# Evidence for Noncirculative Transmission of Pierce's Disease Bacterium by Sharpshooter Leafhoppers

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## ABSTRACT

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Half of the leafhoppers (*Graphocephala atropunctata*) allowed acquisition access on grapevines affected with Pierce's disease (PD) became infective within 2.0 hr, and there was no significant increase in acquisition beyond 24 hr. The median inoculation access period was 3.9 hr. Three of 34 (9%) insects transmitted after 1 hr each for acquisition and for inoculation, which was in close agreement with estimates for which no latent period was assumed. Neither *G. atropunctata* nor *Draeculacephala minerva* retained infectivity after molting. The loss of infectivity after molting and lack of a latent period suggest a noncirculative mechanism of transmission of the PD bacterium by leafhoppers.

*Additional key words*: *Hordnia*, *Graphocephala*, *Draeculacephala*, lucerne dwarf, alfalfa dwarf, almond leaf scorch, rickettsia-like bacteria, stylet-borne.

Pierce's disease (PD) of grapevines usually is lethal to grapevines (*Vitis vinifera*); periodically it has caused serious losses to the California grape industry and it has precluded successful production of bunch grapes in the southeastern USA (5). Bacteria transmitted by xylem-feeding suctorial insects cause PD in grape (2) and also are thought to cause alfalfa dwarf (7) and almond leaf scorch (9) diseases.

Long considered a virus disease, PD has been unusual in its vector-pathogen relationships in several respects: (i) a large but distinct group of vectors (xylem-feeding suctorial insects such as sharpshooter leafhoppers of the subfamily Cicadellinae and spittlebugs [Cercopidae] [3]) (ii) a minimum or required latent period of less than 2 hr, and (iii) persistence of infectivity for long periods (perhaps permanently) in adult vectors (14). Although sharpshooter nymphs can transmit PD (14) apparently no studies of transtadial passage (retention of infectivity by vectors after molting) have been made (12). The purpose of these studies was to investigate the pathway of transmission in the vector by evaluating the effects of molting and of varying acquisition and inoculation access times on transmission of the PD bacterium. A preliminary report has been published (11).

## MATERIALS AND METHODS

The leafhopper *Graphocephala atropunctata* (Signoret) (= *Hordnia circellata* Baker) (16) was collected from natural populations in Berkeley, CA. To test for infectivity with the PD bacterium, *G. atropunctata* used in acquisition tests first were pretested on rooted Pinot Noir cuttings for four days or longer (13). The results with naturally infective insects were not used in analyses of acquisition data. *Draeculacephala minerva* Ball was colonized on barley cultivar Atlas from the progeny of leafhoppers reared from eggs hatched in petri dishes on moist paper (4).

Grapevines with advanced PD symptoms (6) were used as acquisition source plants. Test plants were 4–6-wk-old seedlings of Pinot Noir and Ruby Cabernet grapevines. Insects were caged in 10 × 5 cm diameter plastic and mesh cages on seedlings. Rooted cuttings of Pinot Noir were used in some tests. Test plants and insects were placed in a greenhouse heated to an average temperature of 23 C. After exposure to leafhoppers, test plants were sprayed with dimethoate (Cygon® American Cyanamid Co., Princeton, NJ 08540) 25% WP in water at recommended rates and held in a heated greenhouse. Symptoms of PD normally appeared after 10–14 wk. Grapevines without symptoms of PD for 22–25 wk were recorded as free of PD.

**Acquisition tests**. Fifty to sixty adult and nymphal *G. atropunctata* previously exposed to pretest plants for 4 days or longer were placed for 1, 3, 6, 12, 24, and 48-hr acquisition access periods (AAP) on PD source plants and then caged singly for a 4-day inoculation access period (IAP) on test plants. Leafhoppers allowed the 1-hr AAP were immediately transferred to test plants for 1 hr and then to a second set of test plants for 4 days.

**Inoculation access period (IAP)**. Fifty adults and nymphs of *G. atropunctata* with AAP of 2 days or more were transferred singly to test plants for IAP of 1, 3, 6, 12, 24, 48, and 96 hr. One group was transferred in the sequence of 1, 48, and 12 hr. Because of cumulative mortality to the first group, a second group fed on the same source plants was transferred in the sequence of 3, 24, 96, and 6 hr in order to allow all transfers to be made during daylight hours. Adult *D. minerva* were tested for 6, 12, and 24-hr IAP.

**Effect of molting on vector transmission**. First through fifth instar nymphs with an AAP of 48 hr or more were confined individually on test seedlings. Plants were inspected daily (exceptions noted) and insects transferred approximately weekly or as soon as molting was noted. In one experiment, insects were transferred to new plants every 2 days or as soon as a molt was detected. After access to at least one test plant following molting, test insects usually were transferred to a PD source for 1 day or in a few cases for 2 days and then continued on a sequence of test plants.

## RESULTS

**Effects of AAP and IAP**. *Graphocephala atropunctata*. The percentage of insects that transmitted PD was correlated significantly ($P < 0.01$) with AAP (Fig. 1). Using the probit-log method (15) the median AAP estimated from the regression was 2.0 hr. A similar relationship for IAP estimated a median IAP of 3.9 hr (Fig. 1). Three of 34 (9%) *G. atropunctata* given a 1-hr AAP transmitted in the following hour, thus demonstrating a minimum latent period of no more than 2 hr. As evident from Fig. 1, IAPs of 24 hr or more produced high transmission rates of about 90%, which did not differ significantly ($\chi^2 = 1.37$, $P > 0.5$) from each other.

*Draeculacephala minerva*. Adult *D. minerva* were much less efficient and more sporadic in transmission to grape than were *G.*

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*atropunctata*. In tests using Pinot Noir cuttings with an AAP of 2 days and a 2 to 6-hr IAP, overall transmission efficiency was 24 108 (22%). Three of 16 *D. minerva* transmitted to grape during a 24-hr IAP, but failed to infect a second plant during a 6-day IAP. On the other hand, two insects transmitted to the second but not the first plant. In other serial transfers to grape seedlings, surviving *D. minerva* transmitted to 5 of 32 (16%) plants during a 6-hr IAP but to only 1 of 25 (4%) with a 24-hr IAP.

**Transtadial passage**. In one experiment with nymphs of *D. minerva*, 2 of 12 first or second instar nymphs transmitted before molting and none thereafter. In a second experiment, none of 30 first instar nymphs transmitted before or after molting. Because survival on grape and transmission of the bacterium by *D. minerva* was relatively poor, no further tests of transtadial passage were made with this species.

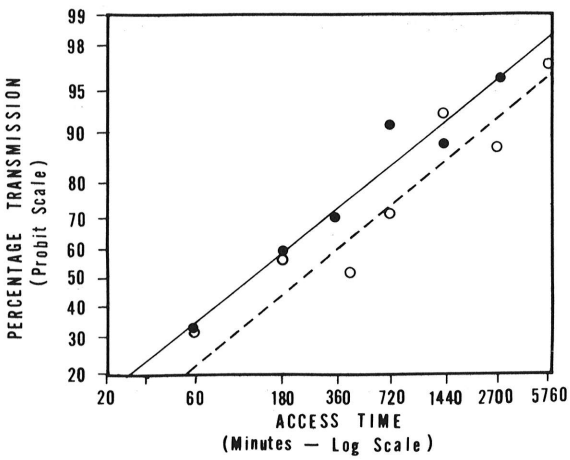


**Fig. 1.** *Graphocephala atropunctata* transmission of Pierce's disease (PD) at various acquisition and inoculation access times. Solid symbols indicate percentages of insects that transmitted during a 4-day test period after six different acquisition access periods on grape. Solid line is regression of acquisition y (probits) = 2.32 + 1.29 x (log minutes); $r^2 = 0.95$. Open symbols indicate percentage (PD) transmission after various inoculation access periods following a 48-hr acquisition access period on diseased grape. Broken line is regression of inoculation y (probits) = 1.99 + 1.27 x (log minutes); $r^2 = 0.88$.

In one experiment with 10 and a second one with eight nymphs, *G. atropunctata* that had been exposed to diseased source plants transmitted to a total of 24 of 69 (35%) test plants before molting. Insects that molted on test plants did not transmit to any of the 57 test plants on which they subsequently were confined. In a third experiment, however, 3 of 9 adult *G. atropunctata* transmitted the PD bacterium to test plants to which they had been transferred after molting. The circumstances under which the three insects that transmitted after a molt differed from other transfers in two ways. First of all, the nine nymphs in this transfer sequence had not been checked for molting in 3 days and thus could have been on the plants for as long as 3 days after molting. Secondly, all of the three insects that transmitted after molting had transmitted PD bacterium to the previous test plants and had remained on the plant on which they molted for 10 to 16 days. Finally, each of the three test plants infected by pathogen transmission via the molted insects required more than 16 wk for PD symptoms to appear, suggesting that a smaller amount of inoculum was transmitted after molting.

To test the possibility that the leafhoppers reacquired the PD bacterium from the plants that had become infected before the insect molted, we inoculated grape seedlings with single *G. atropunctata* transferred from PD-infected source plants and then exposed these plants to *G. atropunctata* adults previously tested for infectivity on grape. None of the pretest plants developed PD, but 5 of 12 pretested leafhoppers transmitted the PD bacterium after a 4-day exposure to grapevines inoculated 7 days before. Thus, *G. atropunctata* was able to acquire the PD bacterium from grapes infected 7 days previously.

We further tested the retention of infectivity after molting by *G. atropunctata* in two subsequent trials. In the first experiment, begun with 50 nymphs fed on PD source plants, nymphs transmitted to 48 of 61 (79%) test plants before molting, but to none of 41 plants after molting. The complete transmission results for seven of these insects are detailed in Fig. 2. In a final trial in which nymphs were transferred every other day or within 24 hr after molting, 13 leafhoppers transmitted to a total of 25 of 67 (39%) plants before molting and to none of 17 test plants after molting within the first eight transfers. One 4th instar nymph never molted in 12 transfers and infected all 12 test plants with the PD bacterium. Two other nonmolting nymphs transmitted to the first test plant but none of eight subsequent test plants. One of these nymphs lived for 21 additional transfers without molting or transmitting.

## DISCUSSION

The lack of a required latent period is implicit in the aphid transmission of nonpersistent or stylet-borne viruses because

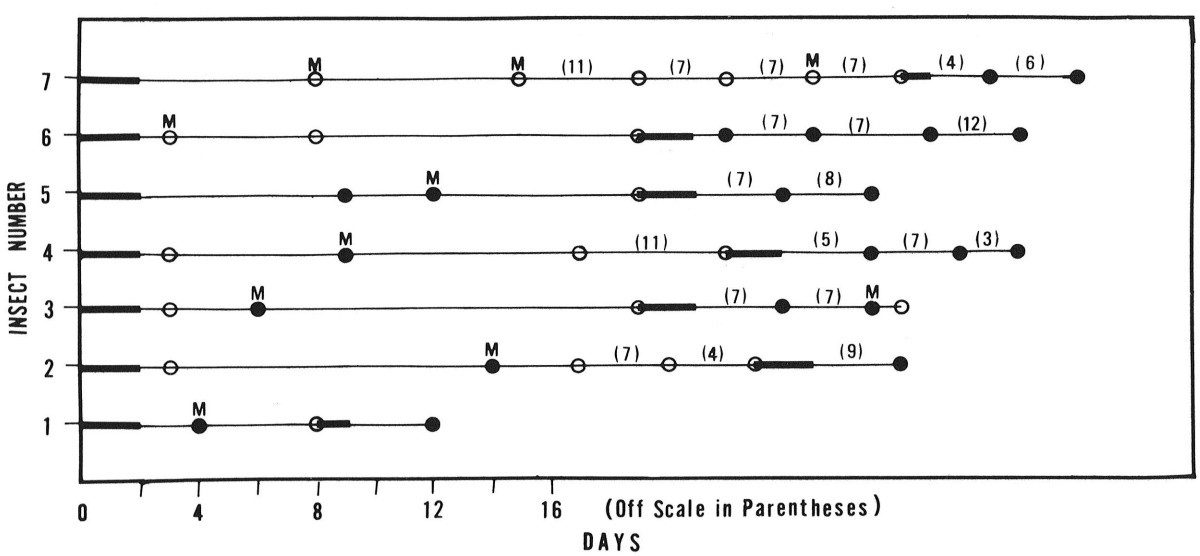


**Fig. 2.** Representative transmission records of individual *G. atropunctata* nymphs: Heavy solid line indicates access period on diseased grape source plant; M = molted on test plant; circles indicate change of test plant; solid circles indicate test plant developed PD symptoms; open circles indicate no PD transmission.

transmission can occur after a brief acquisition probe, transfer, and transmission probe—all accomplished within seconds. With tests of the transmission of the PD bacterium, however, leafhopper feeding behavior precludes using such brief acquisition and inoculation exposures. If there is a required latent period, fewer infective leafhoppers should transmit during the 1 hr following an AAP of 1 hr than those allowed a longer acquisition. In our experiments, the rate of transmission (9%) after a 1-hr AAP and a subsequent 1-hr IAP was not significantly different from the percentage transmission predicted by the product of independently observed hourly acquisition (30%) and hourly transmission rates (30%). We conclude that there is no evidence of a required latent period.

With only three exceptions, none of 110 *G. atropunctata* nymphs transmitted the Pierce's disease bacterium after molting. Leafhoppers could reacquire the bacterium from plants infected only 1 wk previously. The three exceptions to transtadial passage that were noted probably resulted from nymphs reacquiring the PD bacterium from the plants on which they had molted. We conclude that leafhopper vectors of the PD bacterium do not retain infectivity following molting.

The loss of infectivity after molting offers a substantial insight into the mechanism of leafhopper transmission of the PD bacterium. Since the foregut is shed at molting, the inoculum is probably in the foregut or mouthparts. The very short time between acquisition and transmission further suggests that the causal bacteria are not circulated via a blood-salivary route before transmission can occur.

Two other leafhopper-borne pathogens, rice tungro virus (RTV) (8) and maize chlorotic dwarf virus (MCDV) (10) lack transtadial passage and can be acquired and inoculated within hours, but the infectivity of RTV and MCDV diminishes within hours of acquisition. The transmission characteristics of the PD bacterium are similar in several respects to stinkbug (Pentatomidae) transmission of the fungus *Nematospora coryli* (12). In both cases, there is no evidence of latency in the vector and infectivity is retained for prolonged periods but lost after molting. Stinkbugs retained *N. coryli* after feeding on suspensions of inoculum (1). However, a very low percentage (4%) of *G. atropunctata* transmitted the PD bacterium after feeding on suspensions of the bacterium through a membrane or having their mouthparts dipped into colonies of the bacterium (2). These results suggest a biological relationship between vector and pathogen more intimate than simple mechanical contamination of mouthparts.

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