plant disease

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Journal:	*Plant Disease*
Manuscript ID:	PDIS-12-12-1115-PDN
Manuscript Type:	Plant Disease Note
Date Submitted by the Author:	03-Dec-2012
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Keywords:	Viruses and viroids < Causal Agent, tree fruits < Fruit < Crop Type, disease development and spread < Epidemiology < Subject Areas

Little cherry virus 2 is transmitted to sweet cherry by *Pseudococcus maritimus* (Ehrhorn), a new vector of this virus

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Little cherry virus 2 (LChV2; genus *Ampelovirus*, family *Closteroviridae*) is associated with little cherry disease (LCD), one of the most economically destructive diseases of sweet cherry in North America (1). Since 2010, reported incidents of LCD caused by LChV2 has increased dramatically in orchards of Washington State. LChV2 is known to be vectored by apple mealybug (*Phenacoccus aceris*) (3), however the introduction of *Allotropus utilis*, a parasitoid platygastrid wasp (2) as a biological control, kept the population below economic thresh hold. In recent years, populations of grape mealybug, *Pseudococcus maritimus* (Ehrhorn) is increasing in area cherry orchards. Since grape mealybug can transmit *Grapevine leafroll associated virus 3,* also a member of the genus *Ampelovirus,* in grapevine (4), in this study, the potential of grape mealybugs to transmit LChV2 was investigated. A colony of grape mealybugs on myrobalan plum (*Prunus cerasifera*) trees was identified. In a growth chamber, first and second instar crawlers were placed on shoots cut from a sweet cherry tree infected with the North American strain of LChV2 (LC5). After an acquisition period of 7 days, 50 crawlers were transferred to each potted virus-free sweet cherry tree, cultivar 'Bing'. After 1 week, trees were treated with pesticide to eliminate the mealybugs. This process was repeated on two separate groups of trees to yield a total of 21 young cherry trees exposed to potentially viruliferous mealybugs. Two to four months after the inoculation period, leaves were collected from each of the recipient trees and tested by RT-PCR for the presence of virus. To reduce the possibility that the positive reaction in RT-PCR was the result of virus-contamination of mealybug debris on the leaf surface but not transmitted, trees were allowed to naturally defoliate and then given a 3 month dormant period. New growth that emerged was then tested. Two sets of primers: LC26L (GCAGTACG-TTCGATAAGAG) and LC26R (AACCACTTGATAGTGTCCT) (1); and LC2.13007F (GTTC-GAAAGTGTTTCTTGA) and LC2.14545R (CATTATYTTACTAATGGTATGAC) (*this study*) were used to amplify partial replicase (409bp) and complete (1080bp) coat protein genes of LChV2, respectively. Of the total of 21 trees tested, 18 yielded positive results for LChV2. Identity of the amplicons from the six randomly infected trees were cloned and verified by sequencing. Sequences of amplication products from both primer pairs showed ≥99% similarity with LChV2, strain LC5 (GenBank AF416335). The test results confirmed that grape mealybug is an efficient vector of LChV2. The result is very significant for this major production region. Grape mealybug populations are an increasing concern in the tree fruit industry because they are difficult to control in established orchards. The presence of infected orchards that serve as reservoirs of LCD along with this abundant insect pest creates a menacing combination. To our knowledge this is the first report to show transmission of LChV2 by grape mealybug.

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