Sarah J. Pethybridge and Frank S. Hay

Tasmanian Institute of Agricultural Research (TIAR), University of Tasmania, Burnie, Tasmania, Australia

Dez J. Barbara

Warwick HRI, University of Warwick, Wellesbourne, Warwick, United Kingdom

Kenneth C. Eastwell

Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA, USA

Calum R. Wilson

TIAR – University of Tasmania, New Town Research Laboratories, New Town, Tasmania, Australia

Viruses and Viroids Infecting Hop: Significance, Epidemiology, and Management

The hop (*Humulus lupulus*) is a hardy, climbing, dioecious, perennial plant native to Europe, Asia, and North America (172). The genus *Humulus* belongs to the family Cannabaceae and contains three species: *H. japonicus*, *H. lupulus*, and *H. yunnanensis* (32,117). Hops are grown predominantly for their cones (strobiles), which contain glands producing resins, essential oils, and polyphenols (Fig. 1). These compounds are used primarily to add bitterness and aroma to beer. The most important of these compounds for brewing are the alpha acids. Alpha acids are acyl-substituted phloroglucinols, differing from each other only in the nature of the acyl R side chain. They can be separated into humulone (R = isovaleryl), cohumulone (R = isobutryl), adhumulone (R = alpha-methyl butryl), prehumulone, and posthumulone (127). The main properties of alpha acids in relation to beer production are improved foam stability, suppression of gushing, and contributions to bacteriological stability (48). The bitterness of beer is related to stereoisomer formation of each major alpha acid in the brewing process (124). Beta acids are predominantly separated into lupulone, colupulone, and adlupulone. They have limited bittering power but are particularly important because of their bactericidal properties (194). More than 200 other essential oils also occur within the cones, including hydrocarbons and oxygenated and sulfur-containing compounds, which are responsible for the

aroma and flavor of the final product (170). Hops are also grown for their medicinal and soporific effects, as ornamental plants, and as edible delicacies (146).

The areas in which hop can be grown are limited by strict day length and temperature requirements for flowering and hence cone production. Production is generally restricted around 35° latitudes in both hemispheres (32,117). Supplementary artificial lighting has been used to produce hops in areas of lower latitudes, such as in South Africa (196). In 2005, the most significant regions of hop production (i.e., countries with >1,000 ha) were Germany, the Czech Republic, Poland, Slovenia, Ukraine, the United Kingdom, the northwestern states of the United States (Idaho, Washington, and Oregon), and China. Lesser quantities were grown in 14 other European countries. A few other countries grow 500 ha or less, most notably Japan, Argentina, Australia, South Africa, New Zealand, and India (26). The largest area of production was in Germany (17,161 ha producing 34,466.8 metric tons), while 11,956 ha were grown in the United States, producing 24,002 metric tons (26).

Viruses and viroids pose significant constraints to the production of high yields of hop cultivars worldwide. In some countries, such as Australia and New Zealand, these pathogens are considered the only significant pathological problems. This is due to the absence of severe fungal diseases, such as powdery mildew (154) and downy mildew (155). Infections by five viruses and two viroids are or have been widespread and important in commercial hop yards. A further 12 viruses (of which two are poorly characterized) and 1 viroid have been reported in hop; these either have limited distributions or occur only sporadically and are not considered important. The viruses considered generally important are the three carlaviruses, *Hop*

mosaic virus (HpMV) (3,20,94,156), *Hop latent virus* (HpLV) (4,21,55,150,180), and *American hop latent virus* (AHLV) (5,22,149); the ilarvirus *Apple mosaic virus* (ApMV) (27,28,46,64); and the nepovirus *Arabidopsis mosaic virus* (ArMV) (6,9,50,82). The important viroids infecting hops are *Hop latent viroid* (HpLVd) (10,14,24,25,151) and *Hop stunt viroid* (HpSVd) (162–164,201).

Although the problems posed by viruses and viroids for the production of hops are not unique among perennial crops, they are particularly challenging because the rates of spread are often much higher than in other crops such as top fruit. As such, the control measures used with hops present a good case study for the control of these pathogens in crops where long life (hop yards are typically kept for up to 20 years and may last 50 years) means they are repeatedly exposed to infection. The reasons for rapid spread relative to tree fruit crops are unclear, but one might speculate that the very rapid and massive annual growth (the entire aboveground part of the plants is replaced every year, and main stems generally grow more than 5 meters in only 3 to 4 months) in closely spaced plantings favors both mechanical transmission (for HpSVd, HpLVd, and ApMV) and heavy infestations with aphids (for the carlaviruses). The study of hop viruses and viroids also emphasizes the need to fully understand and quantify the effects of these pathogens on yield and/or quality of crops, which are highly dependent on cultivar, pathogen strain, and environmental conditions.

Carlaviruses

HpMV. Hop mosaic disease was first described by Salmon (156), although characteristic symptoms of this disease were recorded as early as 1907 in experimental plots in the United Kingdom (94). HpMV

Corresponding author: Sarah J. Pethybridge, Tasmanian Institute of Agricultural Research (TIAR), University of Tasmania, P.O. Box 3523, Burnie, Tasmania, 7320, Australia; E-mail: sarah_jp@utas.edu.au

has since been found infecting hops in commercial hop yards throughout Europe (144,167), Australia (140), the United States (83), New Zealand (76), China (202), Japan (79), and South Africa (196).

Virions of HpMV are filamentous with modal dimensions of 651 nm by 13.8 nm (3,20). The genome is estimated to consist of ca. 8,800 nucleotides and is encapsulated by a single protein species of molecular mass ca. 34.1 kDa (3,70).

HpMV is commonly found only in *H. lupulus* (3,4) but has also been detected in wild plants of *Urtica urens* in the United Kingdom (3), and in *Stellaria media*, *Chenopodium album*, *Plantago major*, and *Polygonum aviculare* in Germany (20). The significance of these wild hosts is uncertain, but they are thought unlikely to be important for the disease in hops. Infection by HpMV of most modern hop cultivars is generally symptomless, but the effect on hop growth or yield varies in magnitude (140,182). However, in the no longer commonly grown mosaic-sensitive Goldings-type cultivars (i.e., Bramling, Eastwell Golding, Wye Mathon, and J78) HpMV may result in marked symptoms and may be lethal. Leaves of infected mosaic-sensitive plants develop a conspicuous clearing or yellow banding along main veins and curl downward at the margin. Vein clearing is a better diagnostic feature, as the mottle symptom is similar to that induced by some nutrient deficiencies or herbicides (Fig. 2). Infected plants are often stunted, bines tend to fall away from the support string, and plants produce only a small number of cones (182). Some contemporary cultivars with Goldings heritage, such as cv. Chinook, exhibit moderate yield losses and symptom development (147). Different strains of HpMV have been suggested but not fully described (55,92,94).

HpLV. This virus was first described by Schmidt et al. (168) but was thought to be synonymous with HpMV (29) until a latent infection was discovered in a cultivar highly sensitive to HpMV infection (148,150,187). HpLV has been reported in hop yards throughout Europe (54,180), the United States (150), New Zealand (76), Australia (112,140), China (202), South Africa (196), and Japan (79). HpLV has only been found in *H. lupulus* (4,21), with most cultivars remaining symptomless, although sporadic development of systemic chlorotic flecking in cv. Hersbrucker Spät has been described (54).

Virions of HpLV are filamentous with characteristic dimensions slightly larger than HpMV of 674 nm by 14.4 nm. The predicted molecular mass of the capsid protein is 33 kDa, and the genome consists of ca. 8,612 nucleotides in one single-stranded RNA species (4,21,72).

AHLV. AHLV is common in the United States but generally occurs at a lower frequency than other carlaviruses (149).

AHLV was reported at low incidence in New Zealand (76). It has been reported in post-entry quarantine of breeding material in Germany (57), the United Kingdom (4),

and Australia (112) with infected plants destroyed and the virus eradicated (22). Virions are filamentous particles with modal dimensions of 678 nm by 15 nm. The

Fig. 1. Hop cones (strobiles) contain glands producing resins, essential oils, and polyphenols. (Courtesy D. H. Gent)

Fig. 2. Range of symptoms (mild on right and severe on left) on leaves from infection by *Hop mosaic virus* on a sensitive Golding-type hop cultivar.

genome of this virus consists of ca. 8,605 nucleotides with a capsid protein of 34.5 kDa (22). AHLV shares little serological relationship to HpLV, HpMV, or other carlaviruses (22). AHLV has only been found in *H. lupulus* (4,5,22).

Transmission of hop carlaviruses. The hop carlaviruses are principally spread between hop plants by aphid vectors in a nonpersistent manner and by mechanical means (4,58,91). Transmission by seed is not considered important for HpLV (4) or HpMV (20). There are no reports of seed transmission of AHLV.

The most important aphid vector for the three hop carlaviruses in most production regions is the hop-damson aphid, *Phorodon humuli*, (3,58,125,126) due to its high abundance. *P. humuli* is a holocyclic aphid that overwinters on *Prunus* spp. as an egg. Besides acting as a virus vector, it also causes direct damage from feeding on cones and leaves over summer (58). Migration from *Prunus* spp. to hop by the alate form begins generally in early May but depends upon daily temperatures, after which apterous forms are produced on hop (58). The polyphagous potato aphid (*Macrosiphum euphorbiae*) and green peach aphid (*Myzus persicae*) both vector HpMV and HpLV (3,4,47). These additional species are likely to be important in establishing new foci, especially in Australian and New Zealand hop yards where the hop-damson aphid is not present (76,113,139). Studies with the oat aphid (*Sitobion avenae*) failed to transmit HpMV (3). Several other aphid species have been recorded within hop yards (56,134). As most viruses transmitted nonpersistently by aphids have multiple vectors, it is possible that a much wider range of aphids and perhaps transient visitors also play a role in hop carlavirus transmission (133,134).

Spatial patterns of carlavirus epidemics. Rapid spread of HpMV and HpLV in commercial yards occurs where *P. humuli* is endemic. In the United Kingdom, in a block of initially virus-tested (no infections detected) cv. Northern Brewer, HpMV was found in ca. 50% of plants after 1 year (182). In another plot, Adams et al. (7) reported that 42% of plants were infected after 1 year, and that in the second year, 81% of plants were infected. Adams et al. (8) further reported 87% of plants were infected by HpLV and HpMV 3 years after planting with virus-tested material. In the United States, the incidence of HpLV, HpMV, and AHLV in cv. Horizon and cv. Nugget yards 3 years of age was 66, 25, and 34%, respectively (135). Rapid spread after planting with virus-tested material was anticipated from the presence of *P. humuli* (35), but the incidence of HpLV and HpMV in these yards was considerably lower than that of HpLV and HpMV in the United Kingdom and Europe (135).

In comparison with observations in Europe and the United States, carlavirus

spread in Australian and New Zealand hop yards is relatively slow. This can be attributed to the absence of *P. humuli* (76,113,139,140). In Australia, the incidence of these viruses after yard establishment with virus-tested material is dependent upon cultivar (139,140). For example, in yards of the virus-susceptible cv. Victoria, 89 to 98% and 72 to 96% of plants were infected with HpLV and HpMV, respectively, after 7 and 9 years. In contrast, in yards of the relatively resistant cv. Pride of Ringwood of at least 10 years of age, HpLV and HpMV infections were not frequently detected (140).

In areas where *P. humuli* is ubiquitous, strong edge effects and infection gradients are characteristic of carlavirus spread, reflecting the role of *Prunus* spp., such as sloe (*P. spinosa*), plum (*P. domestica*), cherry plum (*P. cerasifera*), and damson (*P. insititia*) as winter hosts of the vector (34). Obstacles such as roads, headlands, or hedgerows have been shown to significantly steepen the infection gradient (8). Chambers et al. (38) reported carlavirus incidence as almost 100% in the outer rows of a cv. Perle yard positioned directly adjacent to a hedgerow and infected block, while virus incidence in inner rows was less than 10%. Infection was reduced significantly in plots of cv. Wye Northdown with guard rows of cv. Northern Brewer to reduce aphid flight incursions.

Spatial and temporal analyses of carlavirus epidemics in Australia have consistently shown aggregation of infected plants along rows with lesser, but significant, aggregation across rows. This has strongly suggested the role of mechanical transmission through agronomic activities such as mowing of basal growth (137). HpLV epidemiology is consistent with very short range spread with minimal influence of inoculum from outside the yard of interest. In contrast, HpMV spread patterns are more consistent with local (within the yard) spread coupled with background (outside the yard) infection, suggesting HpMV was introduced to the yards by aphid vectors, and subsequent localized spread occurred by mechanical means or aphid vectors moving along rows (133,134). In comparative studies in the United States, the spatial distribution of cv. Horizon plants infected by HpLV and AHLV was strongly influenced by plant contact. In contrast, HpMV infection in cv. Horizon and Nugget plants was autocorrelated along rows and unaffected by basal growth contact (135), suggesting that mechanical transmission rather than direct plant contact is important for HpMV within yards. Analyses of the associations between HpLV and HpMV in Australian yards have also consistently suggested a strong positive association across cultivars and locations (136), probably reflecting shared means for localized spread once established in yards (47).

Effect of carlaviruses on yield and levels of brewing organic acids. There are limited data on the influence of individual carlaviruses on hop growth, yield, and product quality due to the difficulty of establishing and maintaining comparative field trials over multiple seasons, and the high natural incidence of co-infections with multiple viruses and viroids. A summary of currently available data is given in Table 1.

As previously mentioned, HpMV infection in mosaic-sensitive cultivars results in a severe and often lethal reaction (182). In many other cultivars, the influence of carlavirus infections is less deleterious and variable. Chambers et al. (38) reported that over 4 years there was minimal difference in cone yield and alpha acid content between plants of cv. Northdown infected by HpLV, compared with those infected with both HpLV and HpMV. Cultivar Challenger, however, was found to yield slightly more in the absence of HpMV. In the United States, significant deleterious effects on agronomic characteristics and yield have been attributed to HpLV and HpMV in cv. Chinook. Both viruses significantly reduced lateral length, leaf weight, and the number of nodes per lateral. The height of plants infected by HpMV was also significantly reduced. All yield parameters (cone yield, dry matter content, the number of cones per plant, and levels of brewing organic acids and oils) were also significantly reduced by these viruses (147).

In Australia, infection by HpMV in cv. Pride of Ringwood reduced yield by 50 to 54% and alpha acid levels by 11 to 19% in each of 2 years (138). Infection by HpLV in cv. Agate was also associated with significant reductions in plant height, losses in dry cone yield varying from 40 to 70%, a 44% reduction in alpha acid levels and the cohumulone portion of the alpha acids, and a 42% reduction in the alpha to beta acid ratio in the first year of production. However, in the second year, no significant reductions in brewing organic acids were detected. In the same study, infection by HpLV and HpMV did not deleteriously affect the agronomic characteristics or yield in cv. Super Pride (139). Significant reductions in yield from individual carlaviruses and their combinations have also been reported in commercial yards in New Zealand (75).

HpMV infection has also been suggested to increase susceptibility of hops to the root rot pathogen *Phytophthora citricola* (141).

ApMV

Infection of hop by an ilarvirus was first suggested following transmission of a virus from hop to cucumber (*Cucumis sativus*), giving rise to symptoms indistinguishable from those induced by isolates of *Prunus necrotic ringspot virus*

(PNRSV) (64). Bock (27) showed this virus was at high prevalence and incidence in commercial yards. Bock (28) described two isolates of PNRSV in hop, designated PNRSV-A (apple) or PNRSV-C (cherry) serotype depending upon the strength of their reactions in Ouchterlony double diffusion tests with antisera prepared against an ApMV isolate from *Malus* spp. and a PNRSV isolate from *Prunus* spp. Fulton (65) further demonstrated that Bock's 'A' isolate was serologically identical to ApMV, while the 'C' isolate was closely related to PNRSV from *Prunus* spp. Barbara et al. (23) also described isolates from hop similar to Bock's 'A' type that reacted strongly with antiserum produced against an apple isolate (ApMV-P) but did not react with antisera against a cherry isolate (PNRSV-G). This study also identified isolates that reacted moderately with both antisera and were similar to Bock's hop 'C' type. However, because these isolates did not react as strongly with ApMV-P antisera as 'A' types, or as strongly to PNRSV-G as isolates from cherry, they redefined Bock's 'C' serotype to 'I' (intermediate). This distinguished this serotype from true 'A' and 'C' types that occur singly or as mixtures only in *Prunus* spp. (cherry and plum) and other Rosaceous species. Only 'A' serotypes have been found in *Malus* spp., while both 'A' and 'I' serotypes occur in hop. Studies from the United States also characterized two isolate types from hop, designated 'HP-1' and 'HP-2'. These were distinguished by differential symptom production in herbaceous indicator species, *Chenopodium*

hybridum and *C. berlandieri*. Further serological testing identified 'HP-2' as an intermediate isolate and 'HP-1' as similar to ApMV (174). Nucleoprotein analysis of ilarvirus isolates also found that isolates from hop were distinct to those from *Prunus* spp. or rose (45). Analysis of RNA sequences of the capsid gene have further substantiated that both ilarvirus serotypes from hops are strains of ApMV, but distinct from those infecting *Malus* spp., and demonstrated that 'Shirofugen' flowering cherry assays may be used to differentiate strains based on hypersensitivity (46). Eppler (59) has identified the cherry serotype of PNRSV in wild hop in Germany, but to our knowledge there are no confirmed reports of PNRSV in cultivated hop. The ilarvirus serotypes infecting hop are therefore now referred to as ApMV-intermediate (ApMV-I) and ApMV-hop (ApMV-H) to reflect the distant serological relationship with PNRSV and the closer but phylogenetically distinct relationship with ApMV from *Malus* spp. (46). No evidence has been found of co-infections between these serotypes in hop (37,46).

ApMV virions consist of three quasi-spherical or slightly pleomorphic particles, ranging in diameter between 25 and 29 nm. Each particle contains one component of the tripartite single-stranded RNA genome of approximately 2.1, 3.0, and 3.5 kb. These particles and the coat protein are all required for infection (23).

Symptoms of ApMV in hop range from chlorotic ringspots that can become necrotic (Fig. 3), oak-leaf line patterns (Fig. 4), to latency. If symptoms are expressed,

they are usually found after rapid changes in temperature (23).

Spatial patterns of ApMV epidemics. ApMV spread in hop yards is generally slow but strongly influenced by cultivar. For example, studies in the United Kingdom have found that ApMV infected 30% of cv. Northern Brewer plants over a 4-year period, but increased more slowly in cv. Wye Northdown (9% of plants over 6 years). In a separate cv. Wye Northdown yard, 26% of plants became infected with ApMV over a 10-year period (183). Studies in New Zealand in a cv. Superalpha yard found no change in the incidence of ApMV over a 3-year period, but in a second yard of the same cultivar, the incidence of ApMV increased by 13% over the same period (76). In Australia, infection by ApMV is also highly variable and dependent on cultivar. In cv. Pride of Ringwood, ApMV was not detected in several 19-year-old yards. However, in cv. Victoria yards, almost 100% of the plants were infected only 7 years after planting with virus-tested material (140). Similar rapid spread of ApMV into newly established yards has also been reported in cv. Agate (139).

Quantitative analyses of the spatial pattern of ApMV epidemics in Australia have consistently suggested significant autocorrelation of infected plants in the same direction as mechanical agronomic operations, such as slashing to control basal growth (137). Spatiotemporal analyses provided further evidence of localized spread of ApMV but could not exclude the influence of inoculum sources from outside the yard (133).

Table 1. Influence of hop viruses on cone yields and brewing alpha acid levels (adapted from Wilson et al. [199])

Cultivar	Yield loss (%)										Ref.
	ApMV ^a		HpMV ^a		HpLV ^a		ArMV-H ^a		Multiple infections		
	Cone	Alpha acids	Cone	Alpha acids	Cone	Alpha acids	Cone	Alpha acids	Cone	Alpha acids	
United Kingdom											
Fuggle	8	0.5	- ^b	-	-	-	39 to 96	4 to 15	-	-	17
Cascade	20	3	-	-	-	-	-	-	-	-	17
Bullion	20	2	-	-	-	-	-	-	-	-	17
Wye Northdown	32	8	-	-	-	-	-	-	-	-	119
Germany											
Hüller Bitter	-	-	-	-	-	-	-	-	34	20	87
Northern Brewer	-	-	0 ^c	0	0	0	-	-	-	26	87
Hersbrucker Spät	-	-	0	0	0	0	-	-	5 to 38	0 to 47	87
USA											
Chinook	-	-	62	0	0	12	-	-	-	-	147
New Zealand											
Superalpha	-	-	-	-	0	0	0	0	0 to 39	0 to 4	75
Australia											
Pride of Ringwood	0 to 50	0 to 10	50 to 53	0 to 18	0 to 20	0	45	-	0 to 39	0 to 12	138
Victoria	0	0	0	0	0	0	0	0	0 to 43	0	138
Nugget	0	0 to 10	0	0	0	0	0	0	0	0	138
Opal	0	0	0 to 27	0	40 to 42	0	-	-	0 to 58	23 to 43	138
Super Pride	-	-	0	0	0	0	-	-	-	-	139
Agate	-	-	0	0	0 to 70	0 to 44	-	-	-	-	139

^a ApMV = Apple mosaic virus; HpMV = Hop mosaic virus; HpLV = Hop latent virus; ArMV-H = Arabis mosaic virus hop strain.

^b - = Not tested.

^c 0 = No significant effect.

Transmission of ApMV. The transmission of ApMV between plants in commercial yards is predominantly by mechanical means (132). This is notable as many other ilarviruses are commonly transmitted in association with thrips and infected pollen (66,67,109). In the ApMV–hop pathosystem, the role of pollen is not considered important for several reasons, including: (i) in the United Kingdom the shift from seeded to seedless hop production by removing male plants was not associated with significant decreases in the rate of ilarvirus infection; (ii) male plants can become infected by ApMV; and (iii) transmission occurs to both flowering and deflowered plants (120,153). The autocorrelated and predominantly local spread patterns suggested by spatial pattern analyses

of ApMV epidemics in the United Kingdom (185) and Australia (133,137) are also not suggestive of transmission by pollen. Although unknown soilborne vectors have been suggested (183,185), mechanical transmission has been demonstrated in glasshouse studies through direct plant contact, transfer of infective sap to leaf and root tissues, simulated pruning, and root grafting, but not simple root contact (60,132). Under field conditions, ApMV transmission can be reduced by preventing contact between neighboring plants early in the season through the use of desiccant herbicides such as paraquat, or similar strategies (132,199).

Effect of ApMV on yield and levels of brewing organic acids. ApMV is regarded as one of the most deleterious viruses to

hop production, but the effect is dependent upon cultivar (summarized in Table 1). It has been reported that ApMV-free cv. Fuggle plants had higher alpha (0.5%) and beta (0.3%) acids and yields (8%) than infected plants (17). Cultivar Cascade plants free of ApMV had 2.8% higher alpha acids, 1.4% higher beta acids, and 20% higher yields. Plants of cv. Bullion had 1.5% higher alpha acids and 20% higher yields when ApMV-free, but beta acids were not affected. Neve and Lewis (118) showed that cones from ApMV-free plants grown in the United Kingdom had 0.7 to 2% higher alpha acid content than infected plants, but with no increase in cone yield. Among three cultivars, Neve and Lewis (119) found ApMV caused an average decrease in yield of 16% and an 11% reduction in alpha acids. Similarly, ApMV reduced cone yield by 32% and alpha acid levels by 8% in cv. Wye Northdown. Neve and Thresh (120) summarized much of this research and showed that five cultivars freed of ApMV had increments in yield of 3 to 21% and alpha levels of 4 to 16%.

In Germany, comparisons between plots of ApMV-infected and virus-tested cv. Hüller Bitter over a 3-year period consistently recorded reductions in alpha acid content of 21, 18, and 22%, respectively, in each year. In a later study, the alpha acid content of ApMV-infected cvs. Hüller Bitter and Northern Brewer was 18 to 26% lower than in virus-tested plants (87).

In Australia, ApMV has been associated with an increase in softwood cutting mortality following propagation and a decrease in cone yield and levels of brewing organic acids. In cv. Nugget, plants infected by ApMV-I had 11% lower alpha acids and 7% lower beta acid levels. In cv. Opal, significant reductions in yield and alpha acid levels were reported with both ApMV serotypes when present in co-infection with the carlaviruses HpMV and HpLV. In cv. Victoria, infection by the individual viruses had no effect in the first year of the study, but significant reductions from some virus combinations were reported, suggesting an exacerbation of the deleterious effects of viruses as plants become older (138). Yield reductions from ApMV-H have also been reported in cv. Superalpha in New Zealand. In this study, these effects were attributed to reductions in individual cone weight and the numbers and weights of bracteoles, and lower alpha acid levels resulted from a smaller number of bracteoles with fewer lupulin glands (75).

ArMV Hop Strain (ArMV-H)

The most common nepovirus found in hop is the hop strain of ArMV (ArMV-H). ArMV-H has been reported in commercial hop yards in several countries, including Belgium (93), the former Czechoslovakia (145), France (54), Germany (104), New Zealand (76), South Africa (196), and the

Fig. 3. Chlorotic rings (right) and necrotic spots (left) on leaves infected by *Apple mosaic virus* in hop cultivar Opal.

Fig. 4. Infection by *Apple mosaic virus* can also cause oak-leaf patterns. Symptoms pictured here are in hop cultivar Willamette.

United Kingdom (188). In Australia (36,197) and the United States (77), there are historical, but no recent reports (112,171) of ArMV-H or diseases attributable to this virus, suggesting it was subsequently eradicated. In Australia, eradication was attributed to a widespread shift from imported English varieties to the locally bred cv. Pride of Ringwood and the absence of the nematode vector (112). In other countries, such as New Zealand, ArMV-H was apparently transmitted from imported English cultivars to locally bred ones and became endemic (76) due to the introduction and establishment of the nematode vector *Xiphinema diversicaudatum* into hop yards (74).

Infection by ArMV-H has been associated with several diseases, including barebine or spidery hop, split leaf blotch, nettlehead, and hop chlorotic disease (189). Symptoms of barebine or spidery hop are apparent in early spring as weak shoot growth, curvature of shoots, and production of small, dark-colored leaves. Symptoms of split leaf blotch include translucent, yellow oily blotches between the leaf veins, leading to leaves splitting upon expansion (Fig. 5A; 190). Cultivar Fuggle is particularly susceptible, exhibiting symptoms referred to as severe blotch. Less severe symptoms (slight blotch) have been reported in other cultivars, but the association of these symptoms with ArMV-H is yet to be ascertained.

Nettlehead was described in detail by Salmon and Ware (157) and is one of the most damaging viral diseases of hop (42,90). Symptoms become progressively more severe until plant death 5 to 6 years after infection (78). Plants with nettlehead often exhibit symptoms of barebine early in the season. Later, plants develop stiff, erect shoots with short internodes that fail to climb and fall away from support strings (Fig. 5B and D). Other symptoms of nettlehead included upward rolling of the leaf margins, vein clearing or mottle, and enations on the abaxial leaf surface (50). Cultivars WGV, Fuggle, and Yeoman are particularly susceptible, while others such as Bramling Cross are tolerant. Understanding the etiology of nettlehead was complicated by the ephemeral nature of symptoms. However, the role of a satellite nucleic acid (SNA) in nettlehead was demonstrated by Davies and Clark (50). Symptom severity was related to the amount and particular species of SNA present (50,51). In Germany (105) and New Zealand (76), infection by ArMV-H was latent. This suggests differences in cultivar susceptibility, variation in the pathogenicity of isolates, or absence of the relevant SNA.

Hop chlorotic disease was first described by Salmon and Ware (158). The disease occurs sporadically and is transmissible via seed, graft, and sap inoculation. Symptoms include severe distortion of developing foliage on the lower parts of

bines (Fig. 5C), with leaves often assuming a characteristic parrot beak shape. The disease is associated with a specific ArMV-H strain that can be differentiated from other strains by the presence of systemic symptoms in *Chenopodium amaranticolor* and *C. quinoa* but not by serology (6,9). The disease has also been reported in Germany, Russia, and Poland (9).

Particles of ArMV are isometric in shape, approximately 25 to 27 nm in diameter (114), with a single capsid protein with a molecular mass of approximately 54 kDa. The genome of nepoviruses is bipartite with both RNA-1 and RNA-2 necessary for infection and multiplication. The ArMV-H associated SNA is 300 nucleotides in length and makes up some 80% of the nucleic acids of purified preparations (50,80). Multiplication of ArMV-H associated SNA is not supported by strains of ArMV from other hosts (51). Wetzal et al. (198) demonstrated a 96% homology between the amino acid sequences of the protein 2A of ArMV-H and ArMV from sugar beet, and suggested the same strain may infect both species.

Transmission of ArMV-H. ArMV-H is transmitted by the dagger nematode, *Xiphinema diversicaudatum* (193). Other nema-

tode species have been implicated in the spread of ArMV; however, the evidence is weak (192). *X. diversicaudatum* is indigenous to the United Kingdom and is widespread in arable permanent pasture, deciduous woodlands, and hop production areas (30). The nematode occurs throughout Europe but is of limited distribution in North America, Australia, and New Zealand (18). *X. diversicaudatum* is a relatively long-lived nematode with a life cycle of up to 3 years, while individual females may live for more than 5 years (143). It can live, but not multiply, in soil without higher plants for 3 years (102). While information on ontogenetic development on hop is lacking, a laboratory study of *X. diversicaudatum* on strawberry (*Fragaria × ananassa*) demonstrated adult females survive approximately 60 weeks, with a reproductive period of 36 weeks and reproductive capacity of 180 to 200 progeny (31). Development from egg to adult took 12 weeks or 1,092 degree-days assuming a basal temperature of 5 °C. Each of the four juvenile stages and adults are found at all times, but there can be an increase in the proportion of mature females during seasonal flushes of new growth, at which time egg-laying occurs with hatch

Fig. 5. A, Leaf symptoms of split leaf blotch caused by *Arabis mosaic virus* hop strain (ArMV-H). B, Symptoms of nettlehead include a failure to climb support strings leading to a falling away of the bines. C, Foliar symptoms of hop chlorotic disease caused by ArMV-H. D, Barebine/nettlehead in a typical spreading patch in cv. Fuggles. The plants in the foreground have weak shoots and few leaves typical of nettlehead affected plants in early spring. Unaffected plants in the background are more vigorous and leafy. (Images A, B, and D, courtesy J. Mike Thresh)

occurring shortly thereafter (178). The rate of reproduction is low, with a mean of 7.3 juveniles per year on strawberry (62), which is a relatively better host of *X. diversicaudatum* than hop (43). However, the hop root system occupies several cubic meters, so even a density of 1 nematode per 200 ml of soil equates to thousands per plant, providing substantial opportunity for virus transmission (189).

Adults and juveniles of *X. diversicaudatum* transmit ArMV with equal efficiency (68) and remain infective for up to 44 and 36 weeks, respectively, in the absence of host plants (193). Infectivity can be retained by nematodes for periods of up to 8 months when they are given access to hosts that are virus immune (69). McNamara and Pitcher (107) found *X. diversicaudatum* was more efficient at transmitting ArMV-H from hop than ArMV from strawberry. The virus is not retained through the molt between stages of the life cycle and is not passed from the female to egg. Detailed studies on the acquisition time necessary for nematodes to become viruliferous with ArMV-H are lacking in hop. However, *X. index* acquired the ability to transmit *Grapevine leaf roll virus* after only 5 minutes (16).

ArMV-H can also be transmitted mechanically. Davies and Clark (50) reported the efficiency of mechanical transmission of ArMV-H from hop to *C. quinoa* or hop was less than 10%. Conversely, Eppler (53) was unable to transmit ArMV-H mechanically between hop plants. Therefore, sap transmission is now considered of minor importance under field conditions. Eppler (53) provided some evidence for pollen transmission of ArMV-H, but further work is required to substantiate this finding. ArMV-H can also be transmitted by dodder and is seedborne in hop (114), with up to 50% of the seedlings from diseased plants infected (189). In seeded crops, where seeds were scattered widely after harvest, infected seedlings were common in spring, allowing nonviruliferous nematodes to acquire ArMV-H (186,189).

Spatial patterns of ArMV-H epidemics.

Early reports of the spread of ArMV-H infected plants describe a range of spatial patterns (78,189). Later, these were attributed to the virus status of the planting material and the spatial distribution of *X. diversicaudatum* (Table 2). In New Zealand, where hop yards are generally uncultivated, *X. diversicaudatum* was detected in six out of eight commercial hop yards examined, with over 90% of samples from which it was detected occurring at yard edges (74). Spread of ArMV-H in a hop yard can be relatively rapid. In the United Kingdom, the incidence of ArMV-H increased from 2.9 to 43.6% after the first 6 years (8) and to 61.8% by year 12 (184) in a hop yard planted with potentially infected material and where *X. diversicaudatum* was widely distributed. This rapid spread is at odds with the small distances with which *X. diversicaudatum* migrate through soil (only 0.3 to 2.0 m annually) (69,103,179). However, more rapid progress of ArMV-H through a yard would be facilitated by systemic movement within the large root systems and the close planting distances, especially within rows, that would allow roots of infected and healthy plants to intertwine, and provide potential for nematode transmission. Adams et al. (8) demonstrated that close spacings facilitated transmission of ArMV-H, with a greater probability of spread from hop plants with symptoms of ArMV-H to the next plant 1 m away within the row (35%) than to those 2 m away within or across the rows (9%). Thresh (181) related the probability of infection to the number of neighboring plants with symptoms and obtained a curvilinear relationship of 26 to 100% for plants with one to eight infected neighbors.

Effect of ArMV-H on yield and levels of brewing organic acids. The effects of ArMV-H on cone yield and levels of brewing organic acids are summarized in Table 1. In the United Kingdom, barebine was associated with yield reductions of 23 to 26% in comparison with healthy plants in

cv. Bullion (191), and cones from diseased plants had alpha acid contents of 6.5% (by weight) in comparison with 7.1% in healthy plants. Plants with barebine or spidery hop recovered following the removal of weak shoots during training, but often developed split leaf blotch or nettlehead later in the season. Severe blotch has been demonstrated to reduce cone yield by up to 50% (189). The yield of nettlehead-affected plants can be reduced by over 75% in susceptible cultivars and in some cases caused yards to be removed (116,122). In Australia, Johnstone (78) reported yield losses of 25 to 45% from nettlehead, with no reduction in alpha acids. In New Zealand, significant yield losses were demonstrated despite the absence of symptoms. In two trials, plants of cv. Superalpha infected with ArMV-H and HpLV or ArMV-H, HpMV, and HpLV had cone yields of 71 and 73% less compared with plants infected with HpLV or HpMV and HpLV, respectively, with no effect on alpha- or beta-acid levels (76).

HpSVd

Hop stunt disease was first identified in hop yards in Japan, initially thought to be associated with infection by a virus and referred to as cedar-shaped hop or dwarf hop (201), but later found to be induced by infection with a viroid (163). The disease has been reported in commercial yards in Japan (160), Korea (89), and the United States in 2004 (K. C. Eastwell, unpublished data).

The viroid consists of covalently closed single-stranded RNA of 294 to 303 nucleotides depending on host and isolate (123). HpSVd has a moderately wide host range and has been found naturally infecting many woody perennial species including plum (*Prunus* spp.), almond (*Prunus dulcis*), citrus (*Citrus* sp.), grapevine (*Vitis* spp.), and pomegranate (*Punica granatum*) (19). Five distinct groups of HpSVd sequence variants have been isolated from different hosts (88). HpSVd isolates from hop have the highest sequence similarity to those from grapevine (162). Experimental infection of hop with HpSVd from several woody hosts has been demonstrated, suggesting possible risk to hop production from natural alternate host reservoirs (86,159).

HpSVd infected plants typically have shortened internodes on the main and lateral bines, reducing plant height to approximately 3 m. Upper leaves appear curled, smaller in size, and chlorotic. Foliar symptoms of HpSVd may vary. In Japan, infected leaves droop from the base creating a characteristic X formation with laterals. Other foliar symptoms associated with HpSVd infection are a yellow-green color on the basal foliage early in the season, while yellow speckling along the major veins may also be present (Fig. 6). However, diagnosis based on symptoms

Table 2. Influence of the presence or absence of *Xiphinema diversicaudatum* and virus status of planting material on the subsequent pattern of spread of *Arabis mosaic virus* hop strain (ArMV-H) in hop yards (adapted from 189)

<i>X. diversicaudatum</i>	Virus status of planting material	
	Virus-tested	Infected
Absent	No infection and spread	Random distribution of diseased plants but no secondary spread
Present and nonviruliferous	No infection and spread	Random distribution of diseased plants followed by slowly expanding foci in areas containing both infected plants and viruliferous vector
Present and viruliferous	Patches of diseased plants with slowly expanding foci in areas where vector is present	Random distribution of diseased plants with no secondary spread in areas devoid of vector, patches of infected plants with slowly expanding foci in areas where vector is present

may be difficult because of their ephemeral nature, and dependence upon cultivar and climate. In general, stunting is more severe in warmer climates (159,160).

Transmission and spatial patterns of spread of HpSVd. Transmission of HpSVd within hop yards is solely through mechanical means and probably facilitated by harvesting or cultural operations such as stringing and mechanical basal growth control. The only method for long-distance spread of HpSVd is through infected propagation material. Within yards, HpSVd spreads along rows, reflecting the role of cultural operations in transmission (162,201).

Effect of HpSVd on yield and levels of brewing organic acids. Plants infected by HpSVd produced fewer and smaller cones with yields 50% lower, and alpha and beta acid levels 50 to 70% lower than in viroid-free plants (164,201). The proportions of the brewing organic acids are also altered by HpSVd. Ratios of beta to alpha acids less than 1:20 are characteristic of healthy plants, whereas ratios greater than 1:44 are typical of HpSVd infection (164). Momma and Takahashi (110) reported that the number of lupulin glands was reduced by more than 60% in cones.

HpLVd

The presence of a second viroid infecting hop was first suggested by Pallas et al. (128) and initially termed "hop viroid-like RNA fast, or HV-f". It was subsequently described and named HpLVd in reference to the latent nature in the majority of hop cultivars (151,152). The viroid is a covalently closed single stranded RNA of 256 nucleotides distinct from HpSVd (with only 45% sequence homology) (151). Although viroids, including HpSVd, generally form complex populations of natural sequence variants called quasispecies, no such variants have been reported for HpLVd.

HpLVd has been reported from commercial yards in the United Kingdom (10,14,24), Germany (84), New Zealand (73), Korea (89), Poland (175), Slovenia (85), Japan (71), Czech Republic (101), Brazil, in material recently imported from the United States (63), United States (115), and Australia (D. Crowle, *personal communication*). In the majority of these areas, the incidence of HpLVd is considered high or ubiquitous. For example, Puchta et al. (152) surveyed 14 yards of cv. Northern Brewer in Germany and found only 1 of 27 samples free of HpLVd. The worldwide distribution of HpLVd is probably due to its efficient mechanical transmission and dissemination in vegetative propagules, the latter being made more likely by HpLVd's latent nature. In addition to the commercial hop, HpLVd also infects *H. japonicus* and stinging nettle (*Urtica dioica*) (84).

Symptoms caused by infection with HpLVd have been described in cv. Omega

in the United Kingdom (25). These included chlorosis, slow growth, and the production of fewer laterals with smaller cones (10,25).

Transmission and spatial patterns of HpLVd spread. The spread of HpLVd in a commercial yard is predominantly localized and to adjacent plants, reflecting the mechanical means of transmission, including harvesting and cultural operations (11,14). The main means by which HpLVd is introduced into commercial yards is in infected propagation material. No evidence for long-distance spread, such as by encapsidation in co-infecting viruses and subsequent spread by aphids, has been found (11). Low transmission efficiency has been reported through pollen transfer or by seed (49,98). Once HpLVd is introduced into commercial yards, the spread can be rapid but is influenced by cultivar susceptibility. High re-infection rates of 65% within 3 years have been described in the Czech Osvald's clone 72 (98). In cv. Omega yards in the United Kingdom, 75% of viroid-free plants became infected within 2 years, while in cv. Wye Northdown, minimal spread was observed (14). Cultivar and region have also been found to significantly affect HpLVd concentration. In the United Kingdom, HpLVd concentration is higher later in the season (being high enough for reliable detection from late spring onward) and generally higher at the

base of the plant (111). The titer of HpLVd is also high in roots (101). In the warmer climate of Germany, HpLVd titer was found to slightly decrease in summer (84).

Effect of HpLVd on yield and levels of brewing organic acids. The effects of HpLVd on cone yield and levels of brewing organic acids are strongly dependent upon genotype. For example, in cv. Omega, cone yield was reduced by 27% and alpha-acids levels by 31%. Moreover, beta-acids levels and oil content were higher in HpLVd-infected plants, suggesting infection may hasten cone maturation (25). Changes to the expression of host components involved in the synthesis of secondary host metabolites due to HpLVd infection were also suggested in trials conducted in the Czech Republic in Osvald's Clones 31, 72, and 114, and in cv. Premiant (130). In these trials, HpLVd infection decreased alpha acids by an average of 40%, whereas beta acids levels, the ratios of humulone to cohumulone and lupulone to colupulone, were not affected. Levels of myrcene, α - and β -pinene were 29, 37.4, and 41.6% higher in infected plants. Levels of the sesquiterpenes, β -caryophyllene, α -humulene, α -copaene, γ -muurolene, β -bisabolene, γ -cadinene, and δ -cadinene were also decreased by 13.7, 13, 14, 18.5, 29, 21.7, and 18.5%, respectively, due to HpLVd infection. These changes in essential oil composition (in-

Fig. 6. Foliar symptoms of *Hop stunt viroid* in hop cultivar Willamette. (Courtesy D. H. Gent)

crease in myrcene and general decrease of sesquiterpenes) are similar to those observed during cone maturation (108), but the associated decrease in alpha acids is not characteristic of this physiological change (130). The effects of HpLVd infection are less marked in cvs. Wye Northdown and Wye Challenger in the United Kingdom, but still resulted in reductions in total alpha-acid production of approximately 20% (through smaller cones and reduced alpha-acids levels, both by ca. 10%) and increases in myrcene levels (24). Infection by HpLVd also significantly reduced rooting of softwood cuttings and establishment (49).

Minor Viruses and Virus-Like Agents Affecting Hop

A range of viruses have been reported infecting hop at either a low prevalence or incidence and in localized regions. For the purposes of this review, we have therefore classified them as minor pathogens, recognizing that in particular pathosystems the status of these diseases relative to others may change. Limited information is available on their effect on cone yield and levels of brewing organic acids (Table 3).

Management of Viruses and Viroids in Hop Production

As with all pathosystems, effective management of disease caused by viruses and viroids in hop production requires a com-

prehensive understanding of both their epidemiology and effects on yield and quality of cultivars in specific environments. This is most effectively demonstrated in the last 50 years of research examining the effect of viruses on different hop cultivars and in various parts of the world. This information clearly demonstrates that the effect of these viruses on yield and quality is strongly influenced by cultivar susceptibility and therefore requires assessment for each newly adopted cultivar. This principle is easily translated to other virus pathosystems. For example, if the effect on yield and/or quality of virus infection is significant but the rate of virus re-infection is slow, management may be achieved by planting with virus-tested material and roguing of any infected plants soon after they are identified. If, however, the effect on production is significant and the rate of re-infection is rapid, alternative methods of management need to be explored. Furthermore, if the cultivar is highly tolerant to infection, then the implementation of management strategies may not be cost effective (131). As strain variation within these viruses, such as ApMV and HpMV, has become more fully described, this adds further complexity in requiring quantitative yield loss information on the various virus strains, cultivars, and environment interactions.

Certification programs. Historically, vegetative propagation has been important

in both disseminating and increasing the incidence of these pathogens. Hop viruses and viroids are passed efficiently from parent to progeny during vegetative propagation. The traditional means of hop propagation involved taking strap cuts. These are obtained by mounding soil over plants during seasonal cultivations in late summer. The buried basal portion of the bines becomes thickened and is cut off in winter for use as planting stock in spring. As strap cuts were taken after the plant had died back, there was usually no visual check on the health of the parent plant. Rapid degeneration of hop planting stocks in the United Kingdom has been attributed to this method of propagation (116). To address this problem, certification programs were established. Such programs seek to eliminate viruses and viroids from the propagation stock and avoid infection during the increase phase of vegetative propagation.

The effectiveness of certification programs has improved along with the sensitivity, reliability, and cost and time efficiency of detection methods. Although they often cause the greatest damage to individual plants, diseases with highly visible symptoms are generally easily detected and therefore more easily rogued, so that overall damage is minimized. Latent infections may cause less damage to individual plants, but they can become very important when inadequate detection allows them to occur at high incidence, leading to more overall damage.

Table 3. Minor viruses and virus-like agents reported affecting hop worldwide

Virus/virus-like agent	Symptoms	Distribution	Literature
<i>Alfalfa mosaic virus</i> (AIMV) <i>Alfamovirus</i>	Systemic chlorotic spots in cv. Styrian	China, former Czechoslovakia	121,202
<i>Cherry leafroll virus</i> (CLRV) <i>Nepovirus</i>	No information	United Kingdom	41
<i>Cucumber mosaic virus</i> (CMV) <i>Cucumovirus</i>	Leaf distortion in cv. Hüller Bitterer. Mottled foliage and shortened internodes in <i>Humulus scandens</i>	Romania, Germany, and United Kingdom	27,96,97,165,166
H-246 Hop virus (unclassified)	Necrotic spots on stems, shoots and leaves. Systemic necrosis and death within 2 to 3 years following artificial inoculation	Romania	95
<i>Humulus japonicus latent virus</i> (HJLV) <i>Iarvirus</i> syn. <i>Humulus japonicus virus</i>	Symptomless	<i>H. lupulus</i> and <i>H. japonicus</i> in China, imported <i>H. japonicus</i> in United Kingdom	12,169
<i>Petunia asteroid mosaic virus</i> (PeAMV) <i>Tombusvirus</i> syn. <i>Tomato bushy stunt virus</i> — <i>petunia</i> strain	Deformed asymmetric leaves with necrotic crinkles, ringspots, line-patterns, chlorotic spotting, perforated leaves, and general chlorosis	Former Czechoslovakia	121,145,173; similar symptoms reported by Chod et al. (40)
<i>Raspberry bushy dwarf virus</i> (RBDV) <i>Idaeovirus</i>	None reported	United Kingdom	33
<i>Strawberry latent ringspot virus</i> (SLRSV) <i>Nepovirus</i>	None reported	Former Czechoslovakia	145; contrary to findings of Valdez et al. (193)
<i>Tobacco mosaic virus</i> (TMV) <i>Tobamovirus</i>	None reported	China	200
<i>Tobacco necrosis virus</i> (TNV) <i>Necrovirus</i>	Necrotic local lesions	Romania, France, and former Czechoslovakia	15,39,54,97
<i>Tobacco ringspot virus</i> (TRSV) <i>Nepovirus</i>	Ringspots	Germany	165
<i>Apple fruit crinkle viroid</i> (AFCVd)	Stunting and severe leaf curling in upper bines	Japan	161
Hop infectious sterility (unknown virus or viruslike agent)	Sterility	Poland	203

Certification programs for hops originated in the United Kingdom in 1943 with a program for the inspection and certification of hop yards for freedom from Verticillium wilt (52). This was a voluntary program run by the Ministry of Agriculture Fisheries and Food (MAFF) that awarded certificates to yards apparently free of wilt. The virus disease nettlehead was also included, but because it was so widespread it was only possible to set "a limit of very low percentage of virus infection" (13,81). This allowed growers to obtain wilt-free planting material, usually as strap cuts from the rooted base of bines in commercial hop yards. In 1947, legislation in the form of the "Progressive Verticillium Wilt of Hops Order" was passed, making wilt a notifiable disease and prohibiting the sale of hop plants from infected yards. In 1949, grade A and B certificates were introduced in a program for the inspection of hop yards. Virus diseases were included in this, and a tolerance for nettlehead of 0.25% and mosaic of 2% was set for grade A plants. This was superseded by the A-plus program in 1955, in which the propagation of pathogen-tested clones was undertaken by specialist propagators isolated from commercial wilt-infested areas.

The practice of growers taking sets at random from established yards during the dormant period was also important in pathogen dissemination to succeeding yards in Australia (197), and probably elsewhere. In 1950, the Department of Agriculture in Australia began the selection and maintenance of three hop cultivars free of virus symptoms for growers to obtain healthy material (197). Johnstone (78) correctly predicted that the future control of nettlehead lay in the production of virus-free (virus-tested) cultivars by heat therapy and meristem tip culture. The advent of mist propagation of softwood cuttings is also believed to have lowered the probability of plants with obvious symptoms being used for propagation purposes. However, apart from some effects of viruses on softwood cutting mortality (138), the spread of viruses causing latent infections or mild symptoms would have continued.

The increased knowledge of the damage due to latent virus infections led to the production of virus-tested clones by heat therapy and meristem tip culture in the United Kingdom in 1966. Vine and Jones (195) exposed plants for 2 to 4 weeks at 35°C and raised tips of up to 5 mm long in a culture media to obtain plants free of ApMV and HpMV, but not HpLV. They found that while excised tips of this size sometimes contained virus, the resulting plants were often virus-free. This indicated that the virus was sometimes eliminated during the culture procedure rather than through excision of virus-tested material. This suggested a balance between virus

replication and degradation in the meristem which was pushed in favor of the latter during culture. Adams (1) also obtained ApMV-free plants by growing excised tips (1 to 5 mm) from plants treated at 35°C for 10 days, but HpMV was not eliminated. However, removal of smaller meristems (0.5 mm) from plants that were not heat-treated did eliminate HpMV. Adams (2) used improved media to enable the culturing of smaller tips (0.3 to 0.8 mm long) than was achieved by Vine and Jones (195), and was able to obtain plants free of HpMV and HpLV without heat therapy. However, ApMV could only be consistently removed by the use of heat therapy. Tips (1 to 5 cm long) taken after 10 to 18 days at 35°C and 16-h day length (and in one instance after only 3 days) developed into plants free of this virus. From 1969, cultivars were released in the United Kingdom free of ApMV and ArMV but still containing HpMV and HpLV. In 1970, some cultivars were released free of ArMV, ApMV, and HpMV, and by 1972 material free of all known viruses became available.

The A-plus program established in the United Kingdom began with the production of virus-tested plants by heat therapy and meristem culture (2). Four mother plants of each main cultivar were then grown on in isolation, and tested for the presence of virus at biennial intervals. Propagators in areas away from the commercial hop yards were supplied with cuttings which they used to establish layer beds for further propagation. These beds were used as parent stock for 10 years before renewal. The established layer beds were inspected twice each season and certified if they reached the required standard of health. Nurseries had to be established on soil tested to be free of *X. diversicaudatum* (44), and had to be at least 30 km away from areas with virulent Verticillium wilt. In addition, wilt-sensitive cultivars could not be grown on the same property as wilt-tolerant cultivars. Procedures such as using disinfectant footbaths, the wearing of special boots, and restricted vehicle entry were adopted to minimize the spread of soilborne pathogens and *X. diversicaudatum*. Plant indexing was conducted by serological testing and mechanical inoculation to *Chenopodium quinoa* and *Cucumis sativus*. The establishment of certification programs in the United Kingdom was primarily aimed at eliminating ArMV and ApMV. These viruses could be controlled by such a program because of their slow reinfection rates. Many countries, including the United States, Australia, South Africa, New Zealand, and most European countries, now produce virus-tested material as part of their plant propagation techniques.

Management of viruses within commercial yards. The most successful example of management of virus diseases of

hop has been the control of ArMV-H by the institution of a fallow period followed by replanting with virus-tested material. Bare fallows of 12 to 18 months, 19 to 20 months, and 20 to 42 months led to reinfection of virus-tested hops of 13 to 24%, 1 to 3%, and 0%, respectively (7,8). Control was mainly through loss of transmissible virus from the nematode population rather than a direct effect on the vector population. In the United Kingdom, a 2-year fallow period was recommended if the yard had a prior history of nettlehead or ArMV-H, irrespective of whether *X. diversicaudatum* had been detected in the yard, because the efficiency of transmission meant that even low numbers of nematodes could contribute to spread. By contrast, growers were recommended to plant virus tested stock immediately if there had been no evidence of nettlehead or ArMV-H in the yard irrespective of the presence of *X. diversicaudatum* because the nematode population was likely to be nonviruliferous (189). This strategy required careful removal of rogue hops as a source of ArMV-H. Only one full crop would be lost if plants were removed directly after harvest and mist-propagated plants or cold stored sets were planted (8,61,106). Nettlehead, barebine, and split leaf blotch were common in hop yards in the United Kingdom until the 1970s, at which time virus-tested material became available through the A-plus program. These diseases are no longer considered limiting to production in the United Kingdom. Many other countries have also adopted high-health programs that have markedly reduced the incidence of ArMV-H and its impact on hop yield. The use of nematicides to reduce ArMV-H spread into virus-tested material has had limited success. Pitcher and McNamara (142) reported a 95 to 100% reduction in populations of *X. diversicaudatum* in the 15 to 30 cm zone in the first year after application of DD (1,3-dichloropropene). However, by the fourth year after replanting with healthy stock, the incidence of ArMV-H was 80%. The lack of efficacy of chemical control has been attributed to the inability of nematicides to penetrate through the heavy soils to depths that hop roots and vector nematodes can penetrate and to the efficiency of transmission by low vector population densities.

Similarly, vector control for carlavirus spread is not economically feasible. Insecticide sprays to control populations of *P. humuli* are frequently used in regions where the aphid is prevalent, but this aims to reduce the damage from insect feeding and is not effective in reducing nonpersistent carlavirus transmission. For the carlaviruses, ApMV, and all viroids, minimizing contact between plants will also significantly reduce within-yard spread. For example, reducing basal growth contact between plants in early spring reduced the

spread of ApMV to adjacent plants by almost half (131).

Management of viroids. The discovery of widespread infections of HpLVd led to efforts to eliminate this pathogen from hop. Meristem tip culture (71,129), thermotherapy (100), and cold treatment (111) have been found to significantly reduce viroid titer. Hataya et al. (71) were successful at eliminating HpLVd from in

fectured material of two hop cultivars by excising tips of 0.2 mm in size. Moreover, culturing of tips of 0.5 mm in size was successful at regenerating shoots free of HpLVd when selected in May and early June, when viroid titer was low (129). Cold treatment conditions found to be optimal for producing plants free of HpLVd were storage at 2 to 4°C in the dark for periods ranging from 8 to 21

months, and subsequent meristem culture of tips less than 0.5 mm in size (111). Optimal thermotherapy conditions were exposure to 36°C for 14 days; however, original titers were restored after 6 months in field conditions (100). The rapid decrease in HpLVd levels following heat treatment was correlated with the induction of a nucleolytic complex which resulted in cleaving of the viroid (100). Heat treat-

Sarah J. Pethybridge

Frank S. Hay

Dez J. Barbara

Kenneth C. Eastwell

Calum R. Wilson

Dr. Pethybridge is a research plant pathologist with the Tasmanian Institute of Agricultural Research (TIAR) at the University of Tasmania's Cradle Coast campus in Burnie, Tasmania, Australia. She completed her undergraduate degree in Agricultural Science with First Class Honours and Doctor of Philosophy degree in plant pathology at the University of Tasmania in Hobart. Her Ph.D. dissertation examined the epidemiology and management of viruses infecting hop in Australia. Her research program focuses on the epidemiology and management of hop and pyrethrum diseases.

Dr. Hay is the leader of the Vegetable Research Group within TIAR, and is based at the University of Tasmania's Cradle Coast campus in Burnie. He completed his Bachelor of Horticultural Science degree with First Class Honours and Doctor of Philosophy degree in plant pathology at Lincoln University in Christchurch, New Zealand. His Ph.D. dissertation was entitled "Studies on the Viruses of Hop in New Zealand". Dr. Hay's research program focuses on the integrated management of diseases of vegetables.

Dr. Barbara is a research leader at Warwick HRI on the Wellesbourne campus of Warwick University in the United Kingdom. After obtaining a Bachelor of Science in biological sciences and a Doctor of Philosophy degree for studies on *Cucumber mosaic virus* (both from the University of Birmingham, UK), Dr. Barbara worked on virus

and viroid diseases of fruit and hop at East Malling Research Station. His interests then broadened to cover other pathogens, and at present his primary focus is on understanding soilborne fungal diseases.

Dr. Eastwell is an associate scientist in the Department of Plant Pathology at the Prosser Irrigated Agriculture Research and Extension Center of Washington State University. Dr. Eastwell obtained his Bachelor of Science with First Class Honours in biochemistry and his Doctor of Philosophy degree in plant biochemistry from the University of Alberta in Edmonton. His research interests have included plant metabolism and protein chemistry. Current research activity focuses on the characterization and management of virus diseases of perennial fruit crops and hop.

Dr. Wilson is an associate professor of plant pathology within TIAR, and is located at the University of Tasmania's New Town Research Laboratories in Hobart. Dr. Wilson obtained his Bachelor of Science with Honours and Master of Science with Distinction, from the University of Canterbury in Christchurch, New Zealand, and his Doctor of Philosophy degree from the University of Western Australia in Perth. His research portfolio is diverse with work across a range of plant pathogens and hosts, but focuses host-pathogen interactions, resistance mechanisms and genetics, epidemiology, and disease management.

ment also led to the accumulation of sequence variants in the HpLVd population. Despite prediction of destabilizing the secondary structure of HpLVd from these mutations, all mutated cDNAs were found to be infectious to hop and evolved into complex progeny populations containing molecular variants maintained at low levels (99).

The management of viroids requires quick identification and removal of infected plants to prevent further spread. The absence of symptoms associated with HpLVd, and the long latent period before visible symptoms of HpSVd and *Apple fruit crinkle viroid* (AFCVd) develop, challenge the ability to contain these viroids. Effective roguing requires the infected plant and several plants adjacent both along and across rows to be removed (161,162,201). Although partial replanting following removal of diseased and buffer plants can be effective, better control has been achieved by removal of all plants in a yard, followed by replanting with certified material. Roguing for viroid infection required all plants to be destroyed by treating with urea and chloropicrin in autumn to ensure the disease was not carried over to the succeeding crop (162). HpSVd can be found in hop residue; however, leaves and cones were found to lose their infectivity after 3 months under normal field conditions (177). In conjunction with roguing, reducing agronomic operations of a mechanical nature which may contribute to HpSVd spread within and between yards will also be beneficial (162). In areas containing infected plants and where mechanical operations are essential, disinfecting equipment for 10 minutes in either formaldehyde, sodium hydroxide, sodium or calcium hypochlorite, or trisodium phosphate has also been demonstrated to be effective at reducing HpSVd spread (162,176,177). Heating blades at 160°C for 10 min was also effective, but 140°C was not. These measures have seen the virtual elimination of HpSVd from the Kirin Brewery yards in Japan (162). Therefore, the three central pieces to the HpSVd control program in Japan are in most ways similar to every viroid control program, regardless of host. These include making and utilizing certified viroid-free material, identification and prompt roguing of viroid-infected plants, and sanitation of farm equipment between yards. However, after 30 years of aggressive action, these practices have failed to eliminate HpSVd but have effectively minimized the deleterious effects of the pathogen on production.

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