapevines. Clusters were tested as possible sources 1, 7, 14, and 21 days (stored at 4 C) after being harvested from vines confirmed as having PD. None of 420 surviving blue-green sharpshooters or 84 green sharpshooters (*Draeculacephala minerva*) from all tests transmitted the bacterium to grape, but 88% of 49 blue-green sharpshooters and 24% of 37 green sharpshooters surviving from these tests and then given a 6-hr access on diseased grapevines subsequently transmitted *X. fastidiosa* to grape. Isolations of *X. fastidiosa* from cluster stems and rachises were successful in only 5 of 24 samples 1 day after harvest. Concentrations of *X. fastidiosa* isolated from stems of diseased clusters were about 10–100 times lower than typical concentrations in grape petioles or leaf veins, decreased each week, and were not recovered after storage for 3 wk. Post-harvest fumigation with sulfur dioxide did not affect rates of recovery of *X. fastidiosa* from grape cluster stems.

Pierce's disease (PD) of grapevines is caused by a xylem-limited bacterium (1), *Xylella fastidiosa* Wells et al (14), that in nature is spread only by insect vectors (2,10). Because most, if not all, xylem-feeding suctorial insects are PD vectors, it is conceivable that an insect vector could acquire *X. fastidiosa* by feeding on grape clusters taken from PD vines and later transmit the bacterium to other plants. No experimental data exist on the potential of grape clusters taken from PD-infected vines to serve as a source for insect transmission. This information would be useful in assessing quarantine measures to prevent the introduction of *X. fastidiosa* in shipments of fresh grapes.

Our objectives were to determine if fruit clusters from grapevines with PD could serve as feeding acquisition sources for efficient insect vectors and to estimate the concentrations of viable (cultivable) cells of *X. fastidiosa* after post-harvest fumigation and storage. To maximize transmission we *(1)* used the most efficient vector for grape to grape transmission, *(2)* used symptomatic clusters (some raisined fruit but with normally turgid stems) to maximize concentrations of *X. fastidiosa, (3)* used nonfumigated as well as fumigated fruits to determine if fumigation affected bacterial survival, *(4)* forced prolonged feeding of vectors on clusters from diseased vines, and *(5)* conducted feeding trials as soon as possible after harvest.

Accepted for publication 21 October 1994.

The blue-green sharpshooter (BGSS), *Graphocephala atropunctata* (Signoret) (= *Hordnia circellata*), was used because it is perhaps the most efficient vector in transmitting *X. fastidiosa* to and from grapevines (11,13). The green sharpshooter (GSS), *Draeculacephala minerva* Ball, was used in some tests in which the number of blue-green sharpshooters tested was less than originally planned. Although it is less efficient in transmitting *X. fastidiosa* to grape, GSS is the most common PD vector species in the table grape production areas of California (4,12,13).

MATERIALS AND METHODS

Grapevines and fruit bunches with Pierce's disease. Grapevines of *Vitis vinifera* L. 'Thompson Seedless' and 'Ruby Seedless' having symptoms of PD and also harvestable fruit were located and tagged in four commercial vineyards in the California's Central Valley (Fresno and Tulare counties) during June and August, 1992. Tagged vines were verified as infected with *X. fastidiosa* by culture of the bacterium on periwinkle wilt medium (1) and by enzyme-linked immunosorbent assays (ELISA) modified from procedures developed by Nomé et al (6). Our "double-sandwich" ELISA assays utilized commercial antisera from Agdia, Inc. (Elkhart, Indiana) or our own antisera produced in rabbits against whole cells of *X. fastidiosa* that was originally isolated from PD grapevines in Sonoma County, California, in 1990. ELISA procedures followed the manufacturer's instructions (Agdia). Optical absorbance readings at a wavelength of 405 nm (Tektronics plate reader) that were at least two and one-half times that of healthy control plant samples were considered positive. Most positive controls (PD grapevine leaf petioles) produced absorbances above 1.0 within 30 min after adding final substrate; those of negative controls were below 0.09. ELISA samples were prepared from approximately 0.1–0.2 g of leaf petioles from test plants. The plant samples were homogenized in 2 ml of sterile phosphate buffered saline in a 16-mm-diameter sterile glass culture tube with a high speed homogenizer (Beckman Polytron model 10-35 with a PT 10-S bit) and not sonicated.

Populations of *X. fastidiosa* in fruit clusters were estimated by dilution plating of plant samples from fruit cluster stems and rachises. Plant samples were first surface sterilized in ethanol (1 min), followed by 1.25% sodium hypochlorite solution (2–5 min), and finally by three rinses in 100 ml of sterile water. The samples were aseptically homogenized as described above for ELISA. The shaft and bit of the homogenizer were sterilized between samples while still running at high speed by immersing the shaft to a depth of about 12 cm in a bottle of 95% ethanol for 10–15 sec followed by consecutive immersions in two bottles of sterile water. The same ethanol and sterile water were used for consecutive samples. Occasionally, contaminant fungi or bacteria appeared in the undiluted sample and less often in the first dilution, but not at higher dilutions, indicating the effectiveness of the bit sterilization procedure. Sterile phosphate buffered saline tubes were also processed between some samples and did not produce contaminants when cultured. This additionally confirms that the sterilization procedure was effective and that there was no carryover of *X. fastidiosa* between samples. Three 20-µl aliquots of undiluted, and the next three 10-fold dilutions of the homogenized, sample were pipetted and spread onto solid periwinkle wilt medium. This liquid inoculum was air-dried under a laminar flow hood (usually 3–5 min), and the plates were incubated in a plastic container at 24–27 C for at least 6–10 days before counting the colonies of *X. fastidiosa*.

Fruit clusters from infected vines were tagged, harvested, and stored immediately in ice chests. At a commercial packing house, some tagged bunches from each site were fumigated for 20 min with 0.5 % sulfur dioxide at 34–39 C. Clusters were transported in an ice chest to

Berkeley, California and stored in plastic bags in a refrigerated room at 4 C. Bunches used for leafhopper acquisition or assays of viable cell concentrations were not fumigated or were fumigated and stored for 1, 7, 14, or 21 days at 4 C.

Leafhopper survival on grape bunches. In separate experiments, the survival of BGSS that had been caged on grape bunches (1 day after harvest) was recorded in replicated (six times) trials with 20 insects per bunch. The insects used in these trials were collected from natural populations in Berkeley. After a 6-hr exposure on bunches with half of the berries removed, the insects were placed on rooted grape cuttings and mortality was recorded 3 days later. Control BGSSs were caged only on grape seedlings.

Leafhoppers free of *X. fastidiosa*. BGSSs were collected from Russian Gulch State Park, California, where we had found hundreds of such insects to be free of *X. fastidiosa* in previous years. Sharpshooters were also collected from Berkeley where levels of natural transmission had been very low (less than 1%) in the past 2 yr. To ensure that any insects infective with *X. fastidiosa* were rendered noninfective, we confined the BGSS nymphs on *Artemisia douglasiana* Besser (California mugwort), a plant species previously found to be not a systemic host of *X. fastidiosa* (B. Hill and A. H. Purcell, *unpublished data*). We examined the insects daily and transferred newly molted adults for at least 3 days (usually much longer) to "pretest plant" seedlings of Pinot noir grapevines or rooted cuttings of Chardonnay grapevines. Molting removes *X. fastidiosa* along with the shed exoskeleton of the insect from transmissible sites in infective sharpshooters (12). All pretest plants and transmission test plants were kept in an enclosed greenhouse for at least 2 mo, then tested by culture and ELISA for colonization by *X. fastidiosa*. Our intent was to discard from consideration any transmission results derived from insects from pretest plants that became infected by *X. fastidiosa*. However, none of the pretest plants that we used became infected with *X. fastidiosa*.

Vector transmission. We removed BGSS from pretest grapevines with a tube aspirator and confined them in a 10-cm-diameter plastic cylindrical cage on grape clusters from *X. fastidiosa*–infected vines for 5–6 hr. To increase insect access to stems, where the bulk of xylem vessels and *X. fastidiosa* inoculum should be located, 30–50% of the fruits from the clusters were removed. After the 5–6 hr interval, the fruit cluster was removed from the cage, and the cage with insects was placed over a Chardonnay test plant in a 10-cm-square pot. The following day, the insects were individually transferred to Pinot noir seedlings grown in 6-cm-square pots. Both of these grape cultivars are very susceptible to PD (7,9). The insects were removed after 5 days and the plants were kept in a heated, insect-proof greenhouse for detection of infection by *X. fastidiosa*.

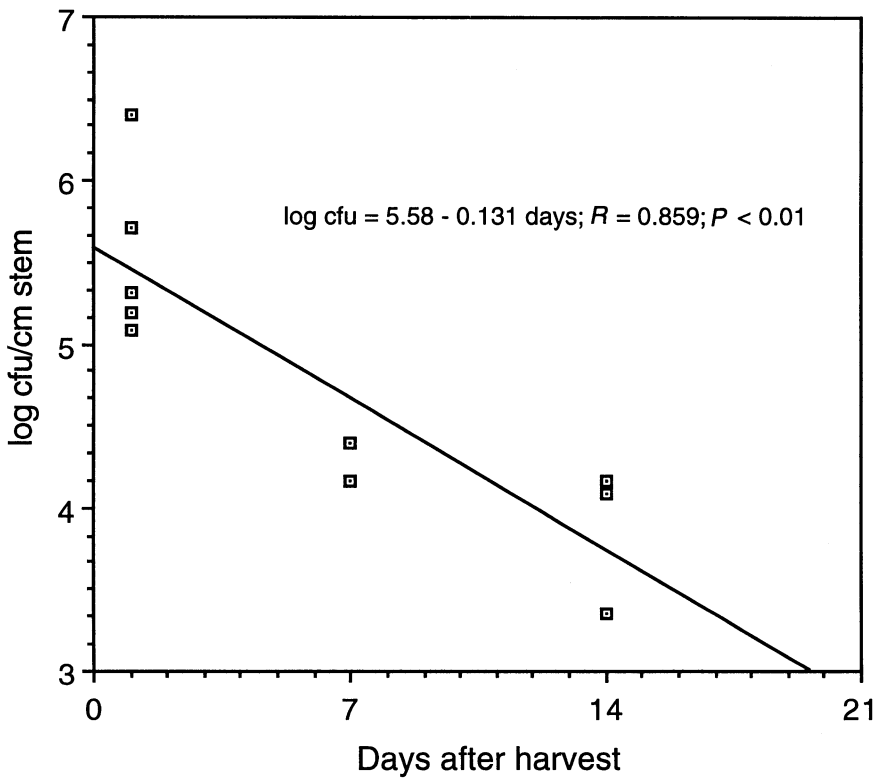

Fig. 1. Regression of cfu of *Xylella fastidiosa* per centimeter of stem from grape clusters after various times during cold storage following harvest. Only positive isolations are included (74 of 84 were negative).

As a positive control, after the last experiment (21 days post-harvest) all surviving insects were given a 6-hr access to potted Pinot noir grapevines that displayed PD symptoms and in which infection by *X. fastidiosa* had been verified by culture. These insects were then transferred individually to grape test plants for 5 days to assess transmission rates from infected grapevines under the same experimental conditions as for acquisition feeding from fruit clusters.

RESULTS AND DISCUSSION

Bacterial viability in grape clusters. *Xylella fastidiosa* was recovered by culture from only 5 of 24 samples of rachises 1 day after harvest (Fig. 1). Numbers of viable *X. fastidiosa,* as estimated from dilution plating, decreased with time spent in cold storage from log 5 to log 6.5 cfu/cm stem after 1 day to no cultivable cells recovered from 12 clusters after 21 days of refrigeration. By comparison, populations of *X. fastidiosa* in grapevine leaf petioles were from log 7 to log 8 cfu/cm. Fumigation did not reduce the numbers of recoverable *X. fastidiosa* in grape clusters. Colonies of *X. fastidiosa* were recovered from 4 of 12 fumigated and 1 of 12 nonfumigated clusters after 1 day in storage. After 7 and 14 days storage, *X. fastidiosa* was recovered from 4 of 36 fumigated and 1 of 12 nonfumigated clusters.

Sharpshooter survival after 6 hr on grape clusters. There was no noticeable residual effect on BGSS survival after 6-hr confinement on freshly harvested (1-day-old) grape clusters. Mortality averaged 5.67% (range 0–14%), and control mortality averaged 10% (range 5–15%).

Insect transmission. None of a total of 420 BGSS or 84 GSS transmitted *X. fastidiosa* to grapevines after 6-hr access for feeding on clusters of fruit from PD grapevines and completing a 5 day inoculation access period (Table 1). Sharpshooters appeared to be feeding, but we could not verify this. The lack of transmission by sharpshooters capable of efficient grape-to-grape transmission of *X. fastidiosa* was especially impressive because the experimental conditions were manipulated to maximize transmission. The same conditions using the same insects, but with PD-grape foliage instead of fruit clusters as acquisition sources, produced high rates of transmission by BGSS (88%). GSS exposed to vines with PD transmitted at a rate of 24%. These conditions were highly artificial, and normal post-harvest handling, transit, and storage would be expected to lower the exposure of harvested clusters to potential vectors. The low and sporadic numbers of cultivable *X. fastidiosa* cells in the stems and rachises of grape clusters perhaps best explains why BGSS was unable to acquire *X. fastidiosa* from fruit clusters.

Although we could not verify how well

Table 1. Transmission[a] of *Xylella fastidiosa* from grape bunches by blue-green sharpshooter (*Graphocephala atropunctata*)

Day no.	Fumigated cv. Thompson Seedless	Fumigated cv. Ruby Seedless	Nonfumigated cv. Ruby Seedless	No. tested	Transmission rate (%)
1	49[b]	48	51	148	0
7	32	41	44	117	0
14	74	53	...	127	0
21	81[c]	31	...	112	0
Positive[d] controls	Blue-green sharpshooters	...	...	49	88
	Green sharpshooters	...	...	37	24

[a]Transmission assessed by sharpshooter acquisition feeding on grape bunches or on diseased vines for 6 hr (controls), followed by exposure to grape test plants.
[b]Numbers of sharpshooters that survived throughout transmission tests. At least 60 insects were placed on each cultivar-fumigation treatment.
[c]Total of 84 green sharpshooters used in place of blue-green sharpshooters.
[d]Insects from 21-day test were exposed for 6 hr to Pinot noir grapevines with PD symptoms, then tested on grape test plants for transmission of *X. fastidiosa*.

the insects fed on fruit stems, feeding on succulent plant tissues appears to be a necessity for xylem feeders (5,10). Prolonged sharpshooter survival on fruit clusters alone probably would not exceed one to several days, but we did not test this directly. Fruit clusters are not attractive feeding sites for sharpshooters in the field (8). The attractiveness to sharpshooters of fruit clusters that have been harvested and stored should be even lower. In our experiments we used clusters with visual symptoms (shriveled fruits) because they should have had the greatest concentration of bacteria and thus should have been the most efficient acquisition host plants (3). The fruits and stems in grape clusters from grapevines with PD were selected from vines with distinctive PD symptoms, including dried or raisined fruits, in order to maximize transmission. Normally clusters with dried or withered fruits would not be harvested because of low quality.

ACKNOWLEDGMENTS.

This research was supported in part by a grant (TGC-CA-B-Ent-4858-H-493) from the California Table Grape Commission. We thank Barry Hill and Reggie Martinez for technical assistance.

LITERATURE CITED

1. Davis, M. J., Raju, B. C., Brlansky, R. H., Lee, R. F., Timmer, L. W., Norris, R. C., and McCoy, R. E. 1983. Periwinkle wilt bacterium: axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. Phytopathology 73:1510-1515.
2. Frazier, N. W. 1965. Xylem viruses and their insect vectors. Pages 91-99 in: Proc. Int. Conf. on Virus and Vector on Perennial Hosts, with Special Reference to *Vitis*. W. B. Hewitt, ed. University of California, Division of Agricultural Science, Davis.
3. Goodwin, P. H., DeVay, J. E., and Meredith, C. P. 1988. Roles of water stress and phytotoxins in the development of Pierce's disease of the grapevine. Physiol. Mol. Plant Pathol. 32:1-15.
4. Hewitt, W. B., Frazier, N. W., and Freitag, J. H., 1949. Pierce's disease investigations. A. J. Winkler, ed. Hilgardia 19:207-264.
5. Houston, B. R., Esau, K., and Hewitt, W. B. 1947. The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. Phytopathology 37:247-253.
6. Nomé, S. F., Raju, B. C., Goheen, A. C., Nyland, G., and Docampo, D. 1980. Enzyme-linked immunosorbent assay for Pierce's disease bacteria in plant tissues. Phytopathology 70:746-749.
7. Purcell, A. H. 1974. Spatial patterns of Pierce's disease in the Napa Valley. Am. J. Enol. Vitic. 25:162-167.
8. Purcell, A. H. 1975. Role of the blue-green sharpshooter, *Hordnia circellata,* in the epidemiology of Pierce's disease of grapevines. Environ. Entomol. 4:745-752.
9. Purcell, A. H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. Phytopathology 71:429-435.
10. Purcell, A. H. 1989. Homopteran transmission of xylem-inhabiting bacteria. Pages 243-266 in: Advances in Disease Vector Research, Vol. 6. K. F. Harris, ed. Springer-Verlag, New York.
11. Purcell, A. H. and Finlay, A. H. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology 69: 393-395.
12. Severin, H. H. P. 1949. Life history of the blue-green sharpshooter *Neokolla circellata*. Hilgardia 19:187-189.
13. Severin, H. H. P. 1949. Transmission of the virus of Pierce's disease by leafhoppers. Hilgardia 19:190-202.
14. Wells, J. M., Raju, B. C., Hung, H-Y., Weisberg, W. G., Mandelco-Paul, L., and Brenner, D. J. 1987. *Xylella fastidiosa* new-genus new-species Gram-negative xylem-limited fastidious plant bacteria related to *Xanthomonas* spp. Int. J. Syst. Bacteriol. 37:136-143.