Recovery of Cranberry Plants Infected With *Tobacco streak virus* and Incidence and Distribution of TSV in the Field

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Abstract

Wells-Hansen, L. D., and McManus, P. S. 2016. Recovery of cranberry plants infected with *Tobacco streak virus* and incidence and distribution of TSV in the field. *Plant Dis.* 100:2257-2265.

Tobacco streak virus (TSV) has been detected in cranberry plants in Wisconsin, New Jersey, and Massachusetts, and is associated with berry scarring symptoms. In the current study, cv. Mullica Queen plants that produced scarred, symptomatic, TSV-positive fruit in one year produced nonscarred, asymptomatic, TSV-positive fruit in subsequent years, consistent with the “recovery” phenomenon previously documented in other ilarvirus-woody plant interactions. In field trials, fruit set and berry weight were significantly reduced ($P < 0.05$) in symptomatic, TSV-positive cranberry shoots but not in recovered, TSV-positive shoots compared with healthy, TSV-negative shoots. Likewise, return bloom in the

first year following berry scarring was not negatively impacted in recovered shoots. Detection of TSV in various plant parts throughout the growing season was more variable in symptomatic shoots than in recovered shoots. Of all plant parts tested, TSV detection was lowest in berries at the time of harvest in both symptomatic and recovered shoots. Minimal increases in incidence of TSV-infected shoots in cranberry beds from one year to the next, and spatial autocorrelation of TSV-infected shoots, suggest that most new infections result from TSV spreading within a bed, rather than frequent introductions of the virus from external sources.

Cranberry (*Vaccinium macrocarpon* Aiton) is an important fruit crop in Wisconsin, ranking first by acreage and economic value. Wisconsin produces approximately 60% of the U.S. cranberry crop, making it the world's leading producer of the fruit (Colquhoun and Johnson 2010; USDA NASS 2015). Cranberry is a woody, low-growing perennial that produces horizontal stolons, hereafter referred to as “runners,” that allow the plant to spread by growing across the surface of the soil. Short vertical shoots, hereafter referred to as “uprights,” arise from runners. Uprights can produce either vegetative or mixed buds, and therefore fruit are borne on uprights, usually biennially (Roper et al. 1993; Vanden Heuvel and DeMoranville 2009).

Tobacco streak virus (TSV), the type member of the genus *Ilarvirus*, emerged as a potential threat for the cranberry industry when it was first detected in association with berry scarring symptoms in infected cranberry plants in Wisconsin in 2012 and later in Massachusetts and New Jersey, other major cranberry production regions (Wells-Hansen et al. 2016). Infected plants exhibit a shock reaction where berries become scarred and disfigured during the early fruit set stage of development, while leaves and other plant parts remain healthy in appearance. Affected berries either abort or mature with severe deformation and stunting (Wells-Hansen et al. 2016). A shock reaction occurs in blueberry (*V. corymbosum*) and sour cherry (*Prunus cerasus*) infected with other ilarviruses, *Blueberry shock virus* (BShV) and *Prunus necrotic ring-spot virus*, respectively (MacDonald et al. 1991; Mink 1995). Blueberry and sour cherry plants recover from, or become tolerant to, these viruses, and infected plants bear normally after recovery from the initial shock symptoms (Bristow et al. 2002; Davidson and George 1965). It is not known if cranberry plants recover from infection with TSV. Likewise, the effect of TSV on yield and yield components in cranberry in years following symptom appearance is not known.

In many crops, TSV is transmitted on vegetatively propagated materials (Ghanekar and Schwenk 1974; Greber et al. 1991). Avoiding this method of transmission is particularly challenging because the

uneven distribution of viruses in woody plants complicates detection protocols used by certification programs (Garrett et al. 1985; Komínek et al. 2009; Rodio et al. 2006; Salem et al. 2003). Moreover, the distribution of viruses in woody plants can fluctuate during the growing season (Salem et al. 2003), making the timing of sampling, as well as plant part sampled, important considerations for propagators and certifying agencies who strive to produce virus-free planting stock and for researchers studying the epidemiology of viral diseases. Historically, cranberry has been vegetatively propagated by mowing uprights from a mature bed and disking the cuttings into sand to establish a new bed. However, with new cultivars introduced over the past decade, nurseries root cuttings in small containers, and the resulting plants are then distributed to growers. If TSV is transmitted through vegetative propagation in cranberry, infection of cuttings or nursery plants could lead to the rapid and widespread dissemination of this pathogen.

We previously reported that the incidence of berry scarring associated with TSV infection varied greatly among farms, and even within a planting it ranged from difficult to detect to nearly 100% in patches measuring 1 m² or less (Wells-Hansen et al. 2016). The incidence of TSV in cranberry uprights is almost certainly greater than the incidence of symptomatic uprights, if cranberry plants recover from TSV, and if recovered plants continue to harbor the virus, as we hypothesize. The spatial distribution of TSV in cranberry beds has not been investigated, but patterns of TSV-infected uprights might shed light on how TSV spreads within a planting.

Additional knowledge on the biology of TSV in cranberry is essential to understand the economic impact that the pathogen will have and to develop effective management strategies for growers and propagators. Therefore, the specific objectives of this research were to: i) document recovery of TSV-infected cranberry plants after the shock phase, ii) investigate the distribution of TSV within cranberry plants throughout the growing season, iii) determine the effect(s) of TSV on yield components in symptomatic and recovered cranberry plants, and iv) investigate the incidence and spatial distribution of TSV in cranberry beds.

Materials and Methods

Virus detection. In the various experiments described below, plant samples were stored at approximately 4°C for up to 10 days until they were processed. Plants were confirmed as TSV-positive by ELISA and/or reverse-transcription (RT)-PCR. Double antibody sandwich (DAS)-ELISA detection was performed using a kit according

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Accepted for publication 11 June 2016.

<http://dx.doi.org/10.1094/PDIS-03-16-0407-RE>
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to the manufacturer's instructions (Agdia, Inc., Elkhart, IN). Leaves from known TSV-negative cranberry plants were included in every plate as negative controls, in addition to the positive control provided with the kit and a buffer-only negative control. For all DAS-ELISA tests, samples were considered positive when the absorbance at 405 nm was greater than three times the average values obtained for the healthy controls as read by a Bio-Rad iMark microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA). For a subset of samples, RNA was extracted from leaves of infected uprights using the Qiagen RNeasy Mini Plant Kit (Qiagen, Valencia, CA), and RT-PCR was

Table 1. Dates on which cv. Mullica Queen cranberry plants from Marshes 1 and 2 were collected for testing of plant parts for *Tobacco streak virus* (TSV)

Growth stage	Sampling date ^a	
	2014	2015
Prefruit set	— ^b	3 May
	—	13 May
Early fruit set	—	24 June
	16 Jul	15 Jul
	23 Jul	22 Jul
	31 Jul	29 Jul
Late fruit set	20 Aug	12 Aug
	27 Aug	31 Aug
Harvest	5 Sep	9 Sep
	17 Sep	16 Sep
	9 Oct	— ^b

^a Dates in bold indicate days on which samples were collected from Marsh 1 only; italicized dates indicate days on which samples were collected from Marsh 2 only. Samples were collected from both marshes on all other dates.

^b Indicates that no samples were collected before 16 Jul 2014 or after 16 Sep 2015.

performed as described previously (Wells-Hansen et al. 2016) using forward and reverse primers (TSV CP F and TSV CP R, respectively; Tzanetakis et al. 2004) that amplify an 867-bp fragment of RNA 3. PCR amplifications were carried out using GoTaq Green Master mix (Promega Corp., Madison, WI) in an Eppendorf master cycler gradient thermal cycler (Eppendorf, Westbury, NY). The thermal cycling profile consisted of an initial denaturation at 95°C for 5 min; followed by 40 cycles at 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and a final extension step at 72°C for 5 min.

Field sites. In experiments described below, studies were conducted on commercial cranberry farms, or “marshes,” in central Wisconsin. For all experiments, a single cranberry bed (cv. Mullica Queen) in which TSV had been confirmed previously was monitored at each marsh. The bed on Marsh 1 was established in 2005 with whole plants reared by a nursery. The grower reported observing berry scarring symptoms in this bed for several years prior to the initiation of our experiments but attributed the symptoms to injury from insects or pesticides. The bed on Marsh 2 was established in 2006 with whole plants reared by a nursery, and the grower reported observing berry scarring symptoms in this bed since approximately 2011. The bed on Marsh 3 was established in 2009 with disked-in vine cuttings pruned from the experimental bed on Marsh 1. Marshes 1 and 2 were sampled for all experiments, whereas Marsh 3 was sampled for the incidence and spatial distribution experiment only. Beds on Marshes 1, 2, and 3 were each approximately 0.5 ha in area, and growers at all marshes applied fertilizers, herbicides, insecticides, fungicides, and water as needed to manage the crop.

Recovery of TSV-infected cranberry plants. In 2012, 37 TSV-positive uprights bearing scarred fruit were tagged with twist ties at Marsh 1. In 2013, 29 and 22 TSV-positive uprights bearing scarred fruit were tagged at Marshes 1 and 2, respectively. In 2014, 51 and 18 TSV-positive uprights bearing scarred fruit were tagged in the same manner at Marshes 1 and 2, respectively. All tagging was arbitrarily

Fig. 1. A diagram illustrating the sample (upright) collection positions within cranberry beds sampled for evaluation of the spatial distribution of *Tobacco streak virus*. Sixteen individual uprights of cv. Mullica Queen were sampled from within 90 or 60 9.29-m² plots in beds on three marshes in 2014 or 2015, respectively. In total, 1,440 and 960 uprights were collected from each marsh in 2014 and 2015, respectively, and all uprights were tested for TSV by DAS-ELISA.

performed as uprights with scarred berries were observed in the field, and plant parts were tested for TSV as they became available throughout the growing season. All tagged uprights were left to overwinter in the field. In 2013, 2014, and 2015, leaves, pollen, and fruit of uprights tagged in the field in 2012, 2013, or 2014, were tested for TSV using DAS-ELISA, and a subset of samples were confirmed with RT-PCR as previously described (Wells-Hansen et al. 2016). All tagged uprights were monitored for symptoms throughout each growing season.

Assessment of return bloom. In 2013, 2014, and 2015, living TSV-positive tagged uprights monitored for recovery (described above) were evaluated for return bloom at Marshes 1 and 2. The number of tagged uprights that produced at least one flower was recorded in each subsequent year following berry scarring. Percent return bloom ([number of flowering uprights/total number of living uprights] × 100) was calculated for both marshes.

Distribution of TSV within cranberry plants. The cranberry growing season was divided into four growth stages for the purposes of this study: prefruit set, early fruit set, late fruit set, and harvest. Prefruit set referred to samples collected prior to fruit set, early fruit set ranged from the end of June through the end of July, late fruit set ranged from the beginning of August through the end of August, and harvest ranged from the beginning of September through October. Dates of all sample collections are provided in Table 1. TSV-positive uprights bearing scarred fruit as well as TSV-positive uprights bearing nonscarred fruit were collected at least biweekly from Marshes 1 and 2. However, none of the uprights tagged to monitor recovery were used for TSV distribution studies. Current-season leaves, previous-season leaves, scarred and nonscarred fruit, roots, stems, and terminal buds from a single upright were tested for TSV by DAS-ELISA.

Assessment of yield components. To determine if TSV affects yield components in symptomatic or recovered uprights, we obtained three categories of uprights: TSV-positive uprights with scarred berries (symptomatic); TSV-positive uprights with nonscarred berries (recovered); and TSV-negative uprights with nonscarred berries (healthy). Because it is impossible to distinguish the latter two categories in the field, a relatively large number of uprights with nonscarred berries was collected to ensure that there would be at least 25 uprights in each of those two categories. In 2013, 52 and 28 uprights bearing scarred fruit were collected from Marshes 1 and 2, respectively, and in 2014, 54 and 75 uprights with scarred fruit were collected from the two marshes. In 2013, 270 and 162 uprights bearing nonscarred fruit were collected from Marshes 1 and 2, respectively, and in 2014, 246 and 465 uprights bearing nonscarred fruit were collected from the two marshes. All collections were made within 2 weeks of commercial harvest in both years. Individual uprights were evaluated for total number of berries, number of scarred berries, number of pedicels (as a late-season surrogate for flowers), and total berry weight. Current-season and previous-season leaves were then tested for TSV by DAS-ELISA.

TSV incidence and spatial distribution in the field. In 2014 and 2015, TSV incidence was spatially mapped at Marshes 1, 2, and 3. On 9 June 2014, a 0.27-ha rectangular grid with systematic sampling points was superimposed on test beds at Marshes 1 and 3. This grid

Table 2. Percent return bloom for *Tobacco streak virus* (TSV)-infected uprights of cranberry cv. Mullica Queen at two locations

Location, year of scarring	% return bloom ^a		
	1st year after scarring	2nd year after scarring	3rd year after scarring
Marsh 1 scarred 2012	82 (33) ^b	13 (24)	83 (6)
Marsh 1 scarred 2013	64 (11)	50 (6)	—
Marsh 1 scarred 2014	26 (47)	—	—
Marsh 2 scarred 2013	73 (22)	31 (16)	—
Marsh 2 scarred 2014	65 (17)	—	—

^a Percent return bloom was determined as the percentage of uprights that produced flowers in successive years.

^b Numbers in parentheses indicate number of uprights observed.

consisted of 90 9.29-m² plots (5 plots × 18 plots) with 2.7 m-wide borders between plots. Each 9.29-m² plot was divided into 16 subplots measuring 0.09 m² each (Fig. 1) so that autocorrelation of TSV-infected uprights could be tested at various scales: 1 subplot × 1 subplot; 2 subplots × 2 subplots; 4 subplots × 4 subplots; and, by combining adjacent plots, 8 subplots × 8 subplots. From each subplot, an individual upright was harvested for a total of 1,440 (90 × 16) uprights per bed, and uprights were tested for TSV using DAS-ELISA. On 3 June, 17 June, and 10 June 2015, a 0.18-ha grid with systematic sampling points was superimposed on each experimental bed at Marshes 1, 2, and 3, respectively. Samples were collected in the same manner as 2014, except that a total of 60 9.29-m² plots were divided into subplots, and a total of 960 (60 × 16) individual uprights per bed were harvested and tested for TSV. Incidence of TSV ([no. uprights that tested TSV-positive/no. uprights sampled] × 100) was determined for each bed in 2014 and 2015.

Statistical analysis. Berry weight (fresh weight of fruit) and fruit set ([number of berries/number of pedicels] × 100) were calculated for each upright collected in the assessment of yield components and subjected to analysis of variance (ANOVA) in R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Means separation was performed according to Fisher's least significant difference test, after appropriate transformations to correct for unequal variances

Table 3. Percent of cv. Mullica Queen cranberry plant part samples from uprights with scarred fruit that tested positive for *Tobacco streak virus* (TSV) by ELISA

Plant part tested	Percent of samples that tested TSV-positive from TSV-positive uprights with scarred fruit		
	Early fruit set ^a	Late fruit set ^b	Harvest ^c
Previous-season leaves	83 (132) ^d	99 (93)	98 (230)
Current-season leaves	21 (138)	83 (99)	99 (251)
Symptomatic berries	99 (143)	33 (104)	4 (251)
Asymptomatic berries	67 (30)	7 (55)	6 (33)
Roots	79 (42)	65 (17)	60 (40)
Stems	94 (35)	100 (96)	95 (57)
Terminal buds	— ^e	70 (20)	96 (55)

^a Early fruit set ranged from the end of June through the end of July.

^b Late fruit set ranged from the beginning of August through the end of August.

^c Harvest ranged from the beginning of September through October.

^d Numbers in parentheses indicate number of uprights tested.

^e Terminal buds were not yet available at the early fruit set stage.

Table 4. Percent of cv. Mullica Queen cranberry plant part samples from recovered uprights with nonscarred fruit that tested positive for *Tobacco streak virus* (TSV) by ELISA

Plant part tested	Percent of samples that tested TSV-positive from TSV-positive uprights with nonscarred fruit			
	Prefruit set ^a	Early fruit set ^b	Late fruit set ^c	Harvest ^d
Previous-season leaves	97 (32) ^e	94 (51)	100 (36)	98 (280)
Current-season leaves	94 (17)	93 (61)	97 (38)	95 (311)
Asymptomatic berries	— ^f	42 (52)	18 (38)	4 (68)
Roots	93 (28)	84 (25)	71 (14)	63 (32)
Stems	100 (3)	91 (11)	88 (24)	85 (47)
Terminal buds	100 (15)	— ^f	100 (8)	85 (20)

^a Prefruit set refers to the time period prior to any fruit set in the field.

^b Early fruit set ranged from the end of June through the end of July.

^c Late fruit set ranged from the beginning of August through the end of August.

^d Harvest ranged from the beginning of September through October.

^e Numbers in parentheses indicate number of uprights tested.

^f Berries were not available at the prefruit set stage, and terminal buds were not yet available at the early fruit set stage.

Fig. 2. Mean berry weight (g) per upright for three categories of uprights collected from two cv. Mullica Queen cranberry marshes in Warrens, Wisconsin, in 2013 and 2014. Berry weight means marked with different letters are significantly different and were separated by Fisher's least significant difference test ($\alpha = 0.05$). Bars represent $\pm$ one standard error of the mean.

Fig. 3. Mean percent fruit set ([no. berries/no. pedicels] $\times$ 100) per upright for three categories of uprights collected from two cv. Mullica Queen cranberry marshes in Warrens, Wisconsin, in 2013 and 2014. Percent fruit set means marked with different letters are significantly different and were separated by Fisher's least significant difference test ($\alpha = 0.05$). Bars represent $\pm$ one standard error of the mean.

($\alpha = 0.05$). Moran's Index (Moran's I) spatial autocorrelation coefficient was used to determine the spatial structure of TSV incidence in each cranberry bed (Moran 1950). Moran's I is a measure of spatial autocorrelation used to infer spatial dependence, and provides a measure of the global spatial autocorrelation of the overall clustering of data (Gent et al. 2004). Data are considered positively spatially dependent (clustering of similar observations in space) if $I > -1/(n-1)$, negatively spatially dependent (neighboring observations dissimilar in space) if $I < -1/(n-1)$, and spatially independent (observations random in space and do not depend on neighboring observations) if $I = -1/(n-1)$. In our study, a positive value for this index in combination with a significant

($P < 0.05$) P value indicated that nearby uprights in a bed had similar infection status, indicating the presence of spatial autocorrelation, or clustering. Moran's I coefficients were calculated at several scales to determine whether spatial dependence existed over a range of distances. Semivariograms were constructed, and results were parallel with results from Moran's I coefficients, and therefore data are not shown. All spatial statistics were performed using R version 3.0.2.

Results

Recovery of TSV-infected cranberry plants. Leaves, pollen, and fruit of all symptomatic uprights that were tagged in 2012, 2013, or

Fig. 4. Spatial distribution of *Tobacco streak virus* (TSV)-positive cranberry uprights (cv. Mullica Queen) at Marsh 1 in 2014 and 2015. Each square represents an individual upright that is either TSV-positive (black squares) or healthy (white squares).

2014 tested positive for TSV by ELISA and/or RT-PCR in the year(s) following scarring. Of 157 uprights tagged, 73 produced fruit in the year following scarring. All 73 uprights that had produced scarred, TSV-positive fruit in 2012, 2013, or 2014, produced nonscarred, TSV-positive fruit in 2013, 2014, or 2015, respectively. After recovery, all observed uprights remained asymptomatic for the duration of the study.

Assessment of return bloom. Average percent return bloom ranged from 26 to 82% in the first year following scarring symptoms, from 13 to 50% in the second year following scarring symptoms, and was 83% in uprights at Marsh 1, the only farm observed in the third year following scarring symptoms (Table 2).

Distribution of TSV within cranberry plants. The percent of cranberry plant parts from uprights with scarred fruit as well as from recovered uprights with nonscarred fruit that tested TSV-positive by ELISA is shown in Tables 3 and 4. In uprights with scarred fruit, TSV was detected in 83% or more of previous-season leaves and stems at all growth stages tested and in current-season leaves at late fruit set and harvest. TSV was detected in 60% or more of roots, stems, and buds at all growth stages tested. TSV was detected in 99% of symptomatic fruit during early fruit set but dropped sharply at later stages. In uprights that had recovered, TSV detection was 84% or greater in most plant parts tested at all growth stages. Exceptions

were berries at all stages (4 to 42% detection) and roots at the time of harvest (63% detection).

Assessment of yield components. Uprights were separated into three categories according to results from TSV testing by DAS-ELISA: scarred, TSV-positive (symptomatic); nonscarred, TSV-positive (recovered); and nonscarred, TSV-negative (healthy). Berry weight and percent fruit set were significantly reduced ($P < 0.05$) in symptomatic uprights compared with recovered and healthy uprights at both marshes in both years (Figs. 2 and 3). There were no significant differences for berry weight or fruit set in recovered uprights compared with healthy uprights at both marshes in both years (Figs. 2 and 3).

TSV incidence and spatial distribution in the field. TSV incidence was 67 and 71% at Marsh 1 in 2014 and 2015, respectively. TSV incidence was 9% at Marsh 2 in 2015, and 1 and 2% at Marsh 3 in 2014 and 2015, respectively. Spatial distribution of TSV-positive uprights for the three marshes in 2014 and 2015 is illustrated in Figures 4, 5, and 6. Values in Table 5 followed by one or more asterisks indicate significant P values, and suggest evidence of spatial dependence, or clustering of TSV infected uprights at several different scales. Evidence of positive spatial dependence of TSV incidence was detected at Marsh 1 in 2014 and 2015 (Fig. 4 and Table 5), Marsh 2 in 2015 (Fig. 5 and Table 5), and Marsh 3 in 2014 (Fig. 6 and Table 5).

Discussion

In this study, we document for the first time the recovery of cranberry plants from TSV infection. The distribution of TSV within ‘Mullica Queen’ cranberry uprights differed between symptomatic and recovered plants, and yield components were not negatively affected once plants recovered, despite plants remaining TSV-positive. In other woody plant-ilarvirus interactions, a partial shock reaction occurs in which only some plant parts/branches exhibit shock symptoms in a given growing season, due to uneven distribution of the virus within the plant (Pscheidt and Harper 2014; Uyemoto and Scott 1992). Usually plant parts that experience shock symptoms once will not show shock symptoms again, although reoccurrence has been reported with BISHV in blueberry in rare cases (Pscheidt and Harper 2014). All of the TSV-infected cranberry uprights observed in our study exhibited fruit scarring symptoms for only one year, and in the timeframe of this study, uprights remained asymptomatic in all years following the year of scarring.

We investigated the distribution of TSV in cranberry plants throughout the growing season to improve our understanding of the disease cycle and to determine if there might be a window of time in which cranberry plant material could be collected to “escape” virus transmission through vegetative propagation, as has been reported for several other plant-virus pathosystems (Preece 2003). Our work revealed that TSV distribution was uneven and changed throughout the growing season in uprights with scarred fruit, while relatively little change in virus distribution was observed in recovered uprights bearing nonscarred fruit. This information led to a better understanding of TSV detection levels within cranberry uprights, and will allow advisement of interested parties on the best times and plant parts to sample if testing for TSV. Unfortunately for growers, it also suggests that there is not a time to take cuttings for propagation that allows “escape” from TSV.

Due to the nature of cranberry cultivation and management in commercial plantings, effects of TSV on yield on a bed-wide scale would be difficult to determine (Pozdnyakova et al. 2005). For that reason, we evaluated several yield components, an approach previously used for identifying the influence of factors on cranberry yield (Roper 2006). The significant decrease of berry weight and percent fruit set in TSV-positive uprights with scarred fruit compared with recovered or healthy uprights is not surprising, since most fruit affected by scarring become shriveled by the time of harvest, and any fruit that do not shrivel fail to develop to full size and are severely deformed. Despite high incidences of TSV in some beds, such as the bed at Marsh 1, this pathogen is unlikely to cause extreme crop losses in any one year, at least in cv. Mullica Queen, because uprights apparently bear normally after they have recovered. In fact, the cranberry grower at Marsh 1, where TSV incidence was 71% in 2015,

Fig. 5. Spatial distribution of *Tobacco streak virus* (TSV)-positive cranberry uprights (cv. Mullica Queen) at Marsh 2 in 2015. Each square represents an individual upright that is either TSV-positive (black squares) or healthy (white squares).

reported no notable yield losses despite observing scarring symptoms for several years.

“Return bloom” refers to flowering on a cranberry upright that fruited the previous year. Return bloom is an important characteristic for cranberry growers and breeders whose goal it is to maximize production. Biennial bearing tendencies occur in cranberry uprights, making return bloom on a bed-wide scale generally low, and fruiting uprights are less likely to develop mixed buds (Baumann and Eaton 1986; DeVetter et al. 2013; Eaton 1978; Eck 1990; Roberts and Struckmeyer 1943; Strik et al. 1991). In our studies, percent return bloom ranged from 26 to 82% in the first year following fruit scarring, which is remarkably high compared with rates of 6 to 41% reported recently for several cultivars in Wisconsin (DeVetter et al.

2013). The reason(s) for this high incidence of return bloom in the first year after scarring is unknown. Cranberries set mixed buds for the following growing season in late June to early July shortly after bloom (Eaton 1978; Strik et al. 1991). The fact that return bloom was high in uprights the year following fruit scarring suggests that development of flower primordia is not impaired even while TSV can be detected at moderate to high frequency throughout the upright and while fruit on the upright are becoming scarred.

Evaluation of incidence and spatial distribution of virus-infected plants within a field can help determine potential sources and vectors of the virus, as well as movement of the virus into and within a field, key considerations in developing disease management strategies. In our study, we do not know when or how TSV was introduced into the

Fig. 6. Spatial distribution of *Tobacco streak virus* (TSV)-positive cranberry uprights (cv. Mullica Queen) at Marsh 3 in 2014 and 2015. Each square represents an individual upright that is either TSV-positive (black squares) or healthy (white squares). Gray squares indicate missing samples.

experimental beds. Nevertheless, at Marsh 1 and Marsh 3 where beds were sampled in two consecutive years, the incidence of TSV increased only minimally from one growing season to the next. This suggests that the progress of virus infection is slow in beds with low (1 to 2% at Marsh 3) or high (67 to 71% at Marsh 1) TSV incidence. However, the infection progress curve might be steeper in beds with intermediate levels of TSV infection, as occurs with another ilarvirus, BISHV, in blueberry fields (Bristow and Martin 1999; Pscheidt and Harper 2014). In the blueberry studies, infection incidence was low for two years following the initial discovery of BISHV in a field, but then increased sharply from 7 to 44%, 16 to 85%, and approximately 20% to approximately 70% from one year to the next in three separate fields before plateauing at incidences greater than 90% (Bristow and Martin 1999; Pscheidt and Harper 2014). Similar infection dynamics have been reported for other pollen-borne viruses of fruit crops (Bulger et al. 1990; Childress and Ramsdell 1987; Davidson and George 1964). However, sampling over additional years would be required to determine if TSV infection progress in cranberry beds follows a similar trend. Additionally, our work was conducted in a single cultivar, 'Mullica Queen,' and the rate of spread of TSV may differ between cultivars as has been observed with BISHV in blueberry (Pscheidt and Harper 2014).

Within two of the three cranberry beds we intensively sampled and analyzed by spatial statistics, we found evidence of clustering (spatial autocorrelation) of TSV-infected uprights at several scales. Our work has shown that TSV infection is systemic in 'Mullica Queen' cranberry plants, and given that a cranberry runner can grow to a length upwards of 2 m (Eck 1990), the clustering we observed at the finest scale (1×1) could be the result of sampling uprights from the same plant. In addition to local clustering observed at the 1×1 level, we observed clustering of TSV-infected uprights at larger scales. These data suggest that in general, uprights in close proximity to a given TSV-positive upright are likely to be TSV-positive, while the infection status of uprights at greater distances from a given TSV-positive upright is not correlated with the infection status of the given upright. Clustering of infected plants has been described for other pollen-borne viruses of fruit crops and has been attributed to spread of viruses within a field (Bulger et al. 1990; Childress and Ramsdell 1987; Davidson and George 1964). Likewise, in our study, spatial autocorrelation, combined with the minimal increase in incidence of TSV from year to year, suggests that spread of TSV from plant to plant within an infected cranberry bed, rather than frequent introduction of the virus from an outside source(s), is responsible for new infections.

In summary, results from experiments conducted over three growing seasons suggest that once cranberry plants recover from TSV, there are no apparent negative effects on productivity. However, our study focused on just cv. Mullica Queen and evaluated only short-term effects of TSV on cranberry, a perennial crop that is expected to remain fully productive for several decades. Further investigation into additional cultivars as well as potential long-term effect(s) of TSV on cranberry,

such as longevity or winter hardiness, would lead to a more complete assessment of the impact of TSV. Because we cannot visually distinguish a recovered, infected plant from a healthy, uninfected plant, it is impossible to eliminate all potential sources of inoculum within an infected cranberry bed, thereby making eradication efforts fruitless and emphasizing prevention as the key management strategy. To that end, our work provides important insight into the incidence and distribution of TSV within affected beds as well as within individual uprights, which will be essential for growers, propagators, researchers, and diagnosticians whose goal it is to avoid the widespread dissemination of TSV on planting stock.

Acknowledgments

We thank our cranberry grower-cooperators for use of field sites. We also thank M. Clayton, A. Cramer, T. German, V. Kartanos, S. Thomas-Sharma, M. Hughan, R. Groves, and J. Polashock for assistance with sample collection, data analysis, and helpful discussions. This research was funded by the National Institute of Food and Agriculture, United States Department of Agriculture, under ID number WIS01653, Wisconsin Cranberry Board, Inc., Ocean Spray Cranberries, Inc., Cranberry Institute, Vaughan-Bascom Professorship of the Department of Plant Pathology, University of Wisconsin-Madison, and the Senator Robert Caldwell Graduate Fellowship of the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

Literature Cited

- Baumann, T. E., and Eaton, G. W. 1986. Competition among berries on the cranberry upright. *J. Am. Soc. Hortic. Sci.* 111:869-872.
- Bristow, P. R., and Martin, R. R. 1999. Transmission and the role of honeybees in field spread of Blueberry shock ilarvirus, a pollen-borne virus of high bush blueberry. *Phytopathology* 89:124-130.
- Bristow, P. R., Windom, G. E., and Martin, R. R. 2002. Recovery of plants infected with Blueberry shock virus (BISHV). *Acta Hortic.* 85-89.
- Bulger, M. A., Stace-Smith, R., and Martin, R. R. 1990. Transmission and field spread of raspberry bushy dwarf virus. *Plant Dis.* 74:514-517.
- Childress, A. M., and Ramsdell, D. C. 1987. Bee-mediated transmission of blueberry leaf mottle virus via infected pollen in highbush blueberry. *Phytopathology* 77:167-172.
- Colquhoun, J., and Johnson, H. 2010. Sustainable Cranberry Production for a Vibrant Future: the Wisconsin Experience. http://www.wiscran.org/media/1322/sustainablecranberry_final_web.pdf
- Davidson, T. R., and George, J. A. 1964. Spread of necrotic ring spot and sour cherry yellows viruses in Niagara Peninsula orchards. *Can. J. Plant Sci.* 44:471-484.
- Davidson, T. R., and George, J. A. 1965. Effects of necrotic ring spot and sour cherry yellows on the growth and yield of young sour cherry trees. *Can. J. Plant Sci.* 45:525-535.
- DeVetter, L. W., Harbut, R., and Colquhoun, J. 2013. Bud development, return bloom, and external bud appearance differ among cranberry cultivars. *J. Am. Soc. Hortic. Sci.* 138:338-343.
- Eaton, G. W. 1978. Floral induction and biennial bearing in the cranberry. *Fruit Var. J.* 32:58-60.
- Eck, P. 1990. *The American cranberry*. Rutgers University Press, New Brunswick, NJ.
- Garrett, R. G., Cooper, J. A., and Smith, P. R. 1985. Virus epidemiology and control. Pages 269-297 in: *The Plant Viruses*. R. I. B. Francki, ed. Plenum Press, New York.
- Gent, D. H., Schwartz, H. F., and Khosla, R. 2004. Distribution and incidence of *Iris yellow spot virus* in Colorado and its relation to onion plant population and yield. *Plant Dis.* 88:446-452.
- Ghanekar, A. M., and Schwenk, F. W. 1974. Seed transmission and distribution of tobacco streak virus in six cultivars of soybeans. *Phytopathology* 64:112-114.
- Greber, R. S., Klose, M. J., and Teakle, D. S. 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.
- Komínek, P., Glasa, M., and Komínková, M. 2009. Analysis of multiple virus-infected grapevine plant reveals persistence but uneven virus distribution. *Acta Virol.* 53:281-285.
- MacDonald, S. G., Martin, R. R., and Bristow, P. R. 1991. Characterization of an ilarvirus associated with necrotic shock reaction in blueberry. *Phytopathology* 81:210-214.
- Mink, G. I. 1995. Prunus necrotic ringspot virus. Page 64 in: *Compendium of Stone Fruit Diseases*. J. M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, and J. K. Uyemoto, eds. American Phytopathological Society, St. Paul, MN.
- Moran, P. A. P. 1950. Notes on continuous stochastic phenomena. *Biometrika* 37:17-23.
- Pozdnyakova, L., Giménez, D., and Oudemans, P. V. 2005. Spatial analysis of cranberry yield at three scales. *Agron. J.* 97:49-57.
- Preece, J. E. 2003. A century of progress with vegetative plant propagation. *HortScience* 38:1015-1025.

Table 5. Moran's Index^a for incidence of *Tobacco streak virus* (TSV) in three cv. Mullica Queen cranberry beds sampled in 2014 and 2015

Location, year sampled	Sampling level			
	1 × 1	2 × 2	4 × 4	8 × 8
Marsh 1, 2014	0.206***b	0.469***	0.150**	-0.128
Marsh 1, 2015	0.164***	0.337***	0.142**	-0.080
Marsh 3, 2014	0.008	-0.053	0.131***	0.301**
Marsh 3, 2015	0.015	-0.068	-0.031	0.1385
Marsh 2, 2015	0.130***	0.182***c	0.273***	0.148**

^a Data are considered positively spatially dependent (clustering of similar observations in space) if $I > -1/(n-1)$, negatively spatially dependent (neighboring observations dissimilar in space) if $I < -1/(n-1)$, and spatially independent (observations random in space and do not depend on neighboring observations) if $I = -1/(n-1)$.

^b ***Indicates $P < 0.001$.

^c **Indicates $0.001 < P < 0.02$.

- Pscheidt, J. W., and Harper, N. 2014. Progression of symptoms on blueberry infected with *Blueberry shock virus*. Plant Health Prog. doi:10.1094/PHP-RS-13-0121
- Roberts, R. H., and Struckmeyer, B. E. 1943. Blossom induction of the cranberry. Plant Physiol. 18:534-536.
- Rodio, M. E., Delgado, S., Flores, R., and Di Serio, F. 2006. Variants in Peach latent mosaic viroid inducing peach calico: uneven distribution in infected plants and requirements of the insertion containing the pathogenicity determinant. J. Gen. Virol. 87:231-240.
- Roper, T. R. 2006. The physiology of cranberry yield. Wisc. Cranberry Crop Mgt. Nwsl. Vol. XIX. Accessed 25 Nov. 2014 from http://scholarworks.umass.edu/cranberry_factsheets/16/.
- Roper, T. R., Patten, K. D., DeMoranville, C. J., Davenport, J. R., Strik, B. C., and Poole, A. P. 1993. Fruiting of cranberry uprights reduces fruiting the following year. HortScience 28:228.
- Salem, N., Mansour, A., Al-musa, A., and Al-nsour, A. 2003. Seasonal variation of *Prunus necrotic ringspot virus* concentration in almond, peach, and plum cultivars. Phytopathol. Mediterr. 42:155-160.
- Strik, B. C., Roper, T. R., DeMoranville, C. J., Davenport, J. R., and Poole, A. P. 1991. Cultivar and growing region influence return bloom in cranberry uprights. HortScience 26:1366-1367.
- Tzanetakis, I. E., Mackey, I. C., and Martin, R. R. 2004. *Strawberry necrotic shock virus* is a distinct virus and not a strain of *Tobacco streak virus*. Arch. Virol. 149:2001-2011.
- USDA National Agricultural Statistic Service. 2015. Cranberries, ISSN: 1948-9013. Retrieved 3 June 2016 from <http://usda.mannlib.cornell.edu/usda/current/Cran/Cran-08-13-2015.pdf>.
- Uyemoto, J. K., and Scott, S. W. 1992. Important diseases of *Prunus* caused by viruses and other graft-transmissible pathogens in California and South Carolina. Plant Dis. 76:5-11.
- Vanden Heuvel, J. E., and DeMoranville, C. J. 2009. Competition between vegetative and reproductive growth in cranberry. HortScience 44:322-327.
- Wells-Hansen, L. D., Polashock, J. J., Vorsa, N., Lockhart, B. E. L., and McManus, P. S. 2016. Identification of *Tobacco streak virus* in cranberry and the association of TSV with berry scarring. Plant Dis. 100:696-703.