Thrips-Facilitated Transmission of Prune Dwarf and Prunus Necrotic Ringspot Viruses from Cherry Pollen to Cucumber

R. S. GREBER and D. S. TEAKLE, Department of Microbiology, University of Queensland, Brisbane, Queensland, Australia 4072, and G. I. MINK, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser 99350

ABSTRACT

Greber, R. S., Teakle, D. S. and Mink, G. I. 1992. Thrips-facilitated transmission of prune dwarf and prunus necrotic ringspot viruses from cherry pollen to cucumber. Plant Dis. 76:1039-1041.

When pollen from sweet and sour cherry trees infected by Prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) was dusted onto cucumber seedlings and the seedlings were caged with eight to 15 *Frankliniella occidentalis* for 1 day, approximately 20% (68 of 332) of the seedlings subjected to the thrips plus infective pollen treatment became infected. Only one of 188 cucumber seedlings caged with cherry pollen only became infected. No infection occurred when thrips were caged without pollen. The rate of transmission varied greatly with the virus isolates used. The five pollen inoculum sources that gave highest transmission (25–75%) were from trees having dual (PDV + PNRSV) infections. Four of the six pollen inoculum sources giving less than 10% infection were from trees infected with either PDV or PNRSV alone. The overall transmission of PDV was four times greater than that of PNRSV. In tests with virus-contaminated sweet cherry pollen that had been stored frozen for 2 yr, PDV but not PNRSV was transmitted by *F. occidentalis* to nine of 24 (37%) cucumber seedlings.

Several attempts to find insect vectors of cherry-infecting ilarviruses have failed or have given inconclusive results (4, 15,17). George and Davidson (5,6) reported some transmission when honey bees and thrips were introduced to a cage containing both healthy sour cherry (*Prunus cerasus* L.) trees and sour cherry trees infected with Prunus necrotic ringspot virus (PNRSV). Mink (13) suggested that honey bees were involved in the long-distance spread of PNRSV from California to Washington sweet cherry (*P. avium* (L.) L.) orchards. However, hand-pollination of sweet cherry trees with infected pollen did not lead to infection with PNRSV or prune dwarf virus (PDV), although seed resulting from the pollination was often infected (14).

Accepted for publication 8 June 1992.

Reports of thrips transmission of tobacco streak virus (TSV) by thrips in South America (3) and North America (10) were followed by the demonstration in Australia (16) that both thrips and infective pollen were required for transmission of TSV to *Chenopodium amaranticolor* Coste & Reyn. Further work in Australia (8) investigated a field epidemic of TSV in tobacco and showed that pollen of *Ageratum houstonianum* Mill. was a potent inoculum for infection of several hosts using *Microcephalothrips abdominalis* (D.L. Crawford). Also the transmission of PNRSV to cucumber (*Cucumis sativus* L.) by means of field-grown plum pollen inoculum and several thrips species has been reported from Australia (7).

In the work described below tests were done to determine if PDV and PNRSV associated with virus-infected sweet and sour cherry pollen could be transmitted to cucumber seedlings by the western flower thrips, *Frankliniella occidentalis* (Pergande).

MATERIALS AND METHODS

Branches with unopened cherry flower buds were cut and used either immediately or after storage at 4 C for up to 4 wk as sources of fresh cherry pollen. The branches were placed in water at room temperature until flowers opened

and anthers ripened, as had been done with plum in previous experiments (7). Collection and cool storage of unopened buds allowed pollen to be available over a period of about 5 wk. Sweet cherry pollen was obtained from a plot of cv. Bing trees previously graft-inoculated in pairs with known isolates of PNRSV and PDV at Prosser, Washington (Table 1). Sour cherry pollen was derived from three cv. Montmorency trees at Wenatchee that had been shown by ELISA to be infected with PNRSV and PDV.

The stored pollen was collected in 1989 from anthers of unopened flowers of Bing trees infected with PDV, PNRSV, or PDV + PNRSV. The anthers were dried overnight at room temperature, and the mixture of dried anthers and released pollen was stored frozen in vials for 2 yr at −20 C. The stored pollen was dusted onto cucumber seedlings either by shaking the pollen through cheesecloth or by dipping a small brush in the preparation and flicking it to dislodge pollen over the seedlings.

Four cucumber seedlings (cv. Boston Pickling) were grown per 100-mm-diameter pot and inoculated when cotyledons were half-expanded. Cages were prepared by cutting the bottoms from plastic pill vials, 60 × 40 mm diameter, to produce cylinders. A cylinder was pushed firmly into the potting mix around each plant. Flowers were examined under a microscope to determine optimum pollen availability. A visible deposit of pollen was then brushed onto the cotyledons of the test plant from two to eight flowers from which petals had been removed.

F. occidentalis were reared on French bean (*Phaseolus vulgaris* L.) plants at 28–35 C. Adults and larvae were brushed onto white paper and aspirated into vials. Used vials were given a sharp bump on the bench to concentrate the thrips on the bottom. Groups of eight to 15 thrips were aspirated from the bottom of vials. Thrips were deposited onto pollen-dusted or nondusted individual cucumber seedlings in cages and the lid was snapped on immediately. Cages were removed after 20–30 hr in the dark at 20–22 C, and plants were sprayed with acephate insecticide at a concentration of 4 g a.i./L. Although caged seedlings of cucumbers had some free surface water when the cages were removed after 24 hr for spraying with acephate, this moisture did not appear to greatly hinder the activity of thrips, which congregated mostly on plant surfaces. Regular checks of the identity of thrips used in the experiments indicated that only *F. occidentalis* were present.

Controls consisted of untreated cucumber plants in cages, plants caged with virus-contaminated pollen only, and plants caged with thrips only. Plants were rated for symptoms between 7 and 21 days after treatment. Each plant was subsequently tested for PNRSV and PDV by enzyme-linked immunosorbent assay (ELISA) either directly or after rub-inoculation of symptomatic tissue extract to a second set of cucumber seedlings (9).

RESULTS

Thrips-facilitated virus transmission to cucumber was obtained in most experiments with *F. occidentalis* and fresh sweet cherry pollen. Infected cucumber seedlings sometimes developed primary lesions on cotyledons followed by chlorotic mottle on true leaves after about 1 wk or cessation of terminal growth. Table 1 shows the combined results of experiments with sweet cherry pollen. Of 332 cucumber plants subjected to the thrips plus infective pollen treatment, 68 (20%) developed viruslike symptoms. Symptomatic tissues from these plants were used to rub-inoculate another set of cucumber seedlings. These seedlings were then shown by ELISA to be infected with PNRSV or PDV (Table 1). Seven seedlings inoculated with pollen of trees 19–22 died before serological tests could be completed.

The rate of transmission varied greatly between pollen inoculum sources. For instance, no transmission resulted from tests with pollen from trees 5 and 6, and only 3% infection was obtained with pollen from trees 17 and 18. In contrast, 50% of test plants were infected when pollen from trees 19 and 20 or from trees 36 and 37 was used and 75% with pollen from trees 23 and 24. Similar transmission rates were obtained from individual trees of the pairs inoculated with the same isolates. The five sources giving transmission rates between 25 and 75% were all dual infections, and only PDV was transmitted from four of the five sources. Four of the six pollen inoculum sources that gave less than 10% transmission were from trees infected with either PNRSV or PDV. Overall, PDV was transmitted four times more frequently than PNRSV.

Two transmissions each of PNRSV and PDV from sour cherry pollen sources were confirmed by ELISA (Table 1). However, no transmission was obtained when pollen from infected *P. mahaleb* L. trees was used as inoculum. Anthers of this species open only slightly, compared with full display of pollen by sweet and sour cherry anthers. Little pollen could be collected even by brushing anthers of the *P. mahaleb* flowers.

Of the control combinations, only one cucumber seedling dusted with pollen was infected (Table 1). This seedling could have been infected by virus entering chance mechanical wounds or, more likely, by stray thrips.

In each of two experiments with stored pollen, PDV was transmitted by *F. occidentalis* from two pollen sources, with nine of 24 cucumber seedlings infected. PNRSV was not transmitted by thrips from any of three pollen sources

Table 1. Transmission of prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV) by means of cherry pollen and *Frankliniella occidentalis*

Cherry pollen source			Cucumber seedling test plants				
	Virus content			Number positive by symptoms	Positive ELISA		Not identified
Tree number[a]	PNRSV	PDV	Number inoculated		PNRSV	PDV	
1 and 2	+	−	4	1	1	0	...
3 and 4	+	−	12	1	1	0	...
5 and 6	+	−	20	0	0	0	...
7 and 8	+	−	24	1	1	0	...
9 and 10	+	−	4	1	1	0	...
29 and 30	+	−	12	1	1	0	...
27 and 28	−	+	20	3	0	3	...
34 and 35	−	+	16	0	0	0	...
11 and 12	+	+	2	0	0	0	...
13 and 14	+	+	34	10	0	10	...
15 and 16	+	+	12	1	0	1	...
17 and 18	+	+	32	1	0	1	...
19 and 20	+	+	22	11	0	8	3
21 and 22	+	+	28	11	2	5	4
23 and 24	+	+	12	9	0	9	...
25 and 26	+	+	4	1	1	0	...
31 and 33	+	+	8	2	2	0	...
36 and 37	+	+	20	10	0	10	...
SC1	+	+	14	2	2	0	...
SC2	+	−	12	0	0	0	...
SC3	−	+	20	2	0	2	...
Total pollen and thrips			332	68	12	49	7
Total pollen only			188	1	1	0	0
Total thrips only			48	0	0	0	0
Total no treatment			80	0	0	0	0

[a]Trees 1–37 = sweet cherry cv. Bing at Prosser, Washington, graft-inoculated in pairs with isolates of PNRSV, PDV, or PNRSV + PDV. Trees SC1–SC3 = sour cherry cv. Montmorency at Wenatchee, Washington, naturally infected with PNRSV, PDV, or PNRSV + PDV.

that contained PNRSV alone or combined with PDV.

DISCUSSION

This is the first report of transmission of PDV by thrips and of the transmission of PDV and PNRSV by *F. occidentalis*. The efficiencies of transmission to cucumber were comparable to those obtained previously using plum pollen and other thrips species (7).

Rates of transmission in different experiments ranged from 0 to 40% (30 of 70 inoculated cucumber seedlings infected). This may be due in part to differences in the virus concentration in the pollen preparations. Other factors may also be involved, such as variations in the susceptibility of different batches of cucumber seedlings.

Although thrips inoculation of cucumber seedlings with PDV and PNRSV has now been demonstrated, this has yet to be extended to *Prunus* trees. Infection of cherry trees was associated with flowering (5), but these early experiments did not establish whether the floral structures provided the infection court, vector habitat, or both. Pollen transfer between adjacent trees could result directly through the activity of bees or other flying insects or possibly by wind movement. Bees were observed to dislodge pollen from ripe cherry anthers; thus, pollen transfer between trees could result directly from this dislodgment on intertwined branches or by wind movement.

Pollen transport by thrips is limited by their small size and the tendency for adult thrips to preen before takeoff. However, thrips can dislodge and scatter pollen (1). Some *F. occidentalis* were found in late cherry flowers at Prosser in 1991 but were not common that year. When groups of *F. occidentalis* were caged with either sweet or sour cherry blossoms, the insects congregated in the bell-shaped gynaecial area around the filament bases, from where they foraged for pollen and also fed on flower parts. During feeding, pollen was pierced by the thrip's mouthparts while the pollen grains were held down against the plant surface. Epidermal cells were often pierced with a faster feeding sequence.

Although pollination in sweet cherry often resulted in seed infection, the mother trees remained healthy (14). However, purified virus rubbed on petals and styles resulted in tree infection (G. I. Mink, *unpublished*). Infection of epidermal cells around the ovary or on cluster bracts mediated by thrips and infected pollen would provide an infection route quite different from that involved in the ovary-limited infection that follows fertilization. Fertilization may play a role in the stabilization of gynaecial tissues to allow further spread of the virus.

The exact origin of the PDV and PNRSV associated with cherry pollen transmitted by thrips to cucumber seedlings is not known. Conceivably, it could be virus external to the pollen (2,11), or it could leak from the pollen cytoplasm, especially after the wall is punctured during thrips feeding (12). Infection could occur by virus lodging against a cucumber leaf cell damaged by thrips feeding. Also, the presence of free water on the leaves in the form of dew might cause some pollen to swell and help distribute virus so that it reached wounds. The ability of *F. occidentalis* to transmit PDV in washings of sweet cherry pollen to cucumber seedlings has been demonstrated (R. Sdoodee, *unpublished*).

The ability of *F. occidentalis* to transmit PDV from sweet cherry pollen stored frozen for 2 years at −20 C is of interest from an epidemiological view. Both PDV and PNRSV are remarkably stable when associated with pollen. Both are readily recovered by mechanical inoculation from pollen stored frozen for 2 yr or more. Because stored pollen is often available to growers through commercial pollen companies, viruses associated with this pollen could play a role in disseminating these viruses even in isolated, virus-free plantings (13,14). The relative stability of PDV and PNRSV associated with infected pollen is undoubtedly important in their transmission by thrips.

ACKNOWLEDGMENTS

We thank W. Howell, W. Cone, and M. Conant for much advice and assistance. Financial support was provided by the IR-2 Project at Washington State University and the University of Queensland. PPNS No. 0119. Project 1262. College of Agriculture and Home Economics Research Center, Washington State University, Pullman 99164.

LITERATURE CITED

1. Anonymous. 1988. *Frankliniella occidentalis*—Biology and control. EPPO Publ. Ser. B, No. 91.
2. Cole, A., Mink, G. I., and Regev, S. 1982. Location of Prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathology 72:1542-1545.
3. Costa, A. S., and da Costa Lima Neto, V. 1976. Transmissao do virus da necrose branca do fumo por *Frankliniella* sp. Congr. Soc. Bras. Fitopatol. No. 9.
4. Fulton, R. W. 1970. Prunus necrotic ringspot virus. No. 5 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England.
5. George, J. A., and Davidson, T. R. 1963. Pollen transmission of necrotic ringspot and sour cherry yellows viruses from tree to tree. Can. J. Plant Sci. 43:276-288.
6. George, J. A., and Davidson, T. R. 1964. Further evidence of pollen transmission of necrotic ringspot and sour cherry yellows virus in sour cherry. Can. J. Plant Sci. 44:383-384.
7. Greber, R. S., Klose, M. J., Milne, J. R., and Teakle, D. S. 1991. Transmission of prunus necrotic ringspot virus using plum pollen and thrips. Ann. Appl. Biol. 118:589-593.
8. Greber, R. S., Klose, M. J., Teakle, D. S., and Milne, J. R. 1991. High incidence of tobacco streak virus in tobacco and its transmission by *Microcephalothrips abdominalis* and pollen from *Ageratum houstonianum*. Plant Dis. 75:450-452.
9. Howell, W. E., and Mink, G. I. 1988. Natural spread of cherry rugose mosaic disease and two Prunus necrotic ringspot virus biotypes in a central Washington sweet cherry orchard. Plant Dis. 72:636-640.
10. Kaiser, W. J., Wyatt, S. D., and Pesho, G. R. 1982. Natural hosts and vectors of tobacco streak virus in eastern Washington. Phytopathology 72:1508-1512.
11. Kelley, R. D., and Cameron, H. R. 1986. Location of prune dwarf and Prunus necrotic ringspot viruses associated with sweet cherry pollen and seed. Phytopathology 76:317-322.
12. Kirk, W. D. J. 1984. Pollen feeding in thrips (Insecta: Thrysanoptera). J. Zool. Soc. London 204:107-117.
13. Mink, G. I. 1983. The possible role of honeybees in long distance spread of Prunus necrotic ringspot virus from California into Washington sweet cherry orchards. Pages 85-91 in: Plant Virus Epidemiology. R. T. Plumb and J. M. Thresh, eds. Blackwell Scientific Publications, Oxford, England.
14. Mink, G. I. 1991. Ilarvirus vectors. Adv. Dis. Vector Res. 9:261-281.
15. Phillips, J. H. H. 1951. An annotated list of Hemiptera inhabiting sour cherry orchards in the Niagara Peninsula. Can. Entomol. 83:194-205.
16. Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by *Thrips tabaci*: A new method of plant virus transmission. Plant Pathol. 36:377-380.
17. Swenson, K. G., and Milbrath, J. A. 1964. Insect and mite transmission tests with Prunus necrotic ringspot virus. Phytopathology 54:399-404.