TITLE: Virus studies in blackberry

RESEARCH OR OUTREACH PROJECT: Research

Principal Investigators:
Gina E. Fernandez
Dept. Horticultural Science
North Carolina State University
170 Kilgore Hall, BOX 7609
Raleigh, NC 27695
Email: Gina_Fernandez@ncsu.edu

Zvezdana Pesic-VanEsbroeck
Dept. Plant Pathology
BOX 7616
North Carolina State University
Raleigh, NC 27695
Email: Zvezdana_Pesic@ncsu.edu

Objectives of the project: This project was part of a multi-institutional project, involving North Carolina State University (NCSU), University of Arkansas, Clemson University, and the USDA-ARS Corvallis, Oregon. Funding was requested from the SRSFC to: 1) determine seasonal and spatial distribution of Tobacco Ringspot Virus (TRSV), Tomato Ringspot Virus (ToRSV), Raspberry Bushy Dwarf Virus (RBDV) and Impatiens Necrotic Spot Virus (INSV) in symptomatic and asymptomatic blackberry plant parts and 2) verify presence/absence of INSV in blackberry.

Justification and Description:

Virus and virus-like diseases have an enormous impact on berry production throughout the world (Ellis et al., 1991; Converse, 1987). They are responsible for many of the special management methods used by nurseries to provide growers with certified planting stock from virus-tested sources. In the U.S., the majority of research concerning virus identification on the species of the genus *Rubus* has been done in the Pacific Northwest and mainly on raspberries. The incidence of blackberry viruses in the eastern and southern regions of the U.S. was until recently, considered relatively uncharacterized.

Published literature on virus detection in *Rubus* has indicated that viruses can be detected at any time when the plant is actively growing (Martin 1997). However, during our surveys conducted in 2001 and 2002, we noted that titer of all viruses seemed to vary during the growing season, i.e. it was highest in the spring, lowest during and after fruiting and then somewhat higher in the fall (Guzmán, unpublished data). In addition, we were able to detect virus in roots and not in other tissues at times during the growing season. Therefore, a need exists for a systematic study detailing when and what part of the blackberry plant to sample for accurate detection of viruses.

Methodologies: Objective 1. Seasonal and spatial distribution of viruses. During the 2003 and 2004 growing seasons we collected tissue samples from 14 symptomatic blackberry plants at the Cunningham Research Station in Kinston, NC. Samples were collected approximately every two weeks from April until August from Arapaho, Apache, Chickasaw, Chesapeake, Triple Crown and Navaho cultivars. The dates in 2003 were 16 April, 30 April, 15 May, 28 May, 11 June, 25 June, 10 July, 23 July and 6 August. In 2004 the dates were 2 April, 12 May, 26 May, 16 June, 24 June, 8 July, 22 July, 5 August and 8 September. Roots, most recent fully expanded primocane leaves, and leaves from the top, middle and bottom portion of the floricanes were collected and taken back to the lab for ELISA (Enzyme-Linked Immunosorbent Assay) tests. We tested all samples for TRSV, ToRSV, RBDV, and INSV. We hypothesized that virus titer was highest in the spring before the plant began to fruit and temperatures were still moderate. We also thought that root tissues may be best for sampling because they are perennial and may therefore be a permanent virus reservoir.
Objective 2. Grafting of blackberry plants that tested positive by ELISA onto indicator plant *Rubus occidentalis* and inoculations with the sap extracted from the same plants onto *Nicotiana benthamiana* have so far generated negative results. We are currently developing protocols for PCR and thrips transmission from virus-infected to healthy blackberry plants. This work will continue in 2005.

Results:

Overall results. In 2003 and 2004 we tested 14 symptomatic blackberry plants parts to determine seasonal and spatial distribution of 4 viruses, TRSV, ToRSV, RBDV and INSV. ELISA tests were conducted on 4,202 samples. This is what we found:

1) All 14 plants tested positive for at least 1 virus in both years. Six plants tested positive for 2 viruses in 2003 but only one plant had 2 viruses in 2004. In 2003, three plants tested positive for 3 viruses and 5 plants were positive 4 viruses. In 2004, 12 plants had 3 viruses, but not a single plant had all 4 viruses.

2) INSV was present in 13 plants both years. ToRSV was in 14 plants in 2003 and was not detected in 2004 in any plant. TRSV was in 7 (2003) and 13 (2004) plants. RBDV was in 5 and 13 plants in 2003 and 2004 respectively

3) The viruses were detected in 8/14, 5/14, 9/14, 6/14, 12/14, 11/14, 9/14, 3/14 and 7/14 plants on the 1st through 9th sampling dates respectively in 2003. 8/14, 10/14, 13/14, 13/14, 13/14, 11/14, 13/14, 5/13, 7/14, 14/14 plants in 2004 on 9 sampling dates.

4) Viruses were detected in roots of all 14 plants, in primocane leaves of 8/14 plants, in the upper third, middle and lower portion of floricanes in 11/14, 13/14 and 10/14 plants in 2003 (complete dataset available upon request). In 2004, viruses were detected in roots of 14/14 plants, 2/14 primocane leaves, 6/14, 7/14 and 3/14 in leaves collected from upper, middle and lower third of floricanes.

Seasonal distribution. Our ability to detect viruses varied by virus and by time of year (Table 1). TRSV could be detected all season, with July and August having the highest incidence of TRSV. ToRSV, had the highest incidence in May and July in 2003, but we did not detect this virus at any time in 2004. September was the best month to sample for RBDV. INSV had the highest incidence in April and May.

Spatial distribution. Spatial distribution of the 4 viruses varied by plant part (Table 2). TRSV could be found in all plant parts, with highest incidence in root pieces. ToRSV,

RBDV and INSV were detected in all of the plant parts, but occurred most frequently in root pieces.

Presence/absence of INSV in blackberry. INSV was detected by ELISA in all 14 blackberry plants in Kinston. However, confirmation of INSV by detection methods other than ELISA continues to be difficult. Grafting of blackberry plants that tested positive by ELISA onto indicator plant *Rubus occidentalis* and inoculations with the sap extracted from the same plants onto *Nicotiana benthamiana* have so far generated negative results. We are currently developing protocols for PCR and thrips transmission from virus-infected to healthy blackberry plants. This work will continue in 2005.

Conclusions:

The detection of viruses in blackberry in the southern U.S. has been challenging. However, we have established that fibrous root pieces are the best part of the plant for detection of TRSV, ToRSV, RBDV and INSV. These root pieces should be collected in either the spring (April-May) or fall (September) and weigh at least 1.0 grams.

The most challenging virus for us to understand is INSV. It has been detected in blackberry plants in the Southern Region of the US by several labs since 2001. However, confirmation of its presence via Koch's postulates is inconclusive. We suspect that one or more viruses yet to be determined may be interacting with the viruses we detected. One of these viruses was recently discovered and named by our colleagues. Leaf samples with chlorotic line patterns were sent to the USDA virology lab in Corvallis, OR and were analyzed using RT-PCR (Tzanetakis et al., 2003). We now know that a new virus, blackberry yellow vein virus (BYVaV) is present in our region.

Finally, our ability to visually identify symptoms and the presence of one or multiple viruses remains a challenge. Virus symptoms varied by cultivar and number of viruses present. We speculate that INSV and these new viruses may play a role in symptom expression. Although we are not seeking additional funding for this project from SRSFC, we will continue to study the cryptic nature of viruses in *Rubus* in the Southern Region.

Impact Statement:

Our research has also shown that the virus "situation" in this region is turning out to be very different from other growing areas. The seasonal and spatial distribution of viruses appears to be highly variable and unlike the scenario that has been established for other growing regions. Our work shows that fibrous root pieces taken in the spring (April-May) are the best means of detecting a virus (TRSV, ToRSV, RBDV and INSV). Root pieces taken in the fall can also yield good results. This will ensure that viruses will be diagnosed accurately and in a timely manner in the future. However, the use of virus-indexed and pathogen-free nursery material remains the essential starting point to ensure the long-term sustainability of the blackberry industry.

Citations:

Martin, R.R., Tzanetakis, I.E., Gergerich, R., Fernández, G. and Pesic, Z.

2004. Blackberry yellow vein associated virus: a new crinivirus found in blackberry. Acta Hort. (ISHS) 656:137-142

Zvezdana Pesic-VanEsbroeck[1], Gina Fernandez[2], Tania Guzman[1] 2004. Identification of Blackberry Viruses in Southeastern U.S. Departments of Plant Pathology[1] and Horticultural Science[2] North Carolina State University, Raleigh, NC 27695, USA. Plant Protection Society of Serbia. 5th CONGRESS OF PLANT PROTECTION. Zlatibor, November 22 - 27, 2004.

Table 1. Seasonal distribution of tobacco ringspot (TRSV), tomato ringspot virus (ToRSV), raspberry bushy dwarf virus (RBDV) and impatience necrotic spot virus (INSV) at two week intervals during two growing seasons.

	TRSV		ToRSV		RBDV		INSV	
	2003	2004	2003	2004	2003	2004	2003	2004
date								
1	3/62	NT	1/69	NT	0/63	NT	25/60	NT
2	2/67	15/60	4/67	0/56	0/67	4/57	0/67	5/56
3	8/69	20/68	14/70	0/67	0/70	5/67	0/70	4/68
4	10/68	28/70	4/68	0/70	0/68	11/70	4/68	10/70
5	7/69	14/70	11/69	0/70	6/69	7/70	0/69	0/70
6	8/70	13/69	3/70	0/70	0/70	1/70	17/70	0/70
7	5/66	19/68	7/67	0/68	0/67	6/64	0/67	3/64
8	4/27	12/67	0/28	0/55	0/27	0/70	0/28	0/66
9	6/28	6/28	7/28	0/28	0/28	0/28	0/28	0/28
10	NT	6/28	NT	0/28	NT	13/28	NT	0/28