

## Detection and Elimination of Viruses in USDA Hop (*Humulus lupulus*) Germplasm Collection

J.D. Postman, J.S. DeNoma and B.M. Reed

United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, Corvallis, Oregon 97333  
United States

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### Abstract

The United States Department of Agriculture (USDA), Agricultural Research Service (ARS) maintains a collection of *Humulus* germplasm at its National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, for the conservation and improvement of hop genetic resources. About 268 clonal accessions and seedlings are maintained as potted plants in a screen house. A 'core' subset of 84 genotypes is also maintained in vitro under cold storage. Clonal accessions were tested by ELISA for *Apple mosaic ilarvirus* (ApMV), *Arabis mosaic nepovirus* (ArMV), *Hop latent carlavirus* (HpLV), *American hop latent carlavirus* (AHLV), *Hop mosaic carlavirus* (HpMV), and *Prunus necrotic ringspot ilarvirus* (PNRV). Ninety-eight hop clones out of 200 were found to be virus infected. HpMV was the virus most commonly detected (67% of infected plants), followed by HpLV (51%), AHLV (50%), and ApMV (28%). ArMV and PNRV were not detected. More than 60% of infected accessions contained 2 or more viruses, and more than 25% contained 3 or more. Cultivars 'Wye Target', 'Vojvodina', 'Shinsuwase', 'Cascade' and 'USDA 21119' were infected with all 4 viruses. Alternating temperature heat-therapy and apical meristem culture were used to eliminate viruses from infected plants. Infected plants were grown for 2 weeks at temperatures alternating between 38°C and 30°C every 4 hours. Apical meristems 0.5mm or smaller were dissected and grown in vitro until large enough to transfer to soil in the glasshouse. Following a natural dormant period, plants were retested by ELISA. Of 182 plants regenerated from heat treated meristems, none tested positive in subsequent virus assays. Presently, the NCGR maintains 125 unique hop genotypes that have tested negative for all of these hop viruses.

### INTRODUCTION

The United States Department of Agriculture (USDA), Agricultural Research Service (ARS) maintains a collection of *Humulus* germplasm at its National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, for the conservation and improvement of hop genetic resources. Hops are also an important commercial crop in Idaho, Oregon and Washington. At least 5 viruses are known to occur in cultivated hops in this part of the United States. Klein and Husfloen (1995) examined commercial hop plantings in the Pacific Northwest for viruses by enzyme linked immunosorbent assay (ELISA) and found that more than half the samples were infected with *Hop latent carlavirus* (HpLV), *American hop latent carlavirus* (AHLV) or *Prunus necrotic ringspot ilarvirus* (PNRV). They detected lesser amounts of *Hop mosaic carlavirus* (HpMV) and *Apple mosaic ilarvirus* (ApMV). In addition to ApMV, HpLV and HpMV, the European Plant Protection Organization (EPPO, 1998) requires testing for *Arabis mosaic nepovirus* (ArMV) as part of any hop certification program.

### Viruses of Hops

Carlaviruses:

- AHLV is known only to infect hop. It is common in the U.S. (Klein and Husfloen,

1995) but in Europe has only been found in breeder collections on introductions from the U.S. It has also been introduced to Australia, New Zealand and Tasmania. AHLV is not associated with disease symptoms. AHLV has been observed to spread in the U.S., presumably by aphids, but has not been observed to spread outside the U.S. (Adams, 1986; Adams and Barbara, 1982b).

- HpLV occurs worldwide and is known only to infect *Humulus*. It is spread by aphids and is not associated with specific disease symptoms (Adams and Barbara, 1982b).
- HpMV occurs worldwide. Natural hosts are *Humulus* and several herbaceous weed species. It is spread by aphids and causes veinbanding, leaf distortion, and poor twining in certain hop cultivars. Cultivars of the Goldings-type are severely affected (Adams and Barbara, 1980). HpMV and HpLV are distinct viruses, but are serologically related (Hataya et al., 2001; Adams and Barbara, 1982a). Hataya et al. (2001) found that of 15 carlaviruses, HpLV had the highest genomic sequence homology to HpMV in 4 different amino acid open reading frames.

#### Iilarviruses:

- ApMV occurs worldwide. It naturally infects several unrelated perennial hosts including *Malus*, *Prunus*, *Aesculus*, *Rosa*, and *Corylus* in addition to *Humulus*. ApMV spreads in hops, but the vector is uncertain. Spatial distribution in hop gardens has suggested mechanical transmission between plants (Eppler and Dahdahbiglou, 1991), and experimental work in field and glasshouse trials by Pethybridge et al. (2002) confirmed the virus could be transmitted by mechanical inoculation, pruning or foliar contact. It is known to spread by pollen or by seed in other species.
- PNRV has a wide host range and occurs worldwide. It spreads by pollen in other hosts, but the vector in hops is uncertain. PNRV is serologically related to ApMV and is associated with the same symptoms as that virus (Smith and Skotland, 1986).

#### Nepoviruses:

- ArMV has a wide host range and has been reported from Europe, Japan, Australia and New Zealand. Occurrence in Australia was only on introduced breeding material that was subsequently destroyed (Pethybridge, personal communication 2004). It is vectored by the nematode *Xiphinema diversicaudatum*, and is associated with the diseases hop split leaf blotch, bare bine, nettle-head and hop chlorotic disease (Adams et al., 1987; Büchen-Osmond, 2002).

○ Isolated infections of *Humulus* by petunia asteroid mosaic tombusvirus and cherry leafroll nepovirus have been reported but these viruses are not considered to be important pathogens of hop (EPPO, 1998). An ilarvirus serologically related to PNRV was detected in England from seedlings of *Humulus japonicus* grown from seed imported from China (Adams et al., 1989). The virus was named *Humulus japonicus* ilarvirus (HJV) and has not been reported elsewhere. Cultivars and selections maintained as plants at NCGR are routinely assayed for the incidence of important viruses commonly found infecting hop. This paper reports on virus incidence and efforts to establish an in vitro core germplasm collection.

## MATERIALS AND METHODS

### NCGR *Humulus* Collection

The NCGR *Humulus* collection represents world diversity of this crop. It includes 254 seed accessions which represent hop populations collected in the wild, and 268 plant accessions. The plant accessions include 161 cultivars or selections and 107 seedlings. Clonal accessions are maintained as potted plants in screenhouses or glasshouses, and 84 are also stored as in vitro cultures at 4°C. In 1997, 80 hop clones were designated as “core” accessions, a subset of the total collection which represents a broad cross section

of the genetic diversity of the genus. Core accessions were the initial focus of virus testing and virus elimination with additional accessions included in subsequent years.

### **Virus Detection**

Two hundred clonal accessions were tested by double antibody sandwich ELISA (Clark and Adams, 1977) for ApMV, ArMV, HpLV, AHLV, HpMV, and PNRV. Antisera to AHLV, HpLV and HpMV were obtained from R. Hampton (USDA-ARS, Corvallis, Oregon) in 1989. Improved HpMV antiserum was obtained from R. Klein (Washington State University, Prosser, Washington) in 1999. Antiserum to ArMV was obtained from Agdia Inc. (Elkhart, Indiana, USA) and American Type Culture Collection (ATCC) (Manassas, Virginia, USA). Antiserum to ApMV was obtained from ATCC (ATCC 254 and ATCC 32). Antisera to PNRV were obtained from R. Klein (hop isolate HP-1) and ATCC (ATCC 22-Fulton G isolate).

### **Virus Elimination**

Alternating temperature heat-therapy and apical meristem culture was used in an attempt to eliminate viruses from infected plants. Plants were grown in a growth chamber at temperatures alternating between 38°C and 30°C every 4 hours. After 2 weeks, shoot tips were collected and surface sterilized in 0.5% sodium hypochlorite. Apical meristems [0.5mm or smaller] were dissected and grown *in vitro* on NCGR *Humulus* medium (Reed et al., 2003) until large enough to transfer to soil in the glasshouse. Following a natural dormant period, plants were retested by ELISA.

## **RESULTS**

### **Virus Detection**

Ninety-eight hop clones of the 200 tested prior to elimination procedures were found to be infected with one or more viruses. HpMV was the virus most commonly detected with 67% of infected plants testing positive for this virus. HpLV, AHLV, and ApMV were detected in 51%, 50%, and 29% respectively. ArMV and PNRV were not detected. Of the 98 infected accessions, 62 were infected with at least 2 and 28 tested positive for at least 3 viruses. All 4 viruses (AHLV, ApMV, HpLV and HpMV) were detected in the 5 cultivars ‘Wye Target’, ‘Vojvodina’, ‘Shinsuwase’, ‘Cascade’, and ‘USDA 21119’.

### **Virus Elimination**

A maximum of 4 sub-clones were generated from meristems for each hop. None of the 182 plants regenerated from heat-treated meristems tested positive for any of the 6 viruses. A single sub-clone was selected to replace each original virus-infected clone in the NCGR core collection.

## **DISCUSSION**

Klein and Husfloen (1995) found that HpLV was the most commonly detected virus in their survey of Pacific Northwest production fields. We found that HpLV was second in incidence to HpMV in hop germplasm received at NCGR from breeder collections. We did not detect PNRV in our hop germplasm, although serotypes of PNRV have been widely reported in hops (Barbara et al., 1978; Bock, 1966; Klein and Husfloen, 1995) and this virus is listed in recommended international indexing procedures for *Humulus* (EPPO, 1998; Martin, 2001). Bock (1966) distinguished at least 3 strains of PNRV with only certain isolates reacting strongly in agar double-diffusion tests to antiserum against a cherry isolate (Fulton G). Barbara et al. (1978) determined that the serotypes of PNRV found in hop did not react in ELISA with the Fulton G antiserum. They suggested that the serotypes found in hop are actually ApMV or a form that is intermediate between PNRV and ApMV. Smith and Skotland (1986) designated two serotypes of PNRV-related viruses from hop in Washington, USA as HP-1 and HP-2.

Their HP-1 was serologically distinct from PNRV Fulton G, but similar to Fulton's 'Cherry' isolate of ApMV. The Washington HP-2 isolate gave only a very slight reaction to PNRV -Fulton G in Elisa, but both HP-1 and HP-2 reacted with Fulton ApMV-Cherry antiserum (Smith and Skotland, 1986). No NCGR hop accessions reacted in ELISA using antisera against PNRV Fulton G or PNRV HP-1; although 28 tested positive using antisera against apple and rose isolates of ApMV. Kanno et al. (1993) detected ApMV virus in 14 of 18 hop gardens examined in Japan, but similarly did not find any hop plants infected with PNRV. Klein (R. Klein, personal communication 1998) was able to detect his entire Washington hop ilarvirus isolates with antiserum against ApMV, but was not able to detect any of these viruses using the PNRV specific monoclonal antibodies available at that time from ATCC.

ArMV is not known to occur in the United States (Adams et al., 1987), however was included in this study as many accessions in United States breeding programs originated in Europe where ArMV is prevalent (Adams et al., 1987). We did not detect ArMV in the USDA hop germplasm collection.

The 4 viruses detected in NCGR hop accessions were easily eliminated using thermotherapy combined with apical meristem culture. Vine and Jones (1969) were able to eliminate HpMV and PNRV, but not HpLV, using shoot tip culture alone with shoot tips up to 5.0 mm in length. Adams (1975) successfully eliminated both carlaviruses HpMV and HpLV from about 80% of infected hop plants using meristem culture alone, but heat therapy combined with meristem culture was required to eliminate the ilarvirus he referred to as PNRV. Hesse and Maier (1994) successfully eliminated 6 viruses from several hop cultivars using in vitro heat therapy combined with meristem culture. Cajza et al. (1997) was also successful at generating a large number of virus-free hop plantlets by preparing meristems both before and after heat therapy. At the USDA hop gene bank 125 *Humulus* genotypes that have undergone the virus elimination protocols described herein are being stored, of which 65% are also stored in vitro. This valuable hop germplasm is freely available to researchers around the world.

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