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# Response of blackberry cultivars to nematode transmission of Tobacco ringspot virus

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*Alisha Sanny<sup>\*</sup>, John R. Clark<sup>†</sup>, and Rose Gergerich<sup>§</sup>*

## ABSTRACT

A study was conducted on eight cultivars of blackberry ('Apache', 'Arapaho', 'Chester', 'Chickasaw', 'Kiowa', 'Navaho', 'Shawnee', and 'Triple Crown'), of which four plants of each were previously determined in the fall of 2001 to have root, but not leaf, infection with Tobacco ringspot virus (TRSV). The objectives of our study were to determine virus effects on plant vigor and the spread of virus infection in the plants. Eight plants of each cultivar, four infected and four free of infection, were grown in pots on a gravel pad for the 2002 growing season, and samples of primocane and floricanes leaves were taken to determine if TRSV had moved to the above-ground portion of the plants. TRSV infection was determined by ELISA tests. At the end of the growing season (October), the plants were harvested and dry weights determined for floricanes, primocanes, and roots to determine virus effects on plant vigor. In all plants that had been shown to have root TRSV infection, the virus was shown to have moved into the top portion of the plants as evidenced by positive ELISA tests on primocane and floricanes leaf tissue. Dry-weight results indicated no significant interaction of virus infection and cultivar, or any main effects of virus on cane or root growth, as all dry weights were similar for infected and non-infected plants. No dramatic leaf symptoms of virus infection were observed on infected plants in our study at any time during the growing season. Further research should focus on possible virus effects on plants that have been infected for a longer period of time to determine if in fact the virus has any effect on plant growth or productivity.

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## MEET THE STUDENT-AUTHOR



*Alisha Sanny*

Clark, Dr. Rose Gergerich, and Scott Karnes, who contributed to my taking learning far outside the classroom, which greatly enriched my college experience.

In May 2003, I graduated *summa cum laude* with a degree in horticulture management and production. As an undergraduate, I was fortunate to have many splendid work and travel opportunities. I spent time working in the horticulture research plots at the Arkansas Agricultural Research and Extension Center, Fayetteville, the Horticulture Display Gardens on campus, and the Plant Pathology Virology lab. The work I did under Dr. John R. Clark at the horticulture farm led to my position as an Adair Intern in the Plant Pathology Department. The research I did during that internship is presented in this paper. As an undergraduate I was also actively involved in the Horticulture Club and the Razorbacks for Christ. In the Horticulture Club, I spent many hours preparing for plant sales, and I was able to travel to Florida for the American Society of Horticultural Science-Southern Region Annual Meeting. The Razorbacks for Christ provided a place to grow spiritually and cultivate leadership skills and friendships. Through the Razorbacks for Christ, I was able to go to Romania and Ukraine on mission trips. My last semester of college was spent at the Scottish Agricultural College in Ayr, Scotland.

I would like to thank everyone, especially Dr. John R.

## INTRODUCTION

There have been 26 virus or virus-like diseases reported for *Rubus* crops (blackberries and raspberries) in the world (Jones, 1986). Viruses cause more damage in the black and purple raspberries and less damage in red raspberries and blackberries (Crandall, 1995). Many viruses that infect blackberry do not produce distinctive symptoms, and reports of virus effects on blackberries are very limited. A recent study on the impact of Raspberry bushy dwarf virus (RBDV) on 'Marion' blackberry in Oregon showed that there was no virus effect on cane number or length in a two-year period, but that there was a significant yield reduction (50%) in RBDV infected plants, along with reduced berry weight (40%) and drupelet number per berry (39%) (Strik and Martin, 2002). Infected plants also showed visual symptoms, including chlorosis, vein clearing, silver discoloration, and malformed small fruit. Newly infected plants did not display such distinct symptoms.

There are approximately 200-400 acres of blackberries grown and marketed locally throughout Arkansas.

Eleven licensed Arkansas nurseries and 29 licensed nurseries in other states and countries propagate University of Arkansas patented cultivars, as well as other cultivars, for national and international markets (Troxell, 2001). The presence of virus symptoms in nurseries and commercial blackberry fields in Arkansas has been a recent cause for concern. A field survey was conducted in Arkansas of blackberry nurseries for TRSV, RBDV, and Impatiens necrotic spot virus (INSV) in 2002. All three viruses were found, but TRSV was found first and was most prevalent (Rose Gergerich, unpublished).

Leaves of blackberry and dewberry plants in North Carolina infected with TRSV showed faint to severe ringspots, mottling, mosaic, stunting, leaf distortion, and yellow line patterns (Rush and Gooding, 1970). However, they usually did not have symptoms on each cane. Virus symptoms on blackberry plants from Arkansas showed chlorosis, oak-leaf patterns, and mosaic (Troxell, 2001; Fig. 1).

Guzman et al. (2002) more recently conducted a virus survey in North Carolina, South Carolina, and Virginia in 2001-2002. In North Carolina, TRSV was found in

57% (257/451) of the symptomatic plants tested. TRSV was identified in 33% (21/62) of the samples from South Carolina, but TRSV was not identified in any of the plantings tested in Virginia. TRSV was detected in 'Apache' and 'Arapaho' most frequently (>50%). It was also detected in 'Chester', 'Chickasaw', 'Lochness', and 'Rosborough'.

TRSV is a nepovirus that was first reported in *Nicotiana tabacum* by Fromme et al. (1927). The first report of TRSV in blackberry was published in 1965 from North Carolina (Stace-Smith, 1987). Rush and Gooding (1970) isolated TRSV from *Rubus allegheniensis*, *R. argutus*, *R. flagellaris*, and an unidentified *Rubus* species in North Carolina. TRSV has a large host range, including both herbaceous and woody plants (Stace-Smith and Hansen, 1974). It occurs throughout North America, especially in the southeastern United States (Rush and Gooding, 1970).

TRSV virions are isometric, not enveloped, and 25-29 nm in diameter. The genome consists of two single strands of linear RNA, both of which are needed for infection (Brunt, et al., 1996). The primary spread of TRSV in the field is by the dagger nematode, *Xiphinema americanum*. The virus does not multiply in the vector and is lost once the nematode molts. It can also be transmitted mechanically, by infected nursery stock, and by pollen (Brunt, et al., 1996).

In the most extensive study of TRSV on blackberry, Troxell (2001) conducted nematode transmission experiments on eight blackberry cultivars (Apache, Arapaho, Chester, Chickasaw, Kiowa, Navaho, Shawnee, and Triple Crown). She infected tissue-cultured plants with TRSV using *X. americanum* transmission, and found all cultivars were susceptible to this virus as determined by sampling roots of exposed plants and testing for TRSV using Protein-A ELISA tests. ELISA tests revealed that TRSV was not present in the leaves of aboveground portions of these plants during the first growing season following nematode transmission of the virus. Symptoms were seen on primocane leaves of infected plants, but these were mild and transient.

Our study was initiated to further evaluate the virus effects on virus-infected or non-infected plants used in 2001 by Troxell (2001). Specifically, we wanted to determine the impact, if any, of TRSV infection in the second year following nematode transmission on blackberry plant vigor and to find if the virus could be found in aboveground plant portions based on ELISA tests.

## **MATERIALS AND METHODS**

In March 2002, four non-infected and four infected plants of each cultivar used in an earlier study (Troxell,

2001) were chosen for this study. The plants had been grown in 3-L plastic pots the previous season, and had been mulched with sawdust mulch over the winter to protect them from winter injury. In March 2002, the plants were removed from the mulch and pruned, leaving two 0.8-m floricanes. Floricanes were staked using 1-m plastic stakes. The plants were moved to a gravel pad at the Arkansas Agricultural Research and Extension Center, Fayetteville, and four plants (replications) of each cultivar/virus infection status combination were arranged in a randomized block design. Each pot was set up in 12.2-m rows on 1.5-m squares (Fig. 2). Starting in May, pots were fertilized every two weeks with one tablespoon Osmocote (19-6-12) (until July) and irrigated overhead twice daily for one hour (until September). Plants were observed from May to November for virus symptoms.

In July 2002, Protein-A ELISA, as described by Edwards and Cooper (1985), was used for the detection of TRSV in the floricanes and primocanes of all plants. Leaf extracts were prepared by grinding floricanes and primocane leaves with a sap extractor (Erich Pollahne Co., Wennigsen, West Germany). The sap was diluted 1:10 (v:v) in extraction buffer (10.3 mM Na<sub>2</sub>SO<sub>3</sub>, 0.5 mM polyvinylpyrrolidone M.W. 40,000 (PVP-40), 0.2% powdered egg albumin, 2% Tween-20 in PBS-T [137.8 mM NaCl, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.68 mM KCl, 0.05% Tween-20; pH 7.4]). Immulon 1 flat-bottom microtiter plates (Dynex Technologies, Inc., Chantilly, Va) were coated with Protein-A (Sigma Chemical Co., St. Louis, Mo.) at 1µg/mL in coating buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 34.88 mM Na<sub>2</sub>CO<sub>3</sub>, 3.08 mM NaN<sub>3</sub>; pH 9.6). Plates were then coated with polyclonal antiserum to TRSV (from University of Arkansas plant virus antiserum collection) that had been diluted 1:1000 with PBS-T. After addition of the diluted leaf extract to duplicate wells of the plate, TRSV antiserum diluted 1:1000 in PBS-T was added to the plates followed by Protein-A alkaline phosphatase conjugate (Sigma) at 1µg/mL in PBS-T). Finally, nitrophenyl phosphate at 1 mg/mL (Sigma) in substrate buffer (0.39 mM MgCl<sub>2</sub>, 3.84 mM NaN<sub>3</sub>, 12.1% diethanolamine; pH 9.8) was added to the wells. Between all steps, plates were washed three times with PBS-T. All reagents were used at 100µL/well, and incubations were at 24°C for 2 h, except for the incubation after antigen addition, which was at 4°C overnight. Absorbance at 405 nm was determined with a 7520 Microplate Reader (Cambridge Technologies, Inc., Watertown, Mass.).

Plants from three replications were defoliated in November 2002 by hand, and the floricanes and primocanes cut at the crown level (soil surface) and dried separately in paper bags for 4 days at 65°C. After canes were

dry, they were weighed and discarded. Roots were washed, dried in the same manner, and weighed. The fourth replication was kept for further observation. Dry weight data were analyzed as a two-factor randomized complete block by JMP (JMP, version 4.0. SAS Institute, Inc. Cary, N.C., 1989-2000).

## **RESULTS AND DISCUSSION**

All plants of all cultivars that had tested positive in ELISA tests in root samples taken in 2001 tested positive for TRSV in primocane and floricanes leaves in July 2002. The ELISA tests also demonstrated that the non-infected control plants continued to be virus free. The finding that virus was present in the leaves indicates that the virus moved from the roots to the aboveground portion of the plants in the second year after nematode transmission. No virus symptoms were observed on the leaves during the study on infected or non-infected plants.

For the dry-weight data, the analysis of variance indicated no significant ( $P=0.05$ ) interaction of infection status and cultivar for the variables measured. Additionally, the main effect of virus infection status was not different for any variables, indicating no virus effect on plant vigor. The dry-weight means were similar for floricanes, primocanes, and roots (Table 1). Although no significant differences were found between infected and non-infected blackberry plants in this study, it is possible the primocanes that are now virus-infected will show symptoms of virus infection when they develop as floricanes next year. Cultivars averaged over plant-infec-

**Table 1. Main effect means (dry weight in grams) for non-infected vs. infected blackberry cultivars.**

Virus status	Primocanes	Floricanes	Roots
Infected	160.1	31.3	590.5
Non-infected	156.5	26.5	640.3
Significance <sup>z</sup>	0.08	0.75	0.30

<sup>z</sup>Significance- F-test,  $P=0.05$ .

tion status were significantly different for dry weight of primocanes, floricanes, and roots (data not shown). This finding was not important for this investigation since our effort was to identify virus effects, not cultivar vigor differences.

The most noteworthy finding of our study was the second-year presence of TRSV in leaf samples. This has a number of implications for management of blackberry virus diseases. First, it is important for nursery growers and regulatory agencies because plants that become infected in the field by nematode transmission may be carrying virus in their roots (the portion of the plant often used for propagation) but testing negative for virus in their leaves in ELISA tests and appearing healthy based on the lack of leaf symptoms. Second, the dynam-

ics of TRSV movement in the plant are clearer from our results. The delay in virus movement from the roots to the aboveground portion of the plant was longer than expected. This area needs further study to determine when virus symptoms commonly appear on aboveground portions of the plant after nematode transmission. The plants that were not destroyed for analysis in this research will be observed next year after another period of dormancy to determine if leaf symptoms develop on the floricanes which became infected with TRSV during the last year.

Field-grown, mature blackberry plants that express viral symptoms often grow well and bear abundant, good quality fruit. This has raised the question: What is the effect of viruses such as TRSV on blackberries? Our data on second-year plants indicate no virus effect on total plant growth. However, the virus may have greater effects the third year as it spreads further in the plant. Also, our plants did not bear fruit, thus we were unable to determine if TRSV-infected plants produced fruit that was malformed, crumbly, or otherwise possibly affected by virus infection. Additionally, if blackberry plants become infected with more than one virus, the combined effects of these viruses often produce severe disease symptoms.

The concerns of blackberry nursery stock producers are somewhat different from those of blackberry fruit producers. Many states and countries have regulatory agencies that restrict the movement of plants that are not certified as virus-tested. Blackberry plants expressing symptoms of virus infection would be denied entrance by such regulatory agencies. Based on the results of the research reported here, the absence of virus symptoms in blackberry leaves should not be used to determine whether blackberry plants are infected with TRSV.

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**Fig. 1.** Symptoms of TRSV infection in 'Arapaho' blackberry.



**Fig. 2.** Blackberry plants in field trials, mid-summer, at the Arkansas Agricultural Research and Extension Center, Fayetteville.