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SHORT RESEARCH NOTE

Iris yellow spot virus found infecting onions in three Australian states

B. A. Coutts[A], *L. A. McMichael*[B], *L. Tesoriero*[C], *B. C. Rodoni*[D], *C. R. Wilson*[E], *A. J. Wilson*[E], *D. M. Persley*[B] *and R. A. C. Jones*[A,F]

[A]Department of Agriculture, Locked Bag No. 4, Bentley Delivery Centre, Bentley, WA 6983, Australia.
[B]Department of Primary Industries, 80 Meiers Road, Indooroopilly, Qld 4068, Australia.
[C]NSW Agriculture, Locked Mail Bag 8, Camden, NSW 2570, Australia.
[D]Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, Ferntree Gully Delivery Centre, Vic. 3156, Australia.
[E]Tasmanian Institute of Agricultural Research, New Town Research Laboratories, 13 St Johns Avenue, New Town, Tas 7008, Australia.
[F]Corresponding author; email: rjones@agric.wa.gov.au

Abstract. *Iris yellow spot virus* (IYSV) was detected for the first time in Australia, infecting onions in three and leeks in one state. Identification was confirmed using sap transmission to *Nicotiana benthamiana*, two IYSV-specific antisera in ELISA, RT–PCR with IYSV-specific primers, and sequence comparison with published IYSV sequences. Spring onion, onion seed and onion bulb crops were all infected, with spring onion being the most severely affected. The virus was also detected in nursery-grown onion and leek seedlings.

Iris yellow spot virus (IYSV; Family *Bunyaviridae*, Genus *Tospovirus*) causes the 'straw bleaching' disease of onions (*Allium cepa)*, and infects other Allium crops, including leeks (*A. porrum*), chives (*A. schoenoprasum*), garlic (*A. sativum*) and some flower species, e.g. iris (*Iris hollandica*), lisianthus (*Eustoma russellianum*) and *Hippeastrum* sp. IYSV is reported from several countries, including Brazil, USA, Israel and the Netherlands (Cortes *et al*. 1998; Gera *et al*. 1998; Pozzer *et al*. 1999; Kritzman *et al*. 2000). Although its onion thrips (*Thrips tabaci*) vector is common throughout Australia (Mound and Gillespie 1997), IYSV is not known to occur.

In 2002, IYSV-like symptoms (Gera *et al*. 1998) were seen in spring onion and onion bulb crops in the Perth Metropolitan region, Western Australia (WA), and in onion seed crops in the Riverina district, New South Wales (NSW) and the Swan Hill district, Victoria (VIC). These consisted of chlorotic and necrotic eye-like or diamond-shaped lesions on the leaves and, especially, the seed stalk. The seed stalk often bent over at the lesion and had generalised chlorosis.

In Perth during 2002, IYSV-like symptoms were widespread in spring onion crops, sometimes causing their abandonment. These symptoms had often been seen in previous years but attributed to 'iron chlorosis', nutrient deficiencies or other disorders (P. Mullins, personal communication). On one property, 10% of leeks also had symptoms of pale bands alongside the leaf mid-rib. Presence of IYSV was confirmed in leaf samples of onions and leeks using ELISA. Each sample was tested using IYSV-specific reagents from both DSMZ (Cat No. D-0528) and Loewe Biochemica (Cat No. 07508), Germany. Samples were tested from one bulb onion, three leek and four spring onion crops in the Wanneroo and Carabooda districts. IYSV was detected in 6/13 random samples of bulb onion, 4/100 random samples from two leek crops, 10/30 random samples from the third leek crop and 1/10–3/10 symptomatic samples from each of the four spring onion crops. In addition, IYSV was detected in 5/6 bulb onion and 4/16 leek seedlings from a vegetable nursery. In these and all subsequent tests, positive IYSV samples always had absorbance values < 10 times that of the healthy onion control.

In NSW during September 2002, a symptomatic sample from an onion seed crop in the Narromine District was sent to Agdia Inc. (USA) and a positive result obtained for IYSV in ELISA (L. Lydon, personal communication). Before this, symptoms typical of IYSV on seed stalks had been observed for several years in onion seed crops in the district. Onion seed crops at Griffith in the Riverina district had a similar history of virus disease (M. Hancock, personal communication). In November 2002, 23 symptomatic seed stalk samples were collected from two seed crops near Griffith. They were tested using the IYSV-specific ELISA

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Table 1. Nucleotide sequence identities (%) of part of the N gene from each of five different *Iris yellow spot virus* isolates

	AF001387	AF271219	VIC-98	NSW-2	VIC-1
AF 001387[A]	100	96	96	95	91
AF 271219[A]		100	94	94	95
VIC-98			100	99	99
NSW-2				100	99
VIC-1					100

[A]GenBank accession no. AF001387 is an isolate from Iris from the Netherlands (Cortes *et al*. 1998), and AF271219 an isolate from Lisianthus from Israel (Kritzman *et al*. 2000).

reagents from Loewe Biochemica, and 18/23 were positive. In Victoria, also in November 2002, four onion seed crops, each on a different property in the Swan Hill district, were inspected for symptomatic plants. Samples from symptomatic seed stalks from all four crops tested positive using the same IYSV-specific reagents. No *Allium* weeds were present. Each weed species infested with onion thrips from within or near the infected crops was sampled. When those from 40 wild lettuce (*Lactuca serriola*), 20 thistle (*Sonchus* spp.), 30 small-leaved mallow (*Malva parvifolia*), 20 paddy melon (*Cucumis myriocarpus*) and 100 wireweed (*Polygonum aviculare*) plants were grouped into batches of ten and tested with the same IYSV-specific reagents, all gave negative results.

Isolates from symptomatic plants in onion seed crops at Griffith (NSW-1 and -2) and Swan Hill (VIC-1) were sap-inoculated to *Nicotiana benthamiana*. This developed symptoms typical of IYSV — necrotic local lesions in inoculated leaves and systemic mottling, necrosis and leaf distortion (Pozzer *et al*. 1999). For each isolate, leaf samples of onion were tested using IYSV antibodies from Loewe Biochemica, *Tomato spotted wilt virus* (TSWV; Family *Bunyaviridae*, Genus *Tospovirus*) from Sanofi (Cat. No. 51267) and tospovirus serogroup IV from DSMZ (Cat No. AS-0118). Samples of *N. benthamiana* were tested with the IYSV-specific antibodies alone. ELISA tests were done using IYSV-infected leaf material obtained from Loewe Biochemica and onion tissue as a negative control. Positive results were obtained using IYSV-specific antibodies with all infected onion and *N. benthamiana* samples, but negative results with the TSWV-specific and tospovirus serogroup IV antibodies.

Sequence data were obtained for virus isolates NSW-2 and VIC-1, and for archived isolate VIC-98 from a symptomatic onion seed stalk from Swan Hill collected in 1998 and stored since as an RNA extract at –20°C. An 823 bp PCR product from the nucleocapsid (N) gene from each of NSW-2 and VIC-1 was amplified using primers IY1 and IY2 (Pozzer *et al*. 1999), then cloned and sequenced using the method of McMichael *et al*. (2002). For VIC-98, cDNA synthesis was primed using random hexamers and the cDNA amplified using the following primers designed to the N gene of a Brazilian isolate of IYSV from onion (GenBank accession no. AF067070): IYSV1S (5′ AGGGTAAAAGC TTCAGAA ATCGAGA 3′), corresponding to nucleotides 13–37, and IYSV1A (5′ CTTGGAGGGATTCTTGG GTTTAG 3′), complementary to nucleotides 782–804. PCR conditions were as described by Jain *et al*. (1998). A PCR product of the expected size (*ca* 790 bp) was amplified from symptomatic tissue and sequenced directly. The cDNA sequences from isolates NSW-2 (GenBank accession no. AY345226), VIC-1 (GenBank accession no. AY345227) and VIC-98 (GenBank accession no. AY341825) were compared with those from recognised isolates of IYSV from Iris in the Netherlands and Lisianthus in Israel (GenBank accession nos AF001387 and AF271219, respectively) using CLUSTALW (Thompson *et al*. 1994) and found to be 91–96% identical at the nucleotide level (Table 1).

Based on symptomatology in onion and leek, reactions in indicator plants, serology, RT–PCR amplification and cDNA sequencing, IYSV presence in Australia was confirmed. IYSV-like symptoms were observed in onions previously in all three affected regions and infection confirmed in an archived sample from 1998, so IYSV has been established for some time. Although surveys are needed to determine its distribution within each state, our observations suggest that infection may sometimes be widespread and causing serious damage to the spring onion industry. Sequential planting of onions in close proximity all-year-round favours cycling of infection from crop to crop. Whether IYSV can be introduced to new locations by planting onion bulbs from infected crops is unknown. However, as all the infected onion seed crops were located at least 10 km from the nearest *Allium* crop, and weed hosts were not found, a plausible explanation for its presence is planting infected bulbs. IYSV detection in nursery-grown onion and leek seedlings indicates contaminated nurseries as another important source. Likely control measures for onion crops include removing volunteer and wild *Allium* spp., destroying discard piles, planting transplants from virus-tested nurseries, avoiding all-year-round overlapping plantings and applying insecticides to kill the vector.

IYSV is the third member of the genus *Tospovirus* to be found in Australia, the two others being TSWV and Capsicum chlorosis virus (Latham and Jones 1997; McMichael *et al*. 2002).

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