CONTROLLING ANTHRACNOSE IN AVOCADO BY ENHANCING NATURAL FRUIT RESISTANCE: THE ROLE OF ROOTSTOCKS AND NUTRITION

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Introduction

In terms of new, long-term sustainable disease control strategies, the most promising and exciting one would have to be the manipulation of the plants natural disease resistance or if you like the 'immunisation' of plants. Plants have evolved highly effective defence mechanisms to protect themselves from attack by pests and pathogens (Ryals *et al*. 1996; Sticher *et al*. 1997). Plants can defend themselves physically and biochemically. Upon attack, plants can defend themselves physically by strengthening up their cell walls by cross-linking pectic polymers and by depositing lignin, callose and suberin. In doing this they may be able to limit or contain the spread of a pathogen. Plants can also defend themselves biochemically by producing phytoalexins, specific antifungal compounds, PR-proteins (pathogenesis-related) such as chitinases, and beta-1, 3-glucanases. These physical and biochemical defences may either be preformed or inducible.

In avocado, a preformed antifungal compound, called diene, has been identified and shown to be involved in the quiescence of the anthracnose pathogen, *Colletotrichum gloeosporioides* (Prusky 1996). This antifungal diene compound is concentrated in the outer layers of the fruit, in the skin, and acts in the first line of defence. However, because antifungals are quite toxic, they naturally decline once the fruit reaches a certain stage of maturity and starts to ripen. This reduction in antifungal concentration during ripening has been found to co-incide with an increase in the susceptibility of the fruit tissue to disease. Once the concentration of antifungals has declined past a certain level, quiescent infections, such as anthracnose, resume development to produce symptoms (Prusky *et al*. 1991).

The induction of the plants broad physiological 'immunity' by a local necrotising pathogen is called systemic acquired resistance, SAR (Kessmann *et al*. 1994). SAR is long lasting and provides broad spectrum protection against further infections by viruses, bacteria, fungi and nematodes. However, this resistance is not usually 100% but may be enhanced by further inductions. Plants can be 'immunised' biologically (eg., non-pathogenic strains), physically (eg., heat, UV light) and chemically (Staub *et al*. 1992). A growing number of chemical elicitors have been found to trigger SAR such as salicylic acid (more commonly known as aspirin), phosphonates, Boost® (functional analogue of salicylic acid) and Messenger®. Some of the benefits of SAR are it is residue free, non-toxic to the environment, it has a very low risk of pathogen resistance developing as the pathogen would have to overcome multiple reactions and thus should provide long-term sustainable control.

The reduction of disease following SAR expression depends on a number of factors such as plant part, plant age, environmental and cultural factors. In this paper, the effects of two cultural factors, rootstock and plant nutrition, on the resistance of 'Hass' avocado fruit to anthracnose will be discussed.

Materials and Methods

The following experiments were conducted in a commercial orchard at Duranbah, northern New South Wales.

1998/99 Season

The postharvest disease susceptibility of avocado fruits from the Guatemalan cv. Hass was compared when grafted to seedling Guatemalan ('Velvick') or Mexican ('Duke 6') race rootstocks in young (3½-years-old) and older (8-years-old) trees. For both studies, trees of the same age grafted to different rootstocks were planted in adjacent rows of the same block of the orchard and thus, were under the same cultural and environmental conditions.

1999/00 Season

The effect of three different nitrogen (N) fertiliser levels on the postharvest disease susceptibility of 'Hass' avocado fruits from 4½-years-old trees grafted to either 'Velvick' or 'Duke 6' seedling rootstocks was compared. The three N levels were;

1. Control – standard application rate of 133 g of ammonium-N/tree/month.
2. Low N – no nitrogen fertiliser applied.
3. High N – double standard application rate of 266 g of ammonium-N/tree/month.

Once fruits reached commercial maturity, they were harvested and ripened at 22^{o}C (65% RH) before being assessed for anthracnose and stem-end rot (*Dothiorella dominicana*). Leaf and fruit samples were also collected for diene and mineral analyses.

Results

1998/1999 Season

Fruits from 3½-years-old 'Hass' trees grafted to 'Velvick' took slightly longer to ripen, had significantly less severe (82% reduction) and a lower incidence (34% reduction) of anthracnose, compared with fruits from 'Hass' trees grafted to 'Duke 6' (Table 1). As such, fruit marketability was significantly improved by 79% for 'Hass' on the 'Velvick' rootstock compared with the 'Duke 6' rootstock (Table 1). 'Hass' fruits from 8-years-old trees grafted to 'Velvick' also had significantly less severe (61%) and a lower incidence (35% reduction) of anthracnose and a higher percentage of marketable fruit (48% improvement) than fruits from trees grafted to 'Duke 6' (Table 1). Levels of stem-end rot (SER) were very low in both studies, and no significant differences in disease severity or incidence between fruits from the different rootstocks were evident (Table 1).

Diene concentrations measured in young and older trees were up to 1.5 times higher in 'Hass' leaves from the 'Velvick' rootstock compared with the 'Duke 6' rootstock (Table 2). Leaf diene concentrations were also measured in leaves from ungrafted seedling 'Velvick' and 'Duke 6' nursery stock trees. In ungrafted nursery trees, 'Velvick' had leaf diene concentrations 3 times higher than 'Duke 6' (Table 2).

Leaves from 'Hass' trees on 'Duke 6' had significantly higher concentrations of N, compared with leaves from 'Hass' on 'Velvick' (Table 3). The leaves from the 'Duke 6'/ 'Hass' combination also had a significantly higher N/Ca ratio and a lower Ca+Mg/K ratio than the 'Velvick'/ 'Hass' combination (Table 3). A significant positive correlation between anthracnose severity and fruit skin N/Ca ratio was also evident (Figure 1).

1999/00 Season

The different N fertiliser rates did not significantly affect the levels of postharvest disease, although there appeared to be a trend evident for less disease with lower N application rates and more severe disease with higher N application rates (Table 4). There was however, a strong rootstock effect and it was again noted that 'Hass' fruits from the 'Velvick' rootstock took longer to ripen, had less severe (49% reduction) and a lower incidence (29% reduction) of anthracnose compared with fruits from trees grafted to the 'Duke 6' rootstock (Table 4). The severity and incidence of SER was also significantly reduced by 85-87% in 'Hass' fruits from trees grafted to the 'Velvick' rootstock compared with the 'Duke 6' rootstock (Table 4). These reductions in disease resulted in an overall improvement in fruit marketability of 65% for the 'Velvick' rootstock compared with 'Duke 6' (Table 4).

Leaves from 'Hass' trees on 'Duke 6' had significantly higher concentrations of N and K and significantly lower concentrations of Ca and Mg which resulted in a lower Ca+Mg/K ratio and a higher N/Ca ratio compared with leaves from 'Hass' on 'Velvick' (Table 5). The concentration of Ca in the leaves was also significantly affected by the different N fertiliser rates. Doubling the rate of N fertiliser significantly reduced the concentration of Ca in the leaves of 'Hass' trees on 'Velvick' compared with the low N (no N applied) treatment (Table 5). A significant positive correlation between anthracnose severity and fruit skin N/Ca ratio was also evident (Figure 2).

Discussion

The greater disease resistance of 'Hass' fruits on the 'Velvick' rootstock is suspected to be related to the higher concentrations of the antifungal diene compound measured in the leaves. Elevating avocado fruit diene concentrations or slowing the decline of dienes during ripening has been shown to reduce anthracnose levels after harvest (Prusky *et al*. 1988).

Leaves of trees grafted on Guatemalan race rootstocks have been reported to have lower concentrations of N and K and higher concentrations of Ca and Mg than Mexican race rootstocks (Haas 1950; Embleton *et al*. 1962). This study found similar effects for N in 1998/99 and for all of the other mineral nutrients in 1999/00. The increased susceptibility of 'Hass' fruits on 'Duke 6' to postharvest diseases may be related to these differences in mineral nutrients as elevated N concentrations and a low N/Ca ratio have previously been associated with poor keeping quality and increased decay of other fruits such as pear (Sugar *et al*. 1992). Excessive N has been reported to have a negative impact on disease severity in some crops (Graham 1983) and is thought to be mediating part of its effect by limiting the uptake of calcium (Ca) into the fruit. By reducing the uptake of Ca, the fruit cell walls may be weakened and therefore more susceptible to attack by fungal pectolytic enzymes (Bateman and Basham 1976).

This N effect on disease was investigated further in the 1999/00 season experiment by applying different rates of N fertiliser to the different rootstock trees. While the differences in postharvest disease were not significant, there was a trend evident for disease to be reduced or increased when lower or higher rates of N were applied, respectively. This experiment is continuing into the 2000/01 season to re-examine this N effect.

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Table 1: The effects of rootstocks on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruits with 5% or less anthracnose severity and no stem-end rot) of 'Hass' avocado fruits harvested from young (3½-years-old) and older (8-years-old) trees and ripened at 22°C (65% RH) during the 1998/99 season. Mean values within columns with the same letter are not significantly different at *P* = 0.05, (n = 6 for young trees and n = 5 or 6 for 'Velvick' or 'Duke 6', respectively).

Rootstock	Shelf life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Young trees						
Velvick	7.0[a]	7.7[b]	61.9[b]	0.3[a]	1.7[a]	66.1[a]
Duke 6	6.7[b]	41.8[a]	93.2[a]	0.6[a]	4.3[a]	13.6[b]
Older trees						
Velvick	9.1[a]	15.6[b]	50.0[b]	1.2[a]	10.1[a]	64.5[a]
Duke 6	8.9[a]	39.5[a]	77.0[a]	1.4[a]	11.7[a]	33.6[b]

Table 2: The effect of rootstocks on the diene concentration of 'Hass' avocado leaves harvested from young (3½-years-old) and older (8-years-old) trees during the 1998/99 season and the diene concentration of avocado leaves harvested from ungrafted seedling nursery trees. Mean values with the same letter are not significantly different at *P* = 0.05, (n = 6 for young and older trees and n = 5 ungrafted nursery trees).

Rootstock	Diene (mg/g $_{FW}$ leaf)
Young trees	
Velvick	2.5[a]
Duke 6	1.7[b]
Older trees	
Velvick	3.3[a]
Duke 6	2.6[b]
Ungrafted nursery trees	
Velvick	0.20[a]
Duke 6	0.06[b]

Table 3: The effects of rootstocks on 'Hass' avocado leaf nitrogen (N), calcium (Ca), magnesium (Mg) and potassium (K) mineral nutrient concentrations harvested from 3½-years-old trees during the 1998/99 season. Mean values within columns with the same letter are not significantly different at *P* = 0.05, (n = 6).

Rootstock	N (% $_{DW}$)	Ca (% $_{DW}$)	Mg (% $_{DW}$)	K (% $_{DW}$)	N/Ca ratio	Ca+Mg/K ratio
Velvick	2.3[b]	2.7[a]	0.6[a]	0.9[a]	0.9[b]	3.6[a]
Duke 6	2.5[a]	2.4[a]	0.6[a]	1.2[a]	1.1[a]	2.5[b]

Table 4: The effects of rootstocks and nitrogen (N) fertiliser on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruits with 5% or less anthracnose severity and no stem-end rot) of 'Hass' avocado fruits harvested from 4½-years-old trees and ripened at 22°C (65% RH) during the 1999/00 season. Mean values within columns with the same case letter are not significantly different at *P* = 0.05, (n = 8 or 10 for 'Velvick' or 'Duke 6', respectively).

Rootstock	Shelf life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Velvick						
Control	9.3[a]	31.5[a]	61.5[a]	0.0[a]	0.3[a]	48.8[a]
Low N	9.3[a]	28.5[a]	62.3[a]	0.0[a]	0.0[a]	48.2[a]
High N	9.3[a]	37.2[a]	68.3[a]	0.1[a]	0.8[a]	43.8[a]
Average	**9.3[A]**	**32.4[B]**	**64.0[B]**	**0.03[B]**	**0.3[B]**	**46.9[A]**
Duke 6						
Control	8.8[a]	69.6[a]	94.0[a]	0.3[a]	2.8[a]	12.0[a]
Low N	8.7[a]	58.6[a]	86.0[a]	0.1[a]	1.4[a]	21.0[a]
High N	8.7[a]	63.4[a]	90.1[a]	0.2[a]	2.7[a]	16.2[a]
Average	**8.7[B]**	**63.9[A]**	**90.0[A]**	**0.2[A]**	**2.3[A]**	**16.4[B]**

Table 5: The effects of rootstocks and nitrogen (N) fertiliser on 'Hass' avocado leaf N, calcium (Ca), magnesium (Mg) and potassium (K) mineral nutrient concentrations harvested from 4½-years-old trees during the 1999/00 season. Mean values within columns with the same case letter are not significantly different at *P* = 0.05, (n = 8 or 10 for 'Velvick' or 'Duke 6', respectively).

Rootstock	N (% DW)	Ca (% DW)	Mg (% DW)	K (% DW)	N/Ca ratio	Ca+Mg/K ratio
Velvick						
Control	2.9[a]	1.6[ab]	0.38[a]	0.54[a]	1.9[a]	3.7[a]
Low N	2.9[a]	1.7[a]	0.36[a]	0.51[a]	1.7[a]	4.2[a]
High N	3.0[a]	1.5[bc]	0.37[a]	0.53[a]	2.0[a]	3.5[a]
Average	**2.9[B]**	**1.6[A]**	**0.37[A]**	**0.53[B]**	**1.9[B]**	**3.8[A]**
Duke 6						
Control	3.0[a]	1.3[cd]	0.30[a]	0.63[a]	2.3[a]	2.7[a]
Low N	2.9[a]	1.2[d]	0.27[a]	0.77[a]	2.5[a]	1.9[a]
High N	3.1[a]	1.4[cd]	0.30[a]	0.70[a]	2.3[a]	2.3[a]
Average	**3.0[A]**	**1.3[B]**	**0.29[B]**	**0.70[A]**	**2.4[A]**	**2.3[B]**

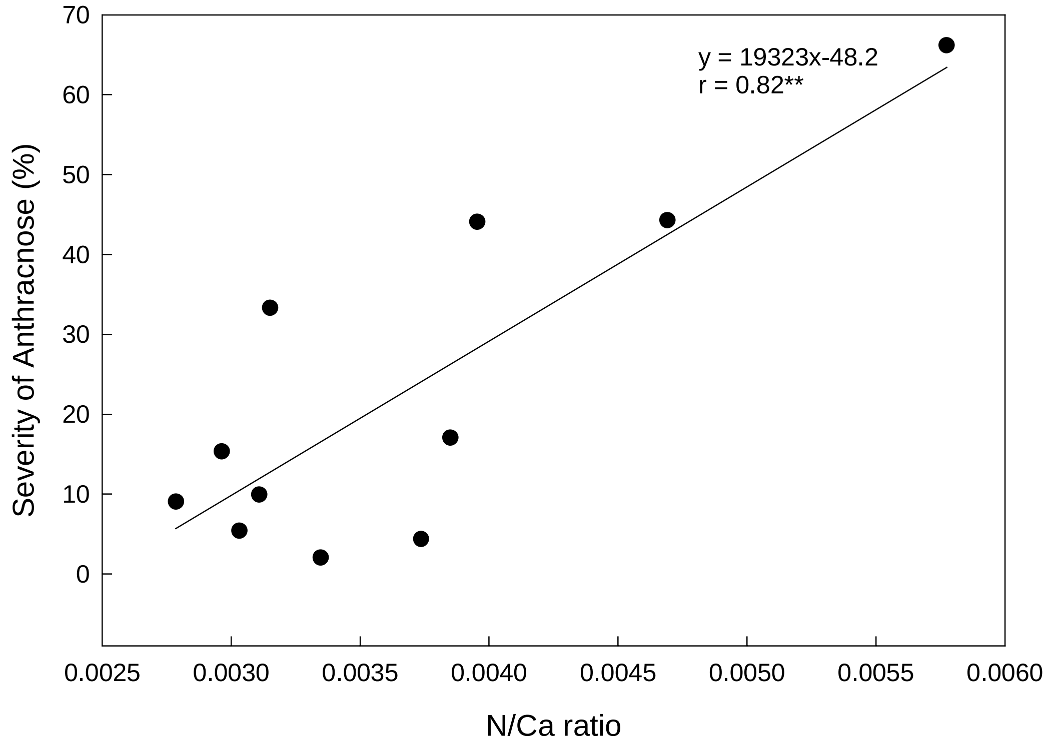

Figure 1. Linear regression relationship between the severity of anthracnose and the N/Ca ratio in the skin tissue of 'Hass' avocado on different rootstocks from the 3½-years-old trees from the 1998/99 season experiment. ** indicates significance at $P < 0.01$.

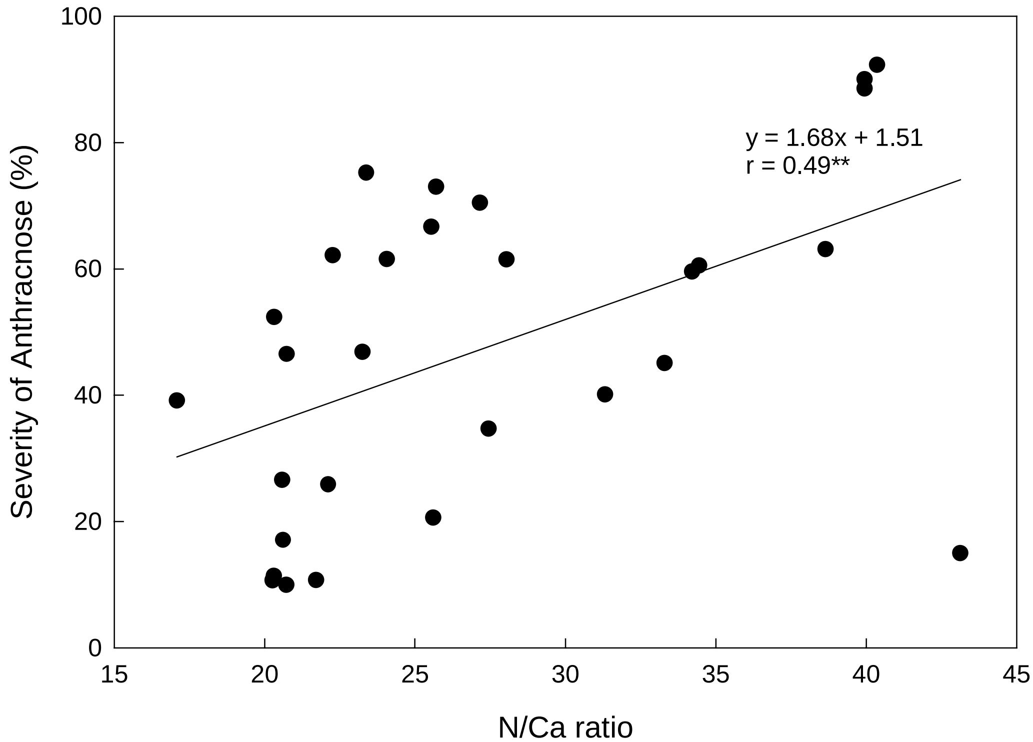

Figure 2. Linear regression relationship between the severity of anthracnose and the N/Ca ratio in the skin tissue of 'Hass' avocado on different rootstocks from the 4½-years-old trees from the 1999/00 season experiment. ** indicates significance at $P < 0.01$.