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AVOCADO FRUIT RESPONSES TO *Colletotrichum gloeosporioides* (Penz) SACC

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Anthracnose caused by the fungal pathogen *Colletotrichum gloeosporioides* causes serious post harvest losses of avocado fruit worldwide. Typically, infections are initiated in the field on unripe fruit, but remain quiescent as germinated appressoria until fruit ripening. Over the past two decades pepper spot, a pre-harvest disease also caused by *C. gloeosporioides*, has been reported on 'Hass' avocado fruit in Australia and South Africa. The aim of this study was to compare molecular characteristics and pathogenicity of *Colletotrichum gloeosporioides* isolates from both anthracnose and pepper spot symptom types in order to determine if these two diseases are caused by different strains of the fungus. In the study, DNA banding patterns were compared, and pathogenicity of isolates was assessed on detached fruit in the laboratory, on leaves and petioles in the glasshouse, and on fruit on trees in the field. The research demonstrated that all isolates, to varying degrees, had some effect on unripe avocado fruit and pedicels on the tree at all stages of maturity as well as on detached ripening fruit. Likewise, all isolates had some effect on petioles of nursery avocado trees. All isolates were pathogenic at the high inoculum levels used in the experiments but they varied in their relative aggressiveness. DNA fingerprinting showed a lot of genetic variability among the isolates.

Keywords: mango, anthracnose, pepper spot, pathogenicity, DNA fingerprinting

RESPUESTAS DE LA PALTA A *Colletotrichum gloeosporioides* (Penz) SACC

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Antracnosis, causada por el hongo patógeno *Colletotrichum gloeosporioides* provoca en el periodo de poscosecha graves pérdidas de palta en todo el mundo. Las infecciones se originan habitualmente en los campos sobre fruta inmadura, pero se mantienen inactivas como apresorios germinados hasta la maduración de la fruta. En las últimas dos décadas el manchado de fruto conocido como Pepper spot, una enfermedad de precosecha también causada por *C. gloeosporioides*, ha sido

registrada sobre palta “Hass” en Australia y Sudáfrica. El propósito de este estudio fue comparar características moleculares y patogenicidad de *Colletotrichum gloeosporioides* aisladas desde ambos tipos de síntomas, Antracnosis y “Pepper spot”, para determinar si estas dos enfermedades son causadas por diferentes variedades del hongo. En el estudio, se compararon patrones de bandeo de ADN, y la patogenicidad de los aislados fue evaluada por separado sobre frutas apartadas en laboratorio, sobre hojas y pecíolos en vivero, y sobre frutas en árboles en el campo. La investigación demostró que todos los aislados, en distintos grados, tuvieron algún efecto sobre palta inmadura y sobre los pedicelos en los árboles, en todos los estados de madurez como también sobre la fruta colectada en maduración. De la misma manera, todos los aislados tuvieron algún efecto sobre los pecíolos de paltos en vivero. Todos los aislados fueron patogénicos en los altos niveles de inóculo usados en el experimento, pero ellos variaron relativamente en su agresividad. La huella del ADN mostró una gran variabilidad genética entre los aislados.

1. Introduction

The interaction of *Colletotrichum gloeosporioides* and avocado is a complex one. When a conidium lands on the surface of the fruit, it adheres and germinates to produce a germ tube, which develops a terminal appressorium. An infection peg then emerges and penetrates into the outer wax layer and the cuticle of the fruit skin where it ceases growth and remains quiescent until fruit ripening (Coates *et al*. 1993). It is believed that the fungus is unable to colonise further due to the presence of preformed antifungal compounds in the fruit peel known as dienes (Prusky *et al*. 1991). During ripening, diene levels decline which correlate with the resumption of fungal growth, and subsequent anthracnose development. This study was initiated due to the recent appearance and perceived spread of localised necrotic lesions (pepper spot), also caused by *C. gloeosporioides*, on ‘Hass’ avocado fruit in Australia (Willingham *et al*. 2000). The pepper spot symptom is different from anthracnose as the symptoms develop on unripe fruit.

Investigations were carried out to see whether there is a relationship between DNA polymorphism and host specificity and variation in pathogenicity (ability to cause disease on a given host) and aggressiveness (relative capacity to cause disease on a given host genotype) in the pathogen populations. Studies were to establish whether the strains of *C. gloeosporioides* causing pepper spot are genetically distinct from those typically causing ripe fruit anthracnose. It was also of interest to know if the strains responsible for disease on avocado fruit are genetically different from those strains causing disease on mango fruit. Previous studies (Hayden *et al*. 1994) have shown that isolates of *C. gloeosporioides* from avocado vary considerably in DNA fingerprint patterns, whereas mango isolates are comparatively more genetically uniform and host specific (Mills *et al*. 1992). Comparisons were made of *C. gloeosporioides* isolated from avocado and mango crops grown in relatively close

proximity. Using DNA fingerprints of isolates collected from a range of sites, it was possible to ascertain the patterns of relationships that exist and, therefore, the potential for spread of the diseases between avocado and mango.

2. Materials and Methods

C. gloeosporioides isolates were collected from avocado (cv. Hass) and mango (cv. Kensington Pride) fruit showing preharvest (i.e. pepper spot symptoms in avocado and tear stain symptoms in mango) and postharvest (anthracnose) symptoms. Five sites across northern New South Wales and south-east Queensland were identified for the avocado collection. Fifty isolates were obtained from each site: 25 anthracnose isolates and 25 pepper spot isolates. Similarly, 50 mango isolates each were collected from two sites in New South Wales and one in Queensland. For pathogenicity studies, 80 *C. gloeosporioides* isolates were selected on the basis of geographic location, host and symptom type. Isolates were obtained from both pepper spot (50%) and anthracnose (50%) lesions of avocado (50 isolates total) and tear stain (50%) and anthracnose (50%) lesions of mango (30 isolates total).

In the laboratory, detached mature 'Hass' avocado fruit and detached seedless cocktail 'Fuerte' avocado fruit were inoculated by pipetting three single droplets (25µL) of spore suspension ($5x10^6$ conidia/mL) of an isolate onto the fruit surface on 3 allocated areas. Fruit were assessed for lesion incidence and diameter. Detached mature 'Brooks' mango fruit were also inoculated. In the glasshouse, avocado branches and leaf petioles on young grafted nursery trees in pots were treated by spraying with spore suspensions ($5x10^6$ conidia/mL) of each isolate. Trees were assessed for pepper spot symptoms. The field trials were carried out on 'Hass' avocado trees. Tagged fruit and pedicels were inoculated by immersion into the appropriate isolate suspension ($5x10^6$ conidia/mL). Fruit were assessed for pepper spot symptoms at fortnightly intervals.

For molecular studies, DNA was extracted from mycelium of isolates using a Machery-Nagel Nucleospin Plant® Kit. Genetic variation among *C. gloeosporioides* isolates was analysed using a modified DNA amplification fingerprinting (DAF) system as described by Bentley and Bassam (1996). The Numerical Taxonomy System (NTSYS) pc version 2.1 (Exeter Software, Setauket, NY, USA) was used to analyse the data.

3. Results and Discussion

DNA fingerprinting showed that isolates from anthracnose and pepper spot lesions on avocado fruit were extremely variable. Molecular characterisation did not distinguish between avocado anthracnose and avocado pepper spot isolates. Mango anthracnose and tear stain (pepper spot) isolates formed a distinct population and were genetically uniform. Unlike some of the avocado isolates, the

mango isolates did not produce a sexual stage in culture suggesting that mango isolates are of clonal origin. This concurs with previous studies in Queensland (Hayden *et al.* 1994). Two of the orchards in the study were growing both avocado and mango in close proximity, allowing inoculum to move freely between the two hosts. Molecular characterisation of isolates from both hosts showed that mango isolates did not occur on avocado fruit and avocado isolates were not found on mango fruit. Thus, cross-infection does not appear to be a problem in the two orchards (Giblin 2006).

On detached, ripening avocado fruit in the laboratory, there were no significant differences between the capacity of avocado anthracnose and avocado pepper spot isolates to cause anthracnose, nor were there significant differences between the capacity of mango anthracnose and mango pepper spot isolates to cause anthracnose on avocado. This was also the case when detached mango fruit were inoculated. There was, however, a clear distinction in disease causing ability on detached avocado fruit between mango isolates and avocado isolates, with disease incidence being lower after inoculation with mango isolates even under these artificial conditions. Some of the mango isolates were not pathogenic at all and many produced only a slight blackened stain on the fruit surface without further necrosis into the tissue, even after 10 days. Of the avocado isolates, the outstandingly aggressive isolates based on mean lesion diameter were from pepper spot symptoms. On mango fruit, lesion incidence and severity was much greater when inoculated with mango isolates. This indicates that there is host specificity by *C. gloeosporioides* (Giblin 2006).

Pepper spots were formed on unripe avocado fruit and pedicels on the tree at all stages of maturity. Likewise, pepper spots developed on petioles of nursery avocado trees, but not their leaves. Most isolates were pathogenic at the high inoculum levels ($5x10^6$ conidia/mL) used in the experiments but they varied in their relative aggressiveness. When all isolates were grouped according to symptom of origin, significantly more symptoms were caused by avocado pepper spot isolates compared with avocado anthracnose isolates on petioles in the glasshouse and on unripe fruit in the field. Mango isolates were only weakly pathogenic on avocado and some were not pathogenic at all, and there were no significant differences in aggressiveness on avocado between mango anthracnose and mango pepper spot isolates (Giblin 2006).

Field observations suggested that pepper spot is more severe in trees with an internal water deficit induced by drought or Phytophthora root rot. Fruit suffering sunburn (high temperature injury) can also be severely affected. Other influences affecting infection by *C. gloeosporioides* and the development of pepper spot in the field, such as rootstocks, nitrogen fertilisation levels, inoculum concentrations, fruit maturity, season and fruit pH, were also considered. It was found that many of the factors influencing anthracnose disease on avocado trees also influenced pepper

spot incidence. Avocado fruit were inoculated with pepper spot isolates of *C. gloeosporioides* throughout the growing season from soon after fruit set until full fruit maturity to assess disease incidence during changing annual seasons (spring through to winter). Growing season and fruit maturity had some effect on pepper spot infection with the incidence of pepper spot infection increasing during the summer months. Disease incidence and severity also increased when fruit were inoculated with an increasing fungal spore concentration (Giblin 2006). This concurred with previous studies of avocado fruit inoculations by Coates (1991) and Alahakoon *et al.* (1994).

When comparing rates of nitrogen fertilisation with pepper spot incidence, it was interesting to find that, opposite to findings on studies of anthracnose (Willingham 2001), pepper spot actually decreased with higher nitrogen levels. Rootstocks have been shown to affect anthracnose disease severity and similar patterns were found for pepper spot incidence in these experiments. The effect of pepper spot infection of fruit on subsequent postharvest anthracnose infection could not be conclusively determined and more work is required to determine whether pepper spot formation triggers any resistance to quiescent infections and/or anthracnose lesion formation.

This study has provided important information about avocado and mango *C. gloeosporioides* populations and their behaviour on avocado fruit. It has also raised interesting questions about the relationship between avocado fruit and the fungus. The formation of the pepper spot symptom seems to be a hypersensitive-like response and demonstrates that avocado is capable of an active host defence response in addition to the usual quiescent infection by *C. gloeosporioides*.

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