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REVIEW

A review of the status of *Avocado sunblotch viroid* in Australia

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Abstract
Avocado sunblotch viroid (ASBVd) can seriously impact both the yield and quality of avocado fruit and is also a serious impediment to international trade because of quarantine conditions imposed by importing countries. The Australian avocado industry was among the first in the world to adopt control measures for ASBVd, an outcome of distinguished research done by Australian scientists during the 1970–80s. The status of ASBVd in Australia has not been recently reviewed and much information is either not published or difficult to find. In this review, this information has been collated and comment made on historical distribution and prevalence, in order to guide future surveys and to allow evaluation of the success of the control programs.

Keywords Avocado nursery voluntary accreditation scheme · ANVAS · Diagnosis · Biosecurity · Incursion pathway · Surveys

Introduction

Only two subcellular pathogens of avocado (*Persea americana*) are known to science, these being *Avocado sunblotch viroid* (ASBVd; genus *Avsunviroid*) and *Potato spindle tuber viroid* (PSTVd; genus *Pospiviroid*). ASBVd is the most economically important of the two with respect to the avocado industry, although PSTVd has a much wider host range and is primarily of importance to the vegetable and ornamental plant industries (Owens and Verhoeven 2017). PSTVd has only ever been recorded infecting avocado in Peru (Querci et al. 1995), and this unusual host-pathogen interaction is thought to be a consequence of the practice of intercropping avocados with potatoes, which are an important reservoir of the viroid. ASBVd is present in Australia, Ghana, Greece, Israel, Mexico, Peru, South Africa, Spain, the United States of America and Venezuela (Kuhn et al. 2017; Lotos et al. 2018). New Zealand, which is the only country that is currently allowed to export fresh avocado fruit to Australia, has declared nationwide freedom from the pathogen (Pugh and Thomson 2009).

Australasian scientists were responsible for many pioneering discoveries on ASBVd. Sunblotch disease was first described in the early twentieth century in California, although the disease was initially attributed to sunburn damage (Coit 1928) and then an infectious agent, probably a virus (Horne and Parker 1931; Whitsell 1952). The hypothesis for a viral aetiology persisted for nearly 50 years, until three independent laboratories in Adelaide, Brisbane and Auckland, observed the presence of viroid-like RNA in diseased plants (Dale and Allen 1979; Palukaitis et al. 1979; Thomas and Mohamed 1979), although infectivity of the RNA and fulfilment of Koch's Postulates were not demonstrated until 2 years later (Allen et al. 1981). Other very significant outputs from this research were the first entire nucleotide sequence for the viroid (Symons 1981), elucidation of the replication cycle (Bruening et al. 1982; Hutchins et al. 1985, 1986; Forster et al. 1988; Bonfiglioli et al. 1994) and development of rapid and sensitive molecular diagnostic methods (Allen and Dale, 1981; Palukaitis et al. 1981).

The economic impacts of ASBVd are manyfold. Apparently asymptomatic strains of the viroid can still reduce yield of 'Hass' trees by 15–30%, and yield reductions are even greater for symptomatic strains, at 50–80% (Saucedo-Carabez et al. 2014). Furthermore, about half of the fruit from symptomatic trees may be scarred and therefore significantly downgraded in quality. ASBVd is also an impediment to trade due to quarantine conditions imposed on the movement of fresh fruit due to the risk of transmitting the viroid in the seed.

Although ASBVd is known to occur in Australia, its status has not been recently reviewed. The aim of this paper is to provide comment on the geographical distribution and

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prevalence of ASBVd in Australia in order to guide future surveys and to assess the success of current control methods.

Origin of ASBVd and possible entry pathway into Australia

Avocado is the only known natural host of ASBVd and a logical corollary is that the viroid originated in the same location as its host, which is the eastern and central highlands of Mexico, through Guatemala to the Pacific coast of Central America and the northern parts of South America (Ayala Silva and Ledesma, 2014). The avocado industry in southern California began using germplasm that was mainly sourced from the cities of Atlixco and Santiago de Querétaro in Mexico but also from Guatemala (Whitsell 1952).

The earliest anecdotal record of sunblotch disease was at the West India Gardens Nursery, Altadena, California, in 1914 (Whitsell 1952), which was owned by Frederick Popenoe. In 1911, Popenoe sent his son Wilson and employee Carl Schmidt on a collecting mission to Atlixco, and they brought back 41 accessions, one of which became the variety 'Fuerte', the mainstay of the infant Californian industry (Bost et al. 2013). In 1948, the California Avocado Society "Committee on Foreign Exploration" surveyed the Rodiles Grove of avocados near Atlixco, and observed sunblotch disease symptoms in 30 year-old 'Fuerte-like' seedling trees (Trask 1948; Whitsell 1952). Credence should be given to these observations, as the expedition members were very familiar with the disease in their home state. This circumstantial evidence suggests that ASBVd could have inadvertently been introduced to California from Mexico by the early botanical explorers. However, it was not until 2009 that ASBVd was officially reported from Mexico (De La Torre et al. 2009) and contrary to historical anecdotal evidence, the only verified records of ASBVd are from Michoacán State, where the pathogen is common in 'Hass' avocados (De La Torre et al. 2009; Saucedo-Carabez et al. 2014; Vallejo Pérez et al. 2017).

During the period 1920–50, ASBVd became very common in southern California and was considered the second most important pathogen of avocado after *Phytophthora cinnamomi* (Whitsell 1952). Consequently, avocado varieties that were sourced from California at this time were at high risk of being infected, often asymptomatically (Wallace and Drake 1962). In 1924, sunblotch disease was recorded for the first time outside of the USA, in Israel, and this was in 'Fuerte' and 'Dickinson' trees that had been imported from California (Whitsell 1952; footnote 2 in Horne and Parker (1931)). Almost certainly ASBVd was introduced into Australia by a similar pathway and possibly at a similar time. Commercial avocado production began in Australia in 1928 at Red Cliffs, Victoria, with the planting of 25 imported trees from California (Cadman and El-Zeftawi 1977). Shortly after, the industry established in south-east Queensland, with the planting of 100 seedling trees on the Blackall Range in 1930, the importation of the first named varieties (grafted 'Fuerte' and 'Nabal' trees) to Redlands Bay from California in 1931 and then the planting of an orchard on Tamborine Mountain in 1933 (Anderson 1977; Whiley 2000).

In 1967, sunblotch disease was reported for the first time in Australia, in a 'Mexicola' tree grown at the NSW Department of Agriculture Tropical Fruit Research Station, Alstonville, NSW, which had been propagated using budwood that had been obtained from Wilson's Nursery on Tamborine Mountain, Queensland, in 1962 (Trochoulias and Allen 1970; T. Trochoulias pers. comm., now retired from NSW Department of Primary Industries). This variety was being trialled as a dwarfing interstock and disease symptoms were observed both on the 'Mexicola' mother tree and 'Fuerte' scions that had been grafted onto 'Mexicola'. In 1973, sunblotch disease was observed at a second location in the avocado varietal collection at the CSIRO Horticultural Research Station, Merbein, about 10 km west of Mildura, Victoria. The infection at Merbein was considered to have originated from a tree (AV-2) that had been imported from California in 1948 and used as a rootstock (Allen, 1981). It is likely that AV-2 was the accession code for 'Mexicola', as Allen (1976) stated that the infection in Merbein could be traced back to a 'Mexicola' tree at Alstonville.

Historical records of ASBVd in Australia

ASBVd appears to have always been very rare in Australia. Initial characterization work was done with viroid isolates from trees that were held at research stations at Merbein or Alstonville and both stations have now closed down. At Alstonville, only a single infected 'Fuerte' tree (isolate SB-2 in Allen and Dale (1981)) was retained for any length of time in the varietal collection, and this tree was destroyed once experimental work was complete in the early 1980s (R. N. Allen pers. comm., now retired from Queensland Department of Agriculture and Fisheries (QDAF)). Viroid isolate SB-1 was maintained in glasshouse-grown plants at Merbein and at the Waite Institute, Adelaide (Bruening et al. 1982; J. W. Randles pers. comm., now retired from University of Adelaide).

Three surveys for ASBVd have been undertaken in Australia. The first of these surveys was undertaken by Palukaitis et al. (1981) and focussed on the Tristates production area, centred on Mildura and including orchards in the Murray River irrigation areas of Victoria, New South Wales and South Australia. ASBVd was only detected in two commercial orchards, in a single 'Hass' tree from Red Cliffs, Victoria, and two 'Zutano AV35' trees from Coomealla, NSW.

The second survey was undertaken by Dale and Allen (1981) and included orchards in northern NSW and south-east Queensland, plants at the quarantine research station in the ACT and a symptomatic Hass tree from the CSIRO Berrimah Research Farm in Darwin (isolate Hass/B). Five infected trees were identified, one of which was asymptomatic (variety 'Carlsbad', held at the ACT quarantine research station) and four that were symptomatic. Two of these symptomatic trees were in commercial orchards: Hass/T from Tamborine Mountain, which was illustrated by Broadley (1991) and Hass/Lg, from an orchard at an unknown location in Queensland. The remaining two symptomatic trees were the aforementioned isolates SB-2 at Alstonville and Hass/B at Berrimah. In an important validation of the biological-indexing scheme of the time, the health status of 17 trees that been accepted as healthy on the basis of graft transmission tests was confirmed by polyacrylamide gel electrophoresis and cDNA hybridization assay.

The third survey was undertaken by Geering and Steele in 2008–2009 using real-time RT-PCR for detection (Geering 2011), and included all commercial orchards on Tamborine Mountain (combined total of 2135 trees tested), as well as the multiplication blocks of two of the largest avocado nurseries in Australia, at Woombye in south-east Queensland and Duranbah in north-east NSW (combined total of 1074 trees tested). None of the trees in the multiplication blocks used for budwood by the nurseries were infected, and only a single infected tree was detected in all of the commercial orchards. Twenty of the nearest neighbours of this infected tree were tested a year apart, and no evidence of spread obtained. The sole infected tree on Tamborine Mountain was cut down and the stump removed in 2010.

In the early 1980s, there was interest in other virus-like diseases of avocado such as black streak disease, and efforts made to find dsRNA replicative intermediates; this was also a de facto test for ASBVd. Allen (1983) analysed dsRNA profiles in 26 avocado trees, each representing a different variety or selection in the Australian Avocado Growers' Federation (AAGF) 'Virus-Tested Tree Registration Programme', and found no evidence for the presence of ASBVd. It should be noted that the sensitivity of detection was only about 20 ng per dsRNA band.

Other than the aforementioned validated records of ASBVd, 12 cases of symptomatic trees from eight different farms have been reported from the Atherton Tableland, Queensland, in the period 1983–1990, mainly in the variety 'Fuerte' but also on 'Sharwill', 'Hass' and 'Rincon' (QDAF Disease Accession Book, Mareeba; K. Grice pers. comm., QDAF). Several infected trees with typical scarring symptoms on the fruit but asymptomatic leaves, were found on a property at Iraak, Victoria, in 2011 (E. K. Dann and A. D. W. Geering unpublished).

ASBVd has never been found in South Australia or Western Australia (R. Van Velsen pers. comm., now retired from South Australian Department of Agriculture and Water Resources; J. W. Randles pers. comm.).

Efforts to control ASBVd in Australia

In Australia, ASBVd has been treated as a quarantine-regulated pathogen since the mid-1960s, when post-entry quarantine testing for the sunblotch disease agent was initiated at Rydalmere, NSW, for new varieties entering the country (R. N. Allen pers. comm.). Indexing was done by side-grafting test scions onto healthy, indicator 'Hass' seedlings, a method recommended by the Californian Avocado Society (Allen and Firth 1980). In 1970, the first, domestic, indexing program commenced in South Australia and from 1973, plants arising from this program were distributed to the industry, although this scheme never progressed (Allen 1976; R. N. Allen pers. comm.). Upon the advent of the NSW Avocado Improvement Committee, an indexing program began at Wollongbar, northern NSW, in 1975, and tested material used to establish an avocado foundation tree block at Somersby Horticultural Research Station, just north of Sydney (Allen 1976). Limited biological indexing was also done by Donald McEwan Alexander for varieties held in the CSIRO Merbein Collection (Allen 1977).

In 1978, a clean planting material scheme for avocados, called the Avocado Nursery Voluntary Accreditation Scheme (ANVAS), was established, which initially was only concerned with ensuring that planting material was free from *Phytophthora cinnamomi* but later, other soil-borne pathogens. This scheme was a first in the world for avocado and served as an exemplar for other countries. The 'Virus-Tested Tree Registration Programme' then began in December 1980 but was amalgamated with ANVAS during the 1990s. The ANVAS Guidelines allowed for the registration of mother blocks containing foundation/nuclear and multiplication trees, which were allocated identification numbers and were tested for ASBVd at least every 5 years in the case of the former or at least every 20 years in the case of the latter (ANVAS Guidelines, September 1996, Australian Avocado Growers' Federation). Following the inception of the 'Virus-Tested Tree Registration Programme', testing for ASBVd has been done by either cDNA hybridization assay or reverse-transcription PCR (Randles et al. 2003; R. Allen pers. comm.; ADW Geering, unpublished).

Although ASBVd is currently not a listed plant pest in any State biosecurity legislation within Australia, there has been a voluntary code adhered to by property owners to destroy infected plants (K. Pegg pers. comm., now retired from QDAF).

Conclusions

A major outcome of the substantial body of research that was done on ASBVd in Australia during the 1970s and 1980s is that control methods were implemented at a very early stage in development of the avocado industry, which undoubtedly has had a major impact in limiting spread of the pathogen within the country. ASBVd appears to have always had a very limited distribution, and records are becoming even less frequent over time, with only two confirmed records since 2000.

Although the Australian avocado industry is about 90 years old, the average tree age is much younger, with less than 1% of the trees predating 1980 (Avocados Australia Ltd. unpublished data). The youth of the tree population reflects both the rapid turnover of plants in the early years due to a high mortality rate from *P. cinnamomi*, and a recent rapid expansion of the industry. The Australian avocado industry is therefore based on trees that have been planted since the introduction of ASBVd-indexing schemes. Based on the scarcity of recent records of ASBVd, it appears that the control methods that were introduced have been very successful.

With the continuation of ASBVd-indexing schemes, it could be expected that ASBVd will be eradicated from Australia. Further surveying of the oldest trees (>30 years old) in the Tristates Region, in northern NSW/south east Queensland and on the Atherton Tableland, is warranted as part of pest-freedom studies, as these trees are the most likely to be infected with ASBVd.

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