AVOCADO SCAB IS NOT PRESENT IN NEW ZEALAND

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Avocado scab is a superficial cosmetic disease of avocados caused by the fungus *Sphaceloma perseae*. Avocado scab was reported as being present in New Zealand in 1991. That record was not validated by New Zealand Phytosanitary Authorities and was therefore not on the avocado pest list. Biosecurity Australia became aware of the record in November 2006 at which time it became a market access issue for New Zealand avocados exported to Australia. Australia does not have this disease. As a consequence the record was re-examined. Using cultural characteristics, spore morphology and DNA sequencing it was shown that the culture upon which the record was based was not *S. perseae*, but was *Phaeosphaeria* sp. (anamorph *Phaeoseptoria* sp.) a fungus common on grasses. This fungus has not been reported to cause disease of avocados, and no symptoms developed following inoculation of young fruitlets. A comparison of herbarium samples of dried skin from the New Zealand specimen upon which the record was based with authenticated herbarium specimens from Florida showed a clear difference in symptomatology. Symptoms in a photograph published with the New Zealand record were similar to those of wind rub reported in Australia. On the basis of these results, the record of avocado scab was shown to be erroneous and the disease is not present in New Zealand.

Keywords: *Sphaceloma perseae*, *Phaeosphaeria* sp., *Phaeoseptoria* sp.

AUSENCIA DE LA VERRUGOSIS DEL AGUACATE EN NUEVA ZELANDA

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La sarna o verrugosis del aguacate es una enfermedad cosmética, causada por el hongo *Sphaceloma perseae*. Esta enfermedad fue señalada por primera vez en Nueva Zelanda en 1991. Sin embargo, dicho registro no fue confirmado por las autoridades fitosanitarias de Nueva Zelanda y por esta razón no fue incluida en la lista de plagas. En Noviembre del 2006, Bioseguridad-Australia se informó de dicho registro y cuestionó el acceso de aguacates importados desde Nueva Zelanda. La presencia de esta enfermedad no esta registrada en Australia y como consecuencia de esta situación se volvió a revisar dicho reporte. En este sentido, se estudiaron la morfología de las esporas y secuencias del ADN. Todo lo cual ha indicado que el registro originalmente descrito no era *S. persea* sino *Phaeosphaeria sp*. (anamorfismo de *Phaeoseptoria sp*.), un hongo comúnmente encontrado en los pastos. Este hongo no está señalado como causante de ninguna enfermedad en aguacate y además no se observó el desarrollo de síntomas

en frutos jóvenes de aguacate inoculados con *Phaeosphaeria sp*. Adicionalmente, se compararon muestras de herbario de cáscara seca de frutas provenientes del registro original de Nueva Zelanda con muestras auténticas de herbario procedentes de Florida, quedando claramente demostradas las diferencias en relación a los síntomas. La fotografía publicada con los síntomas registrados en Nueva Zelanda fue similar a las presentadas por Australia las cuales se asemejan a cicatrices causadas por el viento. Sobre la base de estos resultados, el registro de la sarna o verrugosis del aguacate es considerado erróneo y por lo tanto esta enfermedad no esta presente en Nueva Zelanda.

Palabras Clave: *Sphaceloma perseae*, *Phaeosphaeria* sp., *Phaeoseptoria* sp.

1. Introduction

Avocado scab (*Sphaceloma perseae* Jenkins) was first reported from avocados in a nursery in Florida in 1918 (Stevens, 1918). It has been recorded as present in Africa (Guinea, Morocco, South Africa, Zambia, Zimbabwe), Asia (Philippines, Taiwan), Central America and West Indies (Antilles, Costa Rica, Cuba, Dominican Republic, Guadeloupe, Guatemala, Haiti, Honduras, Jamaica, Nicaragua, Panama, Puerto Rico, Salvador) and in South America (Argentina, Brazil, Guyana, Peru, Venezuela) (Anon., 1986). In 1991 it was recorded as present in New Zealand (Hartill, 1991). The record in New Zealand was based on the identification of a fungus isolated from scars on avocado. In support of that record, two specimens of dried avocado skin with typical symptoms, and a dried culture of the fungus were deposited in the New Zealand Fungal Herbarium (PDD; Landcare Research, Manaaki Whenua). At the same time, a freeze-dried culture was deposited in the International Collection of Micro-organisms from Plants (ICMP; Landcare Research, Manaaki Whenua). The symptom was also photographed and published in Hartill (1991).

Avocado scab causes lesions on the upper and lower surface of young leaves and on the skin of the fruit. On fruit these are generally brown to almost black and up to 3 mm in diameter, sometimes in a circular pattern (Jenkins, 1925, 1934). Lesions are raised, purple brown to dark brown, and circular to oval. The spots are scattered, but many coalesce to form irregular, extended areas that often cover the entire fruit surface (Burnett, 1974; Palmateer, 2006). On leaves lesions are often red, and the centre can fall out to result in holes. Infections on the lower leaf surface initiate at the midrib and veins. Lesions on leaves most often form in the upper part of the tree canopy (Pernezny, 2000), and the upper surface of leaves is more susceptible than the lower surface. In 1-month-old fruit, avocado scab was observed to rupture the epidermis and produce hyaline conidia and conidiophores that formed a dense, velvety covering, dark olive in colour. On the underside of leaves these conidial masses were light brownish olive. The velvety layer was gradually lost by weathering until only a few conidiophores remained by the time the fruit were 4 months old (Jenkins, 1934). After 1 month, leaves became resistant to infection, and fruit became resistant after

they were about half size (Pernezny, 2000). Those avocado varieties reported to be most susceptible to scab are Lula, Hall, and most avocado seedlings. Booth 3, 5, 7, 8; Monroe, Choquette, Trapp, Waldin, and Pollock are moderately susceptible and Booth 1 and Collins are slightly susceptible (Pernezny, 2000). Jenkins (1934) also reported scab symptoms on Fuerte, Trapp, Challenge, Perfecto and Surprise.

The report of avocado scab in New Zealand (Hartill 1991) was not validated by New Zealand Phytosanitary Authorities and was therefore not on the avocado pest list. In December 2006, Biosecurity Australia became aware of the record and immediately placed additional treatment and inspection requirements on the import of New Zealand avocados as the disease is not present in Australia. This paper presents an investigation of the specimens upon which the record in New Zealand is based.

2. Materials and Methods

All aspects of the record of the disease were reviewed. A freeze-dried specimen of the culture ICMP 10613, deposited as *Sphaceloma perseae* (Hartill 1991), was obtained from the International Collection of Micro-organisms from Plants (ICMP; Manaaki Whenua, Landcare Research, Lincoln, New Zealand (Landcare)). This freeze-dried specimen was aseptically divided into two. One half was spread on the surface of a Petri plate containing Difco Potato Dextrose Agar (PDA) with a sterilised bent glass rod. A freeze-dried culture of a related species, *Shaceloma fawcettii* Jenkins CC135, from our collection was also placed on PDA. These were compared by culture and spore morphology. DNA was extracted from the second half of the freeze-dried specimen using the Qiagen DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, CA, USA).

Extracted DNA was used in PCR reactions with the ITS1/ITS4 primer set of White *et al.* (1990) to amplify the Inter-Transcribed Spacer (ITS) regions of the ribosomal RNA gene. The PCR products were then purified by gel electrophoresis (10g L^{-1} agarose) followed by treatment with the Molecular Biochemicals High Pure PCR Product Purification Kit (Roche Diagnostics). Purified PCR products were sent to University of Waikato (Hamilton, New Zealand) for sequencing reactions. Sequence was analysed using BLAST (National Center for Biotechnology Information; NCBI), Vector NTI 8 (InforMax Inc.) and compared with representative ITS sequences from GenBank (NCBI).

The herbarium specimens PDD 58048 and PDD 58615, deposited by W.F.T. Hartill in 1990, were photographed. These were compared with skin specimens of avocado scab, authenticated by Anna E. Jenkins and A.A. Bitancourt in 1940 and 1941, collected from Florida and Cuba and deposited in the Landcare herbarium (PDD 55, 57 and 61).

Avocado fruitlets about 1 month old were inoculated with spores from ICMP 10613 by placing 100 µl of 10^5 spores/ml on each fruitlet. Fruitlets were then placed in a high humidity chamber at ambient temperatures and observed for 6 weeks.

Avocado fruitlets with scars about 1 month old were observed in the field and symptoms were photographed.

3. Results

Growth of the fungus from the sample of ICMP 10613 placed on PDA was visible after 24 hours and the colony grew out over the Petri plate during the succeeding week. The morphology of the dried culture on fungal isolation medium (PDD 58048) was similar to the culture growing from the freeze-dried material. The rate of growth and colony characters were not consistent with *S. perseae* which is a very slow growing fungus that does not have spreading colonies on PDA. *S. perseae* culture morphology is similar to the closely related fungus *S. fawcettii*, which was clearly different from the culture derived from ICMP 10613 (Figure 1). After approximately 2 weeks, ICMP 10613 produced dark masses of spores which were readily identified as *Phaeoseptoria* sp. (Figure 2). This was supported by the ITS sequencing. BLAST analysis showed that the sequence with highest homology to ICMP 10613 was *Phaeosphaeria volkartiana* with 92% identity. *Phaeosphaeria* sp. is the sexual stage of *Phaeoseptoria* sp. (Camara *et al.*, 2002). It is a fungus that causes lesions on grasses and has never been recorded as a pathogen of avocado. Avocado fruitlets inoculated with spores of *Phaeoseptoria* sp. did not show symptoms after 6 weeks in conditions of high humidity, further confirming its lack of pathogenicity to avocado.

Examination of herbarium specimens of avocado scab, PDD 55, 57 and 61, deposited by A.E. Jenkins, who originally described the disease (Jenkins 1925, 1934) showed those specimens were well preserved and symptoms of the disease on leaves and fruit were clearly visible. The symptoms on these authentic specimens were very different from the symptoms on the New Zealand specimen (PDD 58048) that consisted of fruit skin only. They also differed from the symptoms in the photograph used by Hartill (1991) to illustrate the disease (Figure 3). A further New Zealand specimen (PDD 58615) was degraded and symptoms were no longer visible.

Young 'scabbed' fruit in New Zealand avocado orchards were more common on exposed sides of the trees. When examined microscopically, it appeared that the skin had been damaged perhaps by impact of adjacent petioles and leaves when blown by wind. The damaged tissue could not expand as the fruit grew, and cracked forming deep fissures (Figure 4).

There were no leaf symptoms on New Zealand avocado trees from orchards with a high incidence of 'scabbed' fruit. Leaf symptoms, which are diagnostic of avocado scab, have never been observed in New Zealand. The absence of leaf symptoms is further evidence that the disease is not present in New Zealand.

4. Discussion

On the basis of culture and spore morphology, supported by sequence analysis, it was shown that the isolate deposited in the Landcare Herbarium (ICMP 10613) from a New Zealand avocado fruit abrasion was incorrectly identified as *S. perseae*. It should, instead, be re-named as *Phaeoseptoria* sp. Symptoms on fruit specimens deposited in the Landcare Herbarium (PDD 58048) were dissimilar to authenticated Herbarium specimens of avocado scab.

The predominance of scarred fruitlets on the windward side of trees in New Zealand, a close examination of the symptoms, and their similarities to that of

a photograph described as ‘wind rub’ on Australian avocados (Vock, 2001) suggested that the symptoms observed and described by Hartill (1991) were more similar to ‘wind rub’ than to avocado scab. The fruit scars with a chequered appearance on New Zealand and Australian fruit may occur because the damaged tissue cannot expand as the fruit grows, and splits forming deep fissures. These fissures appear to be filled by a light brown corky tissue while the original damaged tissue retains a smoother dark appearance. The more the fruit grows, the more these areas of original damage break up and the space in between is filled by the corky tissue.

The velvety fungal mycelium covering lesions on 1-month-old fruitlets infected with avocado scab reported in Jenkins (1934) was not observed on scars of New Zealand fruitlets. Leaf symptoms have never been seen in New Zealand avocado orchards. Thus, typical symptoms of avocado scab have not been seen in New Zealand orchards.

The photograph in Hartill (1991) was also dissimilar to photographs of avocado scab in more recent publications (Teliz-Ortiz *et al.*, 2003).

The lack of avocado scab symptoms in New Zealand, and the evidence presented in this paper as to the correct identity of the culture ICMP 10613, suggests that New Zealand should be re-classified as being free of avocado scab.

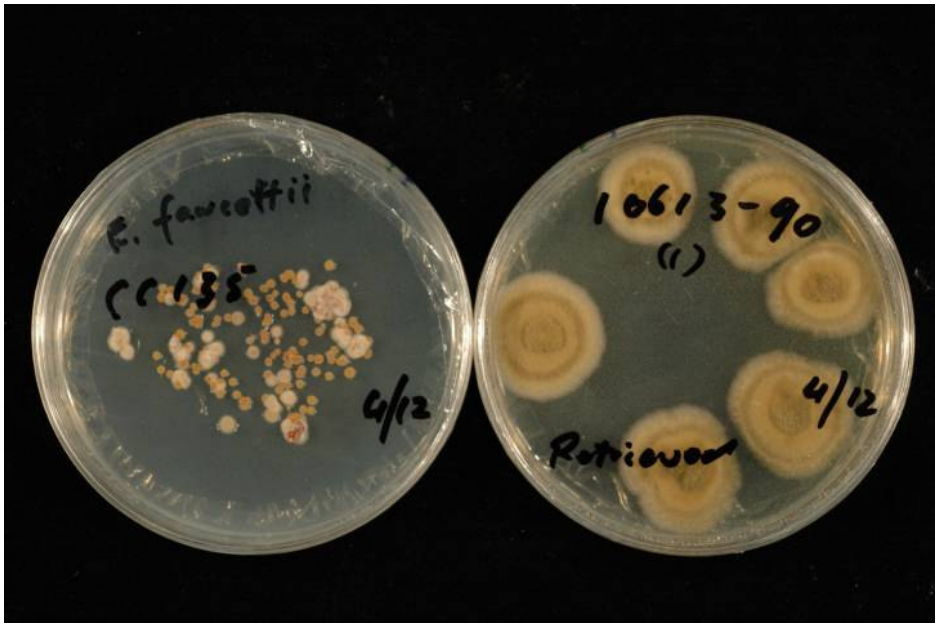

Figure 1 Culture morphology of ICMP 10613 (right) compared with *Sphaceloma fawcettii* CC135 (left). Both cultures are 10 days old growing on Potato Dextrose Agar.

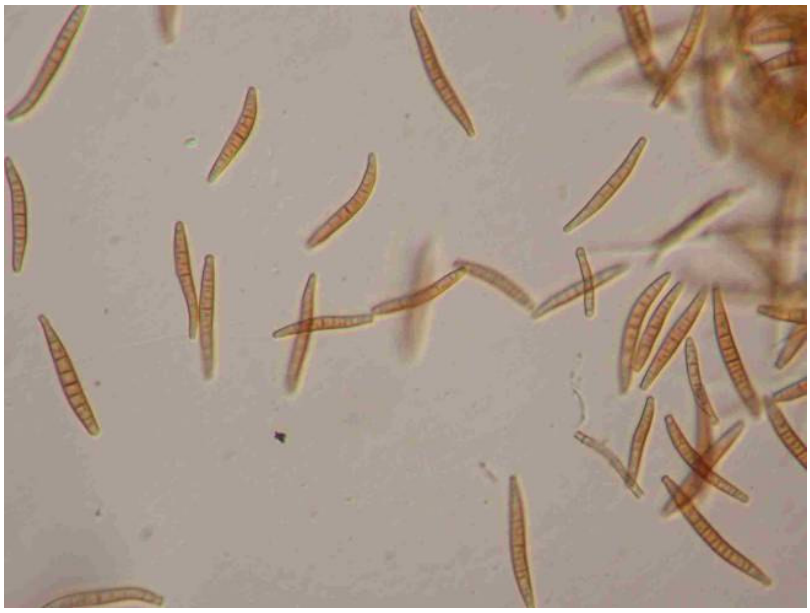

Figure 2. Spores of *Phaeoseptoria* sp. produced by the culture ICMP 10613 after 2 weeks growth on Potato Dextrose Agar.

Figure 3 Herbarium specimen PDD 58048 described as showing Avocado scab symptoms in Hartill (1991) (left) compared with Herbarium specimen PDD 57 authenticated by A.E. Jenkins as Avocado scab symptoms from Florida (right).

Figure 4 Avocado fruitlet less than 1 month old from a New Zealand orchard showing scarring symptoms.

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