

Alaskan *Ribes* L. and *Rubus* L. Plant Species Surveyed for Viruses

N.L. Robertson¹, D.F. Quito-Avila² and R.R. Martin³

¹United States Department of Agriculture, Agricultural Research Service, Arctic and Subarctic Plant Gene Bank, Palmer, Alaska, USA

²Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA

³United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, Corvallis, Oregon, USA

Keywords: currant, wild raspberry, vitivirus, *Ribes nigrum* L., *Ribes rubrum* L., *Rubus strigosus* L.

Abstract

Alaska's domesticated and native *Ribes* and *Rubus* genera have virtually gone unchecked for pathogen detections. Cultivated *Ribes* species are predominantly found in home gardens and landscape areas along highways and in cities. In 2008, while surveying native plants for diseases in North Central Alaska near the town of North Pole, mottled leaves were readily visible on wild raspberries (*Rubus strigosus* L.) growing in agricultural berms and on recent clearings next to forests. In 2009 and 2010, other sites close to the original sites were observed to have similar symptoms. Initial protein extracts from partially purified preparations revealed a putative coat protein about 30 kDa. Mechanical transmission occurred in *Nicotiana benthamiana* and *Chenopodium quinoa*. Virion RNA preparations contained two dominant bands about 5.9 kb and 1.9 kb. Based on these biological parameters and similarities with *Raspberry bushy dwarf virus*, leaf sap and purified virion from infected raspberry and *N. benthamiana* were assayed for RBDV by ELISA and western blot. Leaf samples were also tested specifically for RBDV by RT-PCR. Negative results indicated that RBDV was not the causal agent involved in the native raspberry disease. Commercial raspberries growing near infected wild plants did not have symptoms and did not test positive for viruses. Another virus was discovered and detected from domesticated black and red currants (*Ribes nigrum* L. and *R. rubrum* L.) in 2008 and 2010 on the Kenai Peninsula, Alaska. Diseased plants usually contained leaves with vein-clearing that developed into large spots. Field collected leaf samples tested positive for vitivirus using a RT-PCR assay adapted for vitivirus detection. Direct sequencing of the ca. 200 bp PCR product resulted in nucleotides that were most similar to species in the genus *Vitivirus* when analyzed in BLAST. This is the first time that viruses have been detected in currants and raspberries from Alaska and the first report of a vitivirus from *Ribes* spp.

INTRODUCTION

People living in Alaska have cultivated raspberries (*Rubus* L.), currants, and gooseberries (*Ribes* L.) since the arrival of the first settlers from Russia in the mid-1700s. Today, production of small fruits is limited to home gardens or small plots and the fruit is sold at local farm markets. Since virus diseases of small fruits in Alaska have not been examined, surveys were initiated in July 2008. In North Central Alaska, near North Pole, numerous wild raspberry (*Rubus strigosus* L.) plants growing on agricultural berms contained leaves with noticeable light green/yellow mottle (Fig. 1) accompanied with occasional curling and blistering. Another virus-like disease was discovered in domesticated currants (*Ribes* L.) from a home garden on the Kenai Peninsula near Sterling, Alaska. Leaf symptoms consisted of mosaic with chlorotic spots and vein-clearing (Fig. 3). The aim of this research was to isolate and identify the putative viruses from the two described virus-like diseases in currant and raspberry plants, respectively.

MATERIALS AND METHODS

Leaves were collected from symptomatic and healthy appearing raspberry and currant plants from the North Pole and Sterling sites, respectively, placed in plastic bags, and stored in ice chests for about two days before transferring to 4°C in the laboratory. Collections varied between June and August, and occurred from 2008 through 2010.

Virus Characterization

Partially purified virus preparations were obtained by differential centrifugation of homogenized leaves with 10% PVP-10 (polyvinylpyrrolidone) in buffer (Lane, 1986). Protein and virion RNA extractions from the virus preparations were visualized on Coomassie Blue stained 10% SDS-PAGE and ethidium bromide 1% non-denaturing agarose gel, respectively, to reveal the sizes of the putative viral coat protein and RNA species. Double-stranded RNA was extracted from leaves of *Nicotiana benthamiana* Domin that had been inoculated with virion preparations from the original diseased currant plants (Morris and Dodds, 1979; Tzanetakis and Martin, 2008).

RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

Total RNA was extracted from leaves (RNeasy Plant Mini Kit, Qiagen, Inc.) with buffer modification (Thompson et al., 2003), and used for RT-PCR. Protocols using previously described sets of primers for *Raspberry bushy dwarf virus* (RBDV) (Kokko et al., 1996; Ellis et al., 2005) were used for the raspberry samples. Currant samples were assayed with a nested set of primers for vitivirus detection (Dovas and Katis, 2002).

Serology

ELISA pathoscreen kits (Agdia, Inc) were used as directed for detection of RBDV, *Raspberry ringspot virus*, *Tobacco streak virus*, and *Tobacco ringspot virus* for the raspberry samples. Western blotting incorporated the putative coat protein isolated from raspberry tissue and RBDV antiserum.

Transmission/Experimental Plant Hosts

Virion preparations and leaf extracts from the original diseased plants were inoculated mechanically to seedlings of *Chenopodium amaranticolor* Coste et Reyn., *C. quinoa* Willd., and *N. benthamiana*, and grown in growth chambers with 12 h light at ca. 22°C.

RESULTS AND DISCUSSION

Raspberries

A putative CP ~30 kDa and two prominent single-stranded RNA species ~5.9 and ~1.9 kb were detected from purified virus preparations that were derived from raspberry leaves with the described symptoms, and never from healthy appearing leaves. Inoculated *N. benthamiana* and *C. quinoa* developed mottled and curled leaves (Fig. 2A) or numerous local lesions (Fig. 2B), respectively. Extracts of virus from these experimentally infected plants contained the same prominent CP and RNA species from virion extractions as that of the original raspberry host. Based on the susceptibility to *C. quinoa* and raspberry, and the estimated sizes of the CP and the two RNA species, it was hypothesized that the virus isolated from raspberry was an isolate of RBDV (Jones, 2005). However, all serological assays for RBDV, RpRSV, TRSV, and TSV were negative as well as RT-PCR assays that targeted RBDV. In 2009, commercial raspberries sampled near diseased native raspberries did not have symptoms or did not contain the identifying 30 kDa protein. Native raspberry with virus-like symptoms currently are confined to berms within an estimated 20 km radius near North Pole, Alaska. The incidence of infection varied from nearly every plant to clumps of plants within a berm.

Currants

Routine partial virus purifications from leaf samples of affected red (*R. rubrum* L.) and black (*R. nigrum* L.) currants did not reveal distinct prominent protein(s) or RNA species on gels that were suggestive of a virus entity. However, with these same virus preparations and leaf sap from affected currants, virus was transmitted by mechanical inoculations to *C. amaranticolor*, *C. quinoa*, and *N. benthamiana*. These indicator test plants developed symptoms of mosaic and local lesions on the first two hosts and vein-clearing and large chlorotic spots on the latter (Fig. 4). Double-stranded RNA extractions from symptomatic *N. benthamiana* produced a prominent band ~8.2 kb, and two minor bands ~7.0 and ~6.5 kb. The RT-PCR assay for vitivirus (family *Betaflexaviridae*) detection from field collected red and black currants, and all the experimental hosts gave the predicted final ~200 bp PCR product (Martelli et al., 2007). Preliminary results from direct sequencing the PCR product and BLAST analysis suggested that it was a unique virus and related to members in the genus *Vitivirus* (i.e. *Grapevine virus A*, *Grapevine virus B*, and *Grapevine virus E*). Results from RT-PCR vitivirus assays and minipurifications were all negative for 12 samples of native currants - collected on a beach bank in Cook Inlet near Nikiski on the Kenai Peninsula. Presently, the natural host and location for this virus are domesticated red and black currants confined to three separate plots on a small farm near Sterling, Alaska.

CONCLUSIONS

In 2008, a virus of unknown taxonomic classification was isolated from wild raspberry plants in North Central Alaska, and a new putative vitivirus was discovered in domesticated currants on the Kenai Peninsula. Genomic sequencing and analysis are currently underway for definitive identification and classification of both viruses. The viruses from currant and raspberry were also transmitted mechanically to experimental herbaceous hosts, but appear to be confined to woody hosts in nature. Future transmission experiments involving grafts, insects (ie. aphids and mealy bugs), and seed will help determine the natural spread of these viruses. Antisera production is in progress from purified virus derived from red and black currant isolates. Due to the inherent spread of viruses from propagation practices that incorporate cuttings from infected plants, it is possible that the new Alaska “vitivirus” was derived from imported or domesticated currants. An excellent approach to look for new or novel viruses, is to systematically assay each plant in the *Ribes* L. collection that is maintained in the Arctic and Subarctic Plant Gene Bank (Palmer, Alaska) and National Clonal Germplasm Repository (Corvallis, Oregon). Virus indexing protocols for examination of the *Ribes* collection will incorporate RT-PCR detection assays specifically for vitiviruses. This is the first report of viruses in currants and raspberries from Alaska and the first report of a vitivirus in *Ribes* spp.

ACKNOWLEDGEMENTS

Thanks to Kathryn Brown, Christine Macknicki, Carmel Sergio, and Todd Steinlage for their excellent laboratory work.

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Figures



Fig. 1. Wild raspberry, *Rubus strigosus* L., displaying mottled leaves from a berm near North Pole, Alaska in 2009.



Fig. 2. Experimental hosts displaying leaf curl and mosaic in *Nicotiana benthamiana* (A) and local lesions on *Chenopodium quinoa* (B) after mechanical inoculation of partially purified virus from wild raspberry that was collected from North Pole, Alaska in 2010.



Fig. 3. Black currant, *Ribes nigrum*, leaf collected in 2008 on the Kenai Peninsula, Alaska with vein-clearing and chlorotic spots.

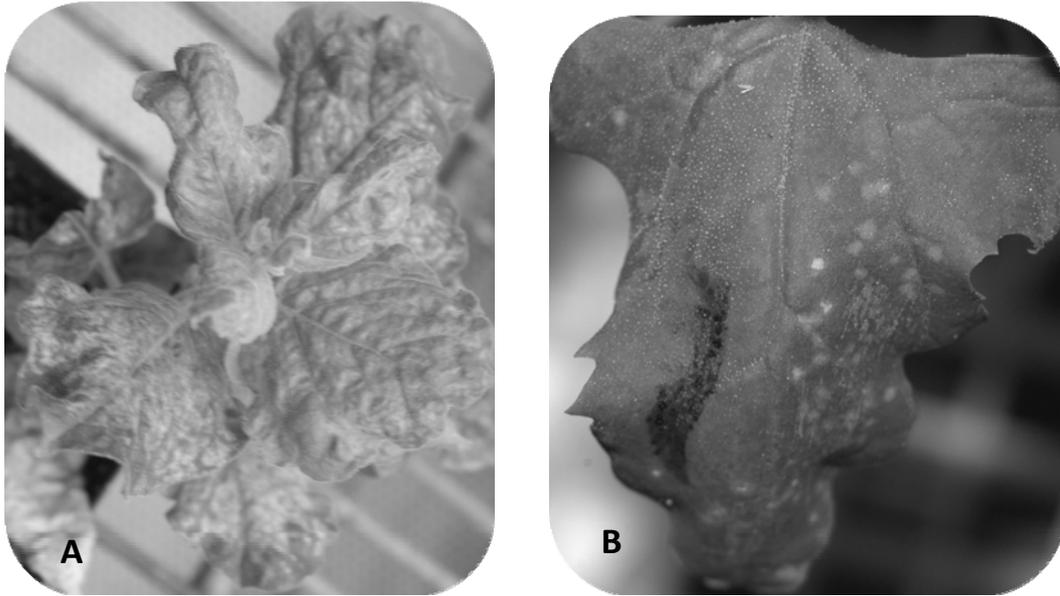


Fig. 4. Experimental hosts displaying mosaic in *Nicotiana benthamiana* (A) and local lesions on *Chenopodium quinoa* (B) from mechanical inoculations of virus from sap of domesticated red currant that was collected in 2010 from the Kenai Peninsula, Alaska.