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Source of *Pseudocercospora macadamiae* inoculum in macadamia trees and its use for characterising husk spot susceptibility in the field

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ABSTRACT

Pseudocercospora macadamiae causes husk spot of macadamia. Husk spot control would be improved by knowledge of the persistence of the pathogen in orchards between seasons, and by characterising macadamia cultivars for susceptibility to infection, premature fruit abscission, and spatial escape from inoculum sources. Field observation of seven macadamia cultivars found a significant exponential relationship between husk spot incidence and the prevalence of fruit pericarp that failed to abscise (sticktights) in the canopy. Viable conidia of *P. macadamiae* were readily (>100 conidia per fruit) produced on sticktights for 30 months. The incidence of husk spot was up to four times greater in trees with sticktights than trees without sticktights. Assessment of the propensity to form sticktights, husk spot incidence and fruit abscission after the insertion/removal of sticktights, demonstrated differences in macadamia cultivars; cv. A16 was susceptible to infection and formation of sticktights, but tolerant of premature abscission; cv. 246 was susceptible to infection, tolerant of premature abscission, and spatially escapes *P. macadamiae* inoculum due to a lack of sticktights; cv. A38 was susceptible to infection, premature abscission, and sticktight formation. Our findings suggest that disease could be reduced by removing sticktights from trees, and that fungicide inputs could be reduced in trees with naturally low numbers of sticktights. In addition we show that sticktights can be used as an inoculum source for field assays to better characterise macadamia cultivars for better informed cultivar selection, and as a tool in breeding programs to screen for germplasm with high levels of resistance.

1. Introduction

In the last 30 years, the cultivation of the Australian native plants *Macadamia integrifolia*, *M. tetraphylla* and their many hybrids for their highly palatable kernels has increased from a cottage industry to a rapidly growing global nut industry with major production areas in Australia, the United States of America (Hawaii), and South Africa. Production is also increasing in countries such as Kenya, Malawi, Zimbabwe, Guatemala, Costa Rica, and Brazil. Production in Australia, the centre of origin of *Macadamia*, is adversely effected by the husk spot disease caused by the fungus *Pseudocercospora macadamiae*. Disease symptoms are characterised by hard, chlorotic and tan to dark brown spots that are confined to the pericarp (husk) of the macadamia fruit (Fig. 1) (Beilharz et al., 2003; Miles et al., 2009). Diseased fruit often abscise prematurely before the kernel oil content exceeds the accepted 72% defining commercial maturity (Akinsanmi et al., 2007). Without adequate disease control, premature abscission due to husk spot is estimated to result in losses of up to $10 million Australian dollars annually, almost 15% of the total annual farm gate value of macadamia production in Australia (Jones, 2004).

In Australia, macadamia anthesis typically occurs in August–September (early spring), with fruit set completing within about 2 weeks (Cull, 1984). Growth of fruit and accumulation of oil within the kernels occurs up to approximately 22–23 weeks post-anthesis (wpa) (McConchie et al., 1996; Trueman et al., 2000), followed at 30 wpa by the abscission of mature fruit from the canopy, and harvesting from the ground (Trueman and Turnbull, 1994). Husk spot can result in premature abscission of fruit as early as 16 wpa (January) (Akinsanmi et al., 2007).

The disease cycle of husk spot is partially understood. Conidia of the pathogen are produced within the visible lesions, with strong

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Fig. 1. Husk spot lesion (arrow) on the pericarp of a living macadamia fruit (left) and a senescent macadamia fruit (centre). Fruit that remain attached to the petiole in the tree canopy after the normal time of abscission become 'sticktights' and can become numerous in the tree canopy (right).

aggregation of diseased fruit in trees suggesting dispersal by water splash (Akinsanmi and Drenth, 2010). Wind dispersal of conidia of *P. macadamiae* has been suggested, but has yet to be experimentally supported (Akinsanmi and Drenth, 2010; Beilharz et al., 2003). Under optimal conditions of 26 °C and in the presence of free water or high humidity, conidia of *P. macadamiae* germinate rapidly and can penetrate the stomata of the macadamia fruit husk within 20 h (Miles et al., 2009). Infection of fruit occurs most readily after fruit set when fruit grow to approximately 3 mm diameter (Akinsanmi et al., 2007; Miles et al., in press). Following entry of the pathogen into the husk tissue, an incubation period of 5–8 weeks elapses before symptoms are first observed on the pericarp in December/January, with sporulation occurring 4–6 weeks later in January/February (Mayers, 1998). Diseased fruit commonly abscise shortly after symptom expression (Akinsanmi et al., 2007).

Perpetuation of the disease cycle through survival of the pathogen between seasons is poorly understood. *P. macadamiae* is only known to occur in the field on the husk of macadamia fruit (Beilharz et al., 2003). When the fruits are infected early in the fruit developmental stage, they typically abscise prematurely (Akinsanmi et al., 2007). Prior to anthesis and fruit set of the succeeding crop, the abscised macadamia fruit are removed from the orchard in the harvesting process (Nagao and Hirae, 1992), leaving no clear source of inoculum in the orchard for the subsequent crop. In some instances, persistence of the pathogen in orchards between seasons could be the result of symptom expression and sporulation on late-maturing cultivars coinciding with anthesis and fruit set of the next crop (Mayers and Giles, 2001). However, this generally requires delayed onset of disease symptoms and/or early anthesis of the following crop, both of which can vary among cultivars and seasons (Mayers and Giles, 2001; Stephenson and Trochoulias, 1994). In cases where there is no coexistence of diseased mature fruit and young susceptible fruit of the next season, it has been hypothesised that the fungus survives on the desiccated pericarps of diseased fruit that have senesced and failed to abscise. The desiccated pericarps are commonly referred to as 'sticktights' (Fig. 1), and can remain within the tree canopy for 1–2 years (Akinsanmi et al., 2007). How sticktights form is not well understood (Hardner et al., 2009), but it is hypothesised to be related to disruption of the abscission layer at the base of the macadamia fruit pedicel. This could be the result of various factors including hormone imbalances, photoperiod, temperature, moisture stress, and diseases (Hardner et al., 2009; Nagao and Hirae, 1992; Taylor and Whitelaw, 2001). Sticktight formation also varies among macadamia cultivars. For example, the cultivars 'A4', 'A16', 'A38' and 'Purvis' are highly prone to sticktights (e.g. Fig. 1), whereas HAES '246', '344', '660' and '800' are generally not prone to sticktights (Hardner et al., 2009; Trueman et al., 2000). Given the prevalence and persistence of sticktights in the canopy of some cultivars, sticktights could be important to the husk spot disease cycle and husk spot management.

Detailed information on the husk spot susceptibility of the ~50 macadamia cultivars commercially grown internationally is not available. Field inoculation studies using macerated *P. macadamiae* cultures grown on artificial media have been used to evaluate cultivars for susceptibility to *P. macadamiae* infection and premature abscission (Mayers and Giles, 2001). However, field observations have deviated from the resulting characterisations of husk spot susceptibility. For example, Mayers and Giles (2001) consider cv. A16 to be moderately tolerant, whereas Bell and Bell (2001) refer to cv. A16 as highly susceptible under commercial orchard conditions. Similarly, cv. 246 is considered susceptible after field inoculation (Mayers and Giles, 2001), yet field observations found trees of cv. 246 adjacent to heavily infested cv. A16 trees to be free of husk spot (Akinsanmi et al., 2007). We hypothesise that this is due to cv. 246 escaping inoculum by not being prone to sticktight formation. This disparity between field observations and the macerated *P. macadamiae* culture inoculation method assessments adds to confusion over which cultivars should be planted to best reduce the risks of losses due to husk spot. Furthermore, inoculation methods based on laboratory-cultured plant pathogens may deviate from natural infection processes leading to differences in disease levels and symptom expression (Hansen et al., 2005). A lack of a field bioassay method that replicates the natural *P. macadamiae* infection process, whilst also being practical and reliable, has hindered detailed characterisation of macadamia cultivars in terms of resistance to *P. macadamiae* infection, resistance to premature abscission, and ability to escape infection. Development of a bioassay for husk spot resistance/susceptibility assessment will enable the selection of appropriate parent cultivars that may be sources of genetically useful traits for future cultivars, thus helping to reduce the current reliance on fungicide application for husk spot management (Akinsanmi et al., 2007, 2008).

Our overall objective for this study was to determine the sources of inoculum in the field and develop methods which would enable assessment of cultivars in terms of husk spot management. In order to test the hypothesis that sticktights are a major source of *P. macadamiae* inoculum in macadamia trees, and are able to be used to manipulate husk spot disease pressure in trees in the field, the specific aims of this study were to determine; (i) the relationship between the prevalence of sticktights in macadamia trees and the incidence of husk spot; (ii) the prevalence and viability of