ETHYLENE PRODUCTION BY *BOTRYTIS CINEREA* AND INFECTED KIWFUIT

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
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$_2$H$_4$) which causes softening of other non-infected fruit in the same tray.

Several fungi are known to produce C$_2$H$_4$ 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 C$_2$H$_4$ in vitro. To achieve this objective, 4 potential precursors of C$_2$H$_4$ (methionine, glutamate, α-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 C$_2$H$_4$ 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 C$_2$H$_4$ in higher plants but it did not result in C$_2$H$_4$ production in *B. cinerea*, either alone or when added with methionine. Although methionine was a substrate of C$_2$H$_4$ production by *B. cinerea*, this production was significantly inhibited by α-aminoxyacetic 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 C$_2$H$_4$ in *B. cinerea*. Cobalt ions (Co**+) added to a culture medium supplemented with methionine, had a temporary inhibitory effect on C$_2$H$_4$ production by *B. cinerea* compared with methionine alone. This inhibitory effect soon disappeared, with the C$_2$H$_4$ 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 C$_2$H$_4$ from methionine but that the biosynthetic pathway appeared to be different from that present in higher plants.
Increased \( \text{C}_2\text{H}_4 \) production in response to stress is a common feature of plants. In an experiment at 20°C, kiwifruit infected with \( B. \text{ cinerea} \) produced more \( \text{C}_2\text{H}_4 \), 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 \( \text{C}_2\text{H}_4 \). Healthy fruit stored with infected fruit in the same tray did not produce \( \text{C}_2\text{H}_4 \). These results suggest that at low temperature \( \text{C}_2\text{H}_4 \) production by infected fruit may not trigger an autocatalytic response from healthy fruit in the same tray. At 0°C, wounding of fruit or \( \text{C}_2\text{H}_4 \) in the environment did not trigger the autocatalytic response in kiwifruit but infection caused by \( B. \text{ cinerea} \) did trigger this response. This suggests that infection may have activated the ACC synthase and ACC oxidase genes of the \( \text{C}_2\text{H}_4 \) pathway which consequently caused an autocatalytic response by the fruit.

A few reports have suggested that the increased \( \text{C}_2\text{H}_4 \) 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 \( \text{C}_2\text{H}_4 \) 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 \( \text{C}_2\text{H}_4 \) in other plant systems. The nature of the \( \text{C}_2\text{H}_4 \) elicitor in \( B. \text{ cinerea} \) 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 \( \text{C}_2\text{H}_4 \) synthesis were present the biosynthetic pathway could not operate in infected tissue. The reason for this is not known but could include inadequate oxygen (\( \text{O}_2 \)) levels for \( \text{C}_2\text{H}_4 \) production in water soaked tissue; presence of a fungal produced toxin which inhibited the action of \( \text{C}_2\text{H}_4 \) 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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