Occurrence of blueberry leaf mottle, blueberry shoestring, tomato ringspot and tobacco ringspot viruses in eleven halfhigh blueberry clones grown in New Brunswick, Canada

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In 1980, a field trial was started to evaluate the horticultural value of 11 halfhigh blueberry clones for New Brunswick conditions. Nine of these certified clonal selections were obtained from the University of Minnesota (Mn 61, 84, 135,327,330,332,350,354,360); one from University of Maine (Me-I) and the last one was a N.B. selection (NBBCL)from a seedling line of Mn3. Recently, clones, Mn 332, Mn 350 and Mn 360 were released as cultivars in Minnesota under names of Northsky, Northcountry and Northblue respectively. All halfhigh clones originated from interspecific crosses of highbush ***Vaccinium corymbosum*** L. and lowbush ***Vaccinium angustifolium*** plants. All plants of these clonal selections were tested for the occurrence of tomato ringspot (TmRSV), tobacco ringspot (TbRSV), blueberry leaf mottle (BBLMV) and blueberry shoestring (BBSSV) viruses using the enzyme-linked immunosorbent-assay (ELISA) procedure.

All plants of 4 clonal selections namely Mn 84, 135, 354 and NBBCL gave negative ELISA results suggesting that these clones might be less susceptible to these four viruses. Clonal selections which tested positive were: (TmRSV – Mn 61, 330,332, 350, 360, Me-I; BBLMV – Mn 327, 330, 332, 350, 360, Me-I; BBSSV – Mn 61, 327,330,360; TbRSV – Mn 350, 360). From a total of 243 plants tested TmRSV was found to be the most prevalent followed by BBLMV, BBSSV and TbRSV, each infecting 13, 11, 6 and 2 plants respectively.

Can. Plant Dis. Surv. 70:2, 113-117, 1990.

En 1980, on a commence un essai en plein champ pour evaluer la valeur horticole de 11 clones de bleuet demi-nains dans les conditions meteorologiques du Nouveau-Brunswick. Neuf de ces selections clonales certifiees provenaient de l'Universite du Minnesota (Mn61, 84, 135, 327, 330, 332, 350, 354, 360), une de l'Universite du Maine (Me-I) et la derniere du Nouveau-Brunswick (NBBCL) d'une lignee de semis de Mn3. Recemment, on a mis au marché les clones Mn 332, Mn 350 et Mn 360 comme des cultivars au Minnesota sous les noms de Northsky, Northcountry et Northblue respectivement. Tous les clones demi-nains provenaient de croisements interspecifiques du bleuet en corymbe ***Vaccinium corymbosum*** L. et du bleuet nain ***Vaccinium angustifolium*** Ait. Tous les plants de ces selections clonales ont ete analysees pour la presence des virus de la tache annulaire de la tomate (TmRSV), de la tache annulaire du tabac (TbRSV), de la marbrure du bleuet (BBLMV) et de la mosa'ique en lacet du bleuet (BBSSV) a l'aide du test immuno-enzymatique ELISA.

Tous les plants de quatre selections clonales soit Mn 84, 135, 354 et NBBCL ont eu des resultats negatifs avec le test ELISA ce qui suggere que ces clones peuvent être moins susceptibles envers ces quatre virus. Les selections clonales suivantes ont eu des resultats positifs: TmRSV – Mn 61, 330,332,350,360, Me–I; BBLMV – Mn 327,330,332,350,360, Me–I; BBSSV – Mn 61, 327, 330, 360; TbRSV – Mn 350,360. Sur un total de 243 plants on a identifie le TmRSV sur 13 plants, le BBLMV sur 11 plants, le BBSSV sur 6 plants et le TbRSV sur 2 plants.

Introduction

Blueberry production in the Province of New Brunswick in Eastern Canada has continued to increase over the past 8-10 years. Various blueberry improvement programs involving interspecific hybridization of lowbush with highbush berries have resulted in the selection of a number of halfhigh clones which have a medium plant stature producing light blue, high quality, high yielding crops. An experimental field trial was started in New Brunswick in 1980 to select a number of halfhigh clones, with desirable characteristics such as high yield, improved fruit quality and high disease resistance.

A number of major viruses and virus-like diseases threaten blueberries in North America (11). Highbush cultivars have been shown to suffer from virus related diseases causing multi-million dollar losses in various parts of the U.S.A. and Canada (4, 5). Since blueberries are woody perennials and represent long-term investments which can' be profitable for many years, the occurrence of even a minor virus disease cannot be ignored.

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Accepted for publication December 18, 1989.

Control measures for various *Vaccinium* viruses have been reviewed (11) and disease symptoms well characterized (8, 9, 10, 11). One of the effective ways to guard against virus diseases is to grow cultivars which are resistant or less susceptible to viruses. The main objective of this study was to determine whether or not the 11 halfhigh clones grown in N. B., on trial basis are susceptible to blueberry viruses. All plants of these clones were ELISA tested for viruses for which the antisera was commercially available and included blueberry shoestring (BBSSV), blueberry leaf mottle (BBLMV), tomato ringspot (TmRSV) and tobacco ringspot (TbRVS). BBSSV is aphid vectored (8); transmission of BBLMV is by honey-bees (2) and TmRSV and TbRSV are vectored by the dagger nematodes *Xiphinema americanum* Cobb or *X. rivesi* (1, 4, 6, 7).

Materials and methods

Experimental plots

In 1980, a field trial was started to evaluate the cultivar potentials of 11 halfhigh blueberry clones for New Brunswick soil and climatic conditions. Nine of these clonal selections were obtained from the Minnesota State University (Mn series), one from the University of Maine (Me-I), and one was a N. B. Selection (NBBCL) from a seedling line of Mn3. All clones originated from interspecific crosses of highbush *V. corymbosum* L. and lowbush *V. angustifolium* plants. The trial was established at the New Brunswick Horticultural Centre, Hoyt, on a sandy soil of low fertility and low organic matter. The clones were planted in a randomized complete block design with 4 replications. Each plot consisted of 6 plants spaced 1.0 m apart in rows spaced 2.0 m apart. The test plot was surrounded by approximately 100 metres of bushes and tall trees on one side and large blueberry fields on the other three sides.

Sampling procedure

In preliminary investigations blueberry buds gave considerably less background absorbance than mature leaves; buds, therefore, were chosen as a suitable tissue for ELISA tests. Approximately 100-120 buds were collected from different locations on each plant in late October, stored at −20°C and assayed during early November. Plants of each clonal selection were ELISA tested in separate batches. Antisera for all viruses was purchased from AGDIA Inc. (30380 Country Road 6, Elkhart, IN, U.S.A.).

Preliminary Tests

Preliminary studies were undertaken to determine optimum experimental conditions for ELISA tests. Five healthy blueberry bud samples (70-80 buds), each weighing 0.5 g were homogenized separately, in 5 mL of extraction buffer. Extracts from 4 samples were spiked separately with 1.0 mL each of BBLMV, BBSSV, TbRSV and TmRSV standards; stored at 5°C and ELISA tested at 1, 6, 17 (overnight), 41 and 65 hr time interval. Extract from the fifth sample was used as a negative standard.

In another experiment, spiked extracts prepared as before were incubated in the antibody coated plates under 3 different conditions: (a) 17 hr (overnight) at 5°C; (b) 2 hr at room temperature; (c) 2 hr at 37°C. Test extracts for (b) and (c) had earlier been stored overnight at 5°C.

When infected plants were identified, identical experiments with respect to (a) storage of bud extract and (b) antibody incubation periods, were conducted on their unspiked bud extracts.

ELISA procedure

The double antibody sandwich ELISA, as described by Clark and Adams (3) was followed1 except that gamma-globulins were labelled with peroxidase and o-phenylene diamine was used as substrate. For analysis 0.2 g of bud tissue (25-40 buds) was homogenized with pestle and mortar in 2 mL of phosphate buffered saline containing 2% polyvinyl pyrrolidone (PVP, mol. wt. 40,000, Sigma) and 0.5% Tween 20. The extracts were placed in plastic covered vials and stored overnight at approximately 5°C and assayed the following morning. One hundred microliters of extract from test samples or negative or positive standards were placed in wells of antibody-coated ELISA plates (AGDIA Inc.). All samples were tested in duplicate wells except negative standards that were used in 4 wells per plate. The plates were placed on a wet paper towel, sealed in plastic bags and kept at room temperature (approximately 25°C) for 2 hours. The plates were emptied, washed thrice with phosphate buffered saline (PBS) – Tween solution, refilled with 100 microliters of enzyme-conjugate and incubated for 2 hr at room temperature. Plates were once again washed with PBS-Tween and refilled with 100 microliters of freshly prepared substrate. After 30 minutes, the reaction was stopped by adding 50 microlitres of 3 M sulphuric acid to each well. Acid addition was essential not only to stop the reaction but also to obtain proper colour development in the assay. Plates were read visually and the absorbance was measured at A490 in an ELISA reader (Titertek Mulutiskan, Type 310 C, Flow Laboratories, Helsinki, Finland).

Results and discussion

Evaluation of experimental conditions

Absorbance (A490 nm) termed "ELISA values" was used to express the virus concentration. These values obtained when spiked bud extracts were ELISA tested after their storage at 5°C for 1-65 hr are presented in Fig. 1. The results suggested that bud homogenates when tested after 17 hr of storage at 5°C did not adversely effect the ELISA values. These values, however, decreased when the storage time was prolonged to 41 hr or beyond.

ELISA values of samples where spiked bud extracts were subjected to 3 different antibody incubation periods are presented in Fig. 2. Absorbance values of these treatment periods, for any particular virus, are almost similar indicating that either of these incubation periods are equally satisfactory for ELISA procedure.

ELISA values of identical experiments conducted on buds of infected plants are presented in Table 1 and 2. The results showed similar trends as were observed with the spiked samples. The bud homogenate stored for up to 17 hr at 5°C did not adversely affect the virus concentration as was evidenced by the ELISA values. The concentration, however, decreased when the extract was stored for 41 hr or more (Table 1). ELISA values of all 3 antibody incubation treatments (Table 2), for each virus, varied in a small range, reinforcing the earlier conclusions that either of these treatments are of equal value for ELISA procedure.

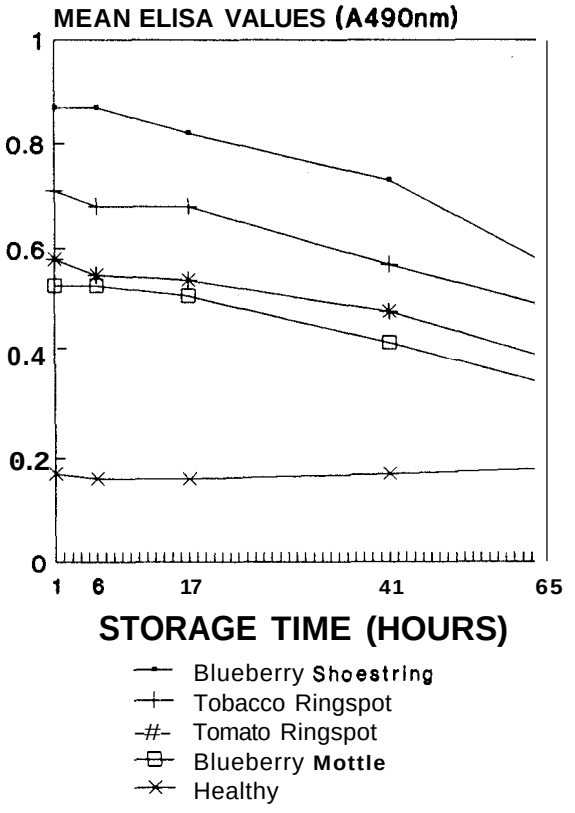

Fig. 1. Effect of storing sample extracts, for 1-65 hr, at 5°C on ELISA values (A490nm). One half gram of healthy blueberry buds (70-80) were homogenized in 5 ml of extraction buffer, spiked with 1.0 ml of virus positive standards and used for ELISA tests. The data are an average of 8 determinations, i.e., 4 each of 2 replicated experiments.

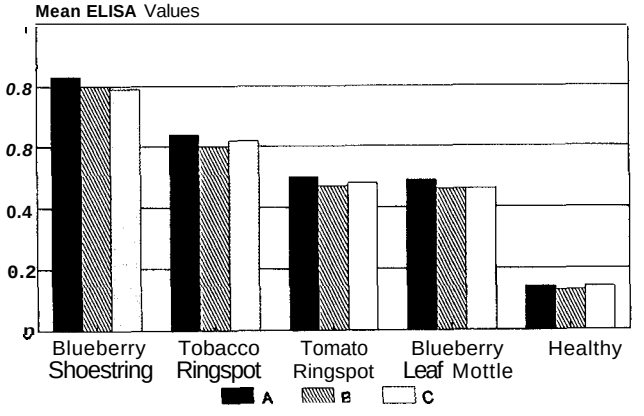

Fig. 2. ELISA values of 3 antibody incubation treatments : (A) Extract placed directly in the antibody coated plate and incubated overnight at 5°C; (B) Extract stored overnight at 5°C then incubated for 2 hr at room temperature; (C) Extract stored as in (B) then incubated for 2 hr at 37°C. Bud samples were extracted and spiked as in Fig. 1.

ELISA results

ELISA test results are presented in Table 3. A few plants in the trial field had died since the 1980 planting; therefore, final plant numbers varied from the initial numbers. The ELISA values of healthy controls of all analyses sets of 11 clonal selections were in the range of 0.10 to 0.21 ($\bar{X} = 0.14$). Test samples showing absorbance value of 0.42 or greater were considered to be ELISA positive.

Mn 332 appeared to be particularly susceptible to TmRSV and BBLMV as 5 of the 24 plants tested were infected individually or with combinations of these viruses. This clone appeared to be less susceptible to BBSSV and TbRSV as all plants tested negative for these viruses.

Mn 360 had the highest rate of infection (24%) and was the most susceptible clone in the lot. This was the only clone which tested positive for all 4 viruses. From a total of 21 plants, 5 were infected: 2 with TmRSV, 1 with BBSSV, 1 with TmRSV and BBLMV and one with TbRSV and BBSSV.

Recently Mn clone 332, 350 and 360 were released as cultivars in Minnesota under the names of Northsky, Northcountry and Northblue respectively. However, all of these clones, especially Mn 360 and 332, although reasonably high yielding under New Brunswick climatic conditions (unpublished information), have been found to be susceptible to the aforementioned viruses. Mn 350 was only moderately

Table 1. Absorbance values* (A490nm) of infected blueberry bud extracts ELISA tested after 1-65 hr of storage at 5°C.

	Time (hours)				
Infecting virus	1	6	17	41	65
Blueberry Leaf Mottle	0.73	0.74	0.70	0.67	0.58
	(0.13)	(0.11)	(0.12)	(0.10)	(0.09)
Blueberry Shoestring	1.10	0.94	0.97	0.83	0.68
	(0.15)	(0.16)	(0.13)	(0.10)	(0.11)
Tobacco Ringspot	0.84	0.86	0.82	0.73	0.54
	(0.13)	(0.15)	(0.16)	(0.13)	(0.12)
Tomato Ringspot	1.50	1.50	1.43	0.89	0.73
	(0.17)	(0.15)	(0.17)	(0.14)	(0.11)

Figures in parentheses represent absorbance values of healthy standards.

* Mean value of 8 determinations, i.e., 4 each of two replicated experiments.

Table 2. Absorbance values' of infected blueberry extracts subjected *to* varying antibody incubation periods.

Infecting virus	Incubation periods: 17 hr* at 5°C (overnight)	2 hr** at room temperature	2 hr** at 37°C
Blueberry Leaf Mottle	0.74	0.70	0.73
	(0.12)	(0.11)	**(0.1**)
Blueberry Shoestring	1.03	0.96	0.93
	(0.17)	(0.15)	(0.17)
Tobacco Ringspot	0.80	0.76	0.73
	(0.15)	**(0.15)**	(0.13)
Tomato Ringspot	1.38	1.26	1.22
	(0.18)	(0.14)	(0.16

Figures in parentheses represent absorbance values of healthy standards.

1 Mean value of 8 determinations, i.e., 4 each of ***two*** replicated experiments.

* Bud extract placed directly in the antibody coated ELISA plate.

** Bud extract stored overnight at 5°C before incubation.

susceptible as only 3 of its plants from a total of 21 tested positive: 1 to TmRSV, 1 to BBLMV and TmRSV and one to TbRSV.

Two clonal selections Mn 327 and 330 were ELISA positive 13 and 15% respectively. The results for Mn 327 were noteworthy because it was the only clone found to be free from TmRSV. Mn 61 and Me-I selections showed 8 and 9% infection respectively, mainly from TmRSV.

Overall TmRSV was found to be the most prevalent virus infecting a total of 13 plants. Except Mn 327, all other virus-positive clones, to some degree, were infected by this virus. The soil of the trial plot is sandy and *Xiphinema americanum* Cobb (TmRSV vector) has been reported to thrive well on soils with high sand content (1). BBLMV was the second most common virus and was detected in 11 plants. All infected clones, with the exception of Mn 61, showed BBLMV presence. BBSSV was present to a moderate extent; a total of 5 plants belonging to 4 different clones (Mn 61, Mn 327, Mn 330 and Mn 360) showed positive reactions for this virus. TbRSV was almost non-existent and was detected only in 2 plants, 1 of Mn 350 and the other of Mn 360.

The ELISA positive plants were eye-indexed for virus symptoms during April to September of the following year. TmRSV was found to infect Mn 61,330,332,350,360 and Me-I. The most conspicuous symptoms appeared on Mn 350; its stems, twigs and branches exhibited circular brownish necrotic spots, chlorotic spots on leaves and some deformed new apical buds. To a lesser extent Mn 332 also showed similar symptoms. The symptoms on other clones were less noticeable, especiallly Mn 360; two of its infected plants, except for a few deformed apical leaves, were almost symptomless.

BBLMV infected Mn 327, 330, 332, 350, 360 and Me-I. Prominent symptoms were exhibited by 332; two from a total of three infected plants clearly showed curled, deformed, mottled and some chlorotic leaves, stunted growth and some dieback of old stems. Similar symptoms of a lesser severity were also exhibited by Mn 327. A few mottled leaves were noticed on Me-I and Mn 360, but Mn 330 and 350 were symptomless.

All BBSSV infected plants of Mn 61, 327, 330 and 360 showed some crescent-shaped leaves. One plant of Mn 360 showed typical BBSSV symptoms. It had some curled up leaves and a few reddish streaks on current and one year old growth. At blossom time a few petals also showed reddish streaks. The presence of BBSSV has also been reported in the adjoining province of Nova Scotia (5).

TbRSV was found only in one plant each of Mn 350 and 360. The infected plants showed some circular brownish spots on stems and branches, necrotic and deformed leaves, stem dieback and stunted growth.

From a total of 11 clonal selections only 4 (Mn 84, 135,354, NBBCL) were found to be completely free from TmRSV, TbRSV, BBLMV and BBSSV. Tlhese selections appear to have adjusted well to the N. B. climatic conditions, considering survival rate of their plants. Plants belonging to Mn 135 and Mn 354 over the **9** year period showed no mortality and this **loss** for Mn 84 and NBBCL was only 1 plant each, suggesting that these clones probably are hardy and not very susceptible to the aforestated viruses. Further work would be needed to determine if these clones are, in fact, resistant to these viruses.

Table 3. Detection by ELISA of BBSSV, BBLMV, TmRSV and TbRSV in eleven halfhigh blueberry clones in New Brunswick.

Clone	No. of plants tested	No. of infected plants	Virus detected	Infected plants Total	Infected plants %
Mn 61	24	1	TmRSV	2	8
		1	TmRSV, BBSSV		
Mn 84	23	-	-	0	0
Mn 135	24	-	-	0	0
Mn 327	24	2	BBLMV	3	13
		1	BBLMV, BBSSV		
Mn 330	13*	1	BBSSV	2	15
		1	TmRSV, BBLMV		
Mn 332	24	1	TmRSV	5	21
		2	BBLMV		
		2	TmRSV. BBLMV		
Mn 350	21	1	TmRSV	3	14
		1	TbRSV		
		1	TmRSV, BBLMV		
Mn 354	24	-	-	0	0
Mn 360	21	2	TmRSV	5	24
		1	BBSSV		
		1	TmRSV, BBLMV		
		1	TmRSV, BBSSV		
Me-1	22	1	TmRSV	2	9
		1	TmRSV, BBLMV		
NBBC1	23	-	-	0	0

• Insufficient number of plants for each replication.

Acknowledgements

The author is thankful to Dr. **R.P.** Singh for sharing his laboratory facilites and to **Mrs.** Anne Dick for typing this manuscript.

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