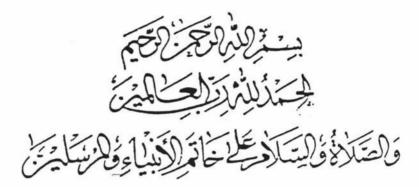
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ETHYLENE PRODUCTION BY BOTRYTIS CINEREA AND INFECTED KIWIFRUIT

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Horticultural Science at Massey University Palmerston North New Zealand

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In the name of Allah

the compassionate, the merciful,

praise be to Allah, Lord of the universe,

and peace and prayers be upon

his final Prophet and Messenger

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ABSTRACT

Botrytis cinerea is an important fungus causing serious losses to field and glass house grown fruits and vegetables and it is also an important postharvest pathogen. As a postharvest pathogen it is responsible for significant quality and economic losses to stored fruits and vegetables on a global scale. In New Zealand, infection by B. cinerea is one of the major causes of postharvest losses to the kiwifruit industry. This may be direct loss of infected fruit or an indirect loss due to secondary effects from the production of ethylene (C_2H_4) which causes softening of other non-infected fruit in the same tray.

Several fungi are known to produce C_2H_4 but B. cinerea has not been reported to do so. One objective of this study was to establish whether B. cinerea is capable of producing C₂H₄ in vitro. To achieve this objective, 4 potential precursors of C₂H₄ (methionine, glutamate, \alpha-ketoglutarate and 1-aminocyclopropane-1-carboxylic acid (ACC)) were added to Pratts modified medium at a range of pH's using two different systems of incubation (shake and static culture). Methionine was shown to be the most efficient precursor of C₂H₄ under both shake and static culture systems, with optimum pH being 3.5 and 4.5 respectively. ACC is known to be a precursor of C₂H₄ in higher plants but it did not result in C₂H₄ production in B. cinerea, either alone or when added with methionine. Although methionine was a substrate of C_2H_4 production by B. cinerea, this production was significantly inhibited by α -aminooxyacetic acid (AOA), indicating that a pyridoxal phosphate (PLP) mediated reaction might be involved. This inhibition was not reversed by addition of ACC suggesting that ACC is not the immediate precursor of C₂H₄ in B. cinerea. Cobalt ions (Co⁺⁺) added to a culture medium supplemented with methionine, had a temporary inhibitory effect on C₂H₄ production by B. cinerea compared with methionine alone. This inhibitory effect soon disappeared, with the C₂H₄ peak in the Co++ treatment reaching the same level as for methionine, only delayed by 2-4 days. This suggests that the ethylene-forming enzyme (EFE) complex in B. cinerea is different from that in higher plant. These results have shown that under defined conditions B. cinerea is capable of producing C₂H₄ from methionine but that the biosynthetic pathway appeared to be different from that present in higher plants.

Increased C_2H_4 production in response to stress is a common feature of plants. In an experiment at 20°C, kiwifruit infected with *B. cinerea* produced more C_2H_4 , than uninfected fruit, even when the latter were physically damaged, or wounded, by drilling a hole through the stem scar. At 0°C, no ethylene was produced by wounded or healthy fruit and only infected fruit were shown to produce C_2H_4 . Healthy fruit stored with infected fruit in the same tray did not produce C_2H_4 . These results suggest that at low temperature C_2H_4 production by infected fruit may not trigger an autocatalytic response from healthy fruit in the same tray. At 0°C, wounding of fruit or C_2H_4 in the environment did not trigger the autocatalytic response in kiwifruit but infection caused by *B. cinerea* did trigger this response. This suggests that infection may have activated the ACC synthase and ACC oxidase genes of the C_2H_4 pathway which consequently caused an autocatalytic response by the fruit.

A few reports have suggested that the increased C_2H_4 production in response to infection may arise from noninfected tissue at the periphery of infection. Use of slices from different parts of infected kiwifruit has shown that most ethylene was produced by the healthy tissue immediately ahead of the infection front. This suggests that in these tissues a transmissible signal was produced which could be acting as an elicitor of C_2H_4 production. Such an elicitor may have been a compound produced by the fungus itself, or it may have been produced as a result of secreted fungal enzymes acting on cell wall polysaccharides. Pectic and xyloglucan oligomers derived from polysaccharides are known to induce C_2H_4 in other plant systems. The nature of the C_2H_4 elicitor in B. cinerea infected kiwifruit tissue has not been determined, but some possibilities have been discussed.

Little or no ethylene was produced by infected kiwifruit tissue while ACC and ACC oxidase levels were no less than in healthy tissue. This suggests that the entire ethylene biosynthetic pathway was intact in these infected tissues. While all the individual components necessary for C_2H_4 synthesis were present the biosynthetic pathway could not operate in infected tissue. The reason for this is not known but could include inadequate oxygen (O_2) levels for C_2H_4 production in water soaked tissue; presence of a fungal produced toxin which inhibited the action of C_2H_4 enzymes or receptors; or lack

of EFE activity in tissue where membrane integrity was destroyed as a result of infection.

This work has provided an opportunity to study in more detail the effect of *B. cinerea* infection on localized kiwifruit tissue. Although this study did not answer all the questions it has answered some difficult and interesting ones.

This study has shown that *B. cinerea* can form ethylene from methionine using a non ACC pathway and that ethylene production is enhanced ahead of the infection front but ceases in diseased tissue. The questions raised by this study which requires further research are the steps involved in ethylene production by *B. cinerea* and the mechanism by which ethylene production is enhanced ahead of the infection front.

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