

# Visual inspections of nursery stock fail to protect new plantings from *Blueberry scorch virus* infection

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## ARTICLE INFO

### Article history:

Received 4 October 2010

Received in revised form

1 February 2011

Accepted 6 February 2011

### Keywords:

*Carlavirus*

Virus transmission

Propagation

*Vaccinium corymbosum*

Highbush blueberry

ELISA

## ABSTRACT

*Blueberry scorch virus* (BIScV) is one of the most pervasive pathogens of highbush blueberry. The virus is aphid-vectored and exhibits a latent period between infection and symptom expression in the host plant of up to 5 years. In many cases, we have observed BIScV symptom expression in new fields that appears inconsistent with aphid-vectored introduction and spread. It was, therefore, speculated that the virus may be introduced through infected nursery stock. To examine this possibility, we first surveyed selected nurseries to determine if mother plants, used for propagation by cuttings, were BIScV-infected. Two nurseries were found to harbor symptomless, infected, mother plants (cv. Duke). Cuttings from one nursery were collected from infected and non-infected plants and rooted in propagation beds. The survival and infection of cohorts from each mother plant were determined one year after planting. A significantly greater proportion of cuttings survived from non-infected mother plants (0.7) than from infected mother plants (0.5). Of the cohort from infected mother plants that survived, 40% tested positive for BIScV. We also surveyed the distribution of infected, symptomatic plants in recent 'Duke' plantings that originated from nurseries with BIScV-infected mother plants and compared distribution with older plantings with more advanced BIScV outbreaks. In all cases, the distribution of BIScV symptom development in young fields was random, which is consistent with introduction from planting stock. Older plantings showed a strong clustered distribution, which is consistent with aphid transmission. This study identifies infected nursery stock as an important source of BIScV dissemination and underscores the importance of having symptomless mother plants virus tested.

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## 1. Introduction

*Blueberry scorch virus* (BIScV) is one of the most pervasive pathogens of highbush blueberry (*Vaccinium corymbosum* L.). The disease was first described in New Jersey as Sheep Pen Hill disease (Stretch, 1983) and was named blueberry scorch in Washington where the causal agent was characterized as a *Carlavirus* (Martin and Bristow, 1988). The disease is now common across the north-eastern and northwestern growing regions of North America, and it was found in Europe for the first time in 2005 (Ciuffo et al., 2005). In 2009, BIScV was detected in field-grown blueberry plants in Michigan, the largest blueberry-producing state in the U.S. (<http://magissues.farmprogress.com/mif/MF09Sep09/MF18to35.html>, accessed 9/29/2010).

BIScV exhibits a latent period in plants that may extend to years (Bristow et al., 2000), which is typical of viruses in the *Carlavirus* group. The virus is transmitted in a non-persistent fashion by aphids including *Illinoia pepperi* and *Ericaphis fimbriata* (Bristow et al., 2000). Once established in a field, spread occurs most commonly along a row where bushes contact each other (A.W. Stretch, personal communication) or in a radial or clustered pattern (Bristow et al., 2000). This is typical of an aphid-vectored virus although aphids in the alate (winged) stage can potentially move further distances. There has been no satisfactory explanation for movement into newly planted fields although the role of reservoirs of the virus in or near fields such as alternate hosts has been speculated (Wegener et al., 2004, 2007).

Blueberry shoestring virus (BSSV) is transmitted by aphids in a persistent, circulative manner (Ramsdell, 1995). It has been shown that BSSV field-distribution is clumped and tends to spread bush to bush (Lesney et al., 1978). This distribution is likely due to transmission by apterous (non-winged) aphids which are present throughout most of the growing season and only a small

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percentage of the aphids are alate early in the season (Morimoto and Ramsdell, 1985). Field incidence of mild yellow-edge virus, an aphid-transmitted virus in strawberries, was also observed to occur in runs or clumps, but direct measurement of infected plants showed a random pattern suggesting that alate aphids were also involved in field spread (Converse et al., 1979).

A routine survey of BLSv symptom expression in several fields in New Jersey revealed that older fields typically exhibited the expected clumped or down-the-row pattern of symptom expression whereas the distribution in some young fields appeared to be more random. We hypothesized that the mechanism of introduction and/or spread of the virus in young fields differs from that in older fields, perhaps by introduction via infected nursery stock.

Highbush blueberry plants are asexually propagated through hardwood or softwood cuttings (Mainland, 2006). The cuttings are usually collected from mature 'mother' plants that may be BLSv-infected and thus spread the virus through rooted cuttings. Standard management practices for mother plants include annual pruning and harvesting of shoots for propagation. Typically, all of the overwintering aerial growth is removed each spring so that only new growth is present for propagation stock during the following season. This practice was implemented to allow identification of phytoplasma-infected mother plants, but we contend that it masks BLSv symptom expression because flower buds do not form on current season growth. Thus, BLSv-infected mother plants will remain symptomless and may serve as an important source of virus dissemination.

The objectives of this study were to 1) to map the incidence of BLSv in selected fields to verify the distribution pattern, 2) to determine if BLSv was present in asymptomatic mother plants; and 3) to determine if cuttings from BLSv-infected mother plants would root, survive and transmit the virus under typical propagation conditions.

## 2. Materials and methods

### 2.1. Determination of field incidence

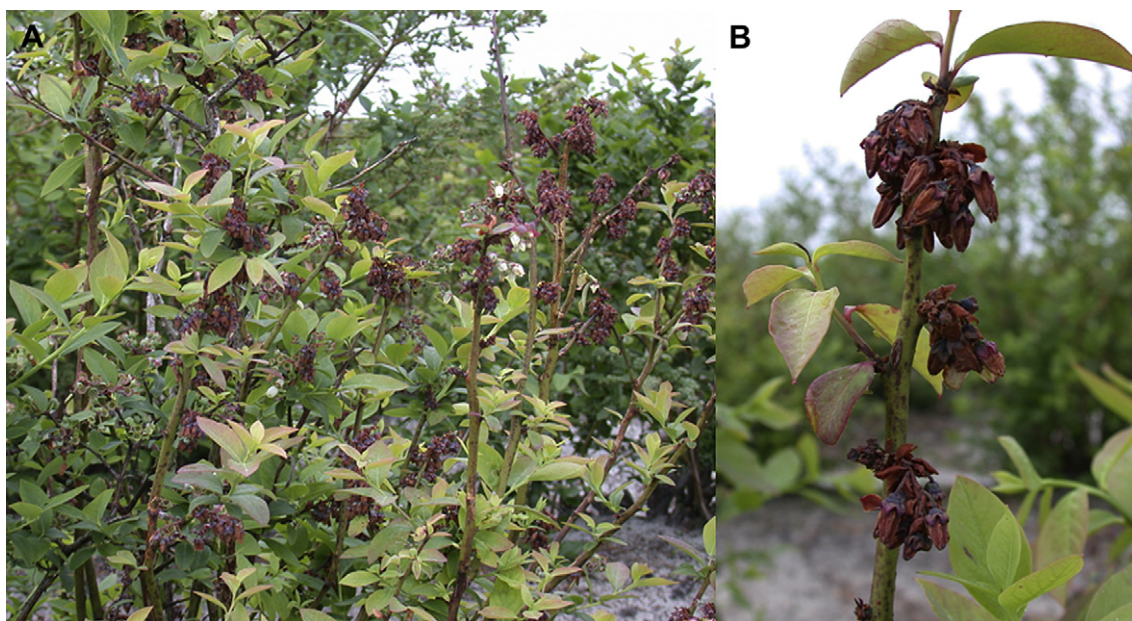
Plants showing typical floral symptoms of BLSv infection (Fig. 1) were flagged and their locations recorded using a Trimble Geo XT

GPS (Trimble Navigation Limited, Sunnyvale, CA). The location data were corrected using the Differential Correction Wizard in GPS Pathfinder Office ver. 3.1 (Trimble Navigation Limited). The location data were exported to ArcView 3.1 (ESRI, Redlands, CA) using NJ State Plane, NAD 83, coordinate parameters. Digital color infrared (CIR) orthophotography was provided by the New Jersey Department of Environmental Protection, Bureau of Geographic Information Systems (<http://www.state.nj.us/dep/gis/>). The CIR orthophotography was 30.48 cm pixel resolution so individual plants could easily be identified and the GPS data were within 61 cm (personal observation) making it possible to assess the distribution of symptomatic plants. Five fields were inventoried for the incidence of symptoms of BLSv (Table 1 and see for example Fig. 2). Three of the fields (FF1, GF1 and GF3) were planted with the highbush blueberry cultivar Duke 3–4 years prior to sampling. The other fields (BF1 and GF2) were planted with the cultivar Elliott. Blueberry scorch symptoms were noted in these fields in 1995 and the growers had not initiated an infected plant removal program when the inventory was conducted in 2003.

To determine the dispersion indices for each field, the distribution of BLSv-infected plants was evaluated by placing quadrats over the field using the ArcView extension Sample 3.03 (Quantitative Decisions, Rosemont, PA). Quadrats were 15.24 m square and were continuous over the fields (Fig. 2). The number of symptomatic plants in each quadrat was determined by assigning the location of each point to the appropriate quadrat (ArcView 3.3 map calculator). The data were analyzed using the Indices of Dispersion (quadrat counts) module in Ecological Methodology ver. 7.0 (Exeter Software Co. Setauket, NY).

### 2.2. Mother plants and determination of BLSv infection

Eighteen asymptomatic plants (cv. Duke) were selected from a single mother-block in a commercial nursery (Table 1). The mother plants were tested in the summer of 2002 for BLSv by ELISA using Agri-Check detection kits (Hydros, Inc., Falmouth, MA) (DeMarsay et al., 2004). Subsequent ELISA tests were performed in standard plate assays as described by Martin and Bristow (1988) using the antisera described by DeMarsay et al. (2004).



**Fig. 1.** Symptoms of Blueberry scorch virus infection on the highbush blueberry, cultivar Duke. Mother plants used for propagation do not flower and therefore do not exhibit obvious symptoms of virus infection. A) Overall appearance of bush showing 'scorched' flowers; and B) Close up of blighted blossoms.

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