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ETHYLENE PRODUCTION BY *BOTRYTIS CINEREA* AND INFECTED KIWIFRUIT

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of the requirements for the degree of
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
لِحَمْدِ اللَّهِ رَبِّ الْعَالَمِينَ
وَالصَّلَاةِ وَالسَّلَامِ عَلَى خَاتَمِ الْأَنْبِيَاءِ وَالرَّسُلِ

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 C_2H_4 *in vitro*. To achieve this objective, 4 potential precursors of C_2H_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_2H_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_2H_4 in higher plants but it did not result in C_2H_4 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 C_2H_4 in *B. cinerea*. Cobalt ions (Co^{++}) added to a culture medium supplemented with methionine, had a temporary inhibitory effect on C_2H_4 production by *B. cinerea* compared with methionine alone. This inhibitory effect soon disappeared, with the C_2H_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_2H_4 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 $20^\circ C$, 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 $0^\circ C$, 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 $0^\circ C$, 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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TABLE OF CONTENTS

	PAGE
ABSTRACT	i
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF PLATES	xvii
CHAPTER 1: GENERAL INTRODUCTION	
<i>B. cinerea</i> as pathogen	2
Ethylene biosynthesis in higher plants	6
Production of ethylene by micro-organisms	8
Stress ethylene in higher plants	15
Harmful effects of ethylene on stored kiwifruit	16
Plant defence mechanisms	16
Cell wall degrading enzymes produced by <i>B. cinerea</i>	21
Elicitors of ethylene	22
Postharvest control of <i>B. cinerea</i>	23
CHAPTER 2: GENERAL MATERIAL AND METHODS	
Plant material	26
Fruit supply	26
Fruit harvest	26
Fruit inoculation	26
Establishment of <i>B. cinerea</i> isolate	27
Single spore isolate	27
Growth medium	27
Incubation conditions	27
Spore suspension	28
Gas measurement	28

Measurements of quality parameters	29
Firmness	29
Soluble solids	29
pH and titrable acidity	29
Chemicals	29
CHAPTER 3: ETHYLENE PRODUCTION BY <i>BOTRYTIS CINEREA</i>	30
Introduction	30
Materials and methods	32
Preparation of cultures	33
Determination of ethylene	33
Results	34
Discussion	71
CHAPTER 4: ETHYLENE PRODUCTION BY INFECTED KIWIFRUIT	78
Introduction	78
Materials and methods	85
<i>B. cinerea</i> Growth on kiwifruit and on malt agar	85
Intact kiwifruit 1991	85
Kiwifruit slices 1991	87
Intact kiwifruit 1992	88
Kiwifruit slices 1992	90
Tetrazolium test	90
Histochemical studies	90
ACC content in <i>B. cinerea</i> infected kiwifruit	91
Plant material	91
Preparation of tissue extracts	92
Assay method	92
ACC oxidase activity in <i>B. cinerea</i> infected kiwifruit	93
Plant material	93
<i>In vivo</i> ACC oxidase activity	93
<i>In vitro</i> ACC oxidase activity	94

Assay of <i>in vitro</i> ACC oxidase activity	94
ACC oxidase activity in slices of kiwifruit	94
Compositional changes in kiwifruit infected with <i>B. cinerea</i>	94
Results	95
1991 Experiments	98
1992 Experiments	106
Discussion	133
CHAPTER 5 FINAL DISCUSSION	141
Final conclusion and future direction	153
REFERENCES	156
APPENDIX 1 Area under the curve.	211
APPENDIX 2 Methods used for the calculation of ethylene, ACC and titratable acidity	212

LIST OF TABLES

TABLE	PAGE
1.1 Fungal and physiological problems of kiwifruit.	3
1.2 Ethylene production by plants in response to fungal infection	17
3.1 Mycelial dry weight (g) of <i>B. cinerea</i> and final pH in basal medium supplemented with a range of methionine concentrations in shake cultures after 7 days incubation at 22°C.	39
3.2 Mycelial dry weight (g) of <i>B. cinerea</i> grown in basal medium with or without possible ethylene precursors at a range of pH levels in shake cultures after 7 days incubation at 22°C.	43
3.3 Final pH of basal medium in which <i>B. cinerea</i> was grown at a range pH levels with or without possible ethylene precursors in shake culture after 24 days incubation at 22°C.	44
3.4 Mycelial dry weight (g) of <i>B. cinerea</i> in basal medium at a range of pH levels with and without possible ethylene precursors in static culture after 24 days incubation at 22°C.	48
3.5 Final pH of basal medium in which <i>B. cinerea</i> is-grown at a range of pH levels with and without possible ethylene precursors in static culture after 24 days incubation at 22°C.	49
3.6 Phosphate concentration (mM) in basal medium with or without 35 mM methionine after 24 days incubation in shake culture at 22°C.	56

- 3.7 Mycelial dry weight (g) of *B. cinerea* and final pH in the medium with and without a range of possible ethylene precursors and/or inhibitors in shake culture after 8 days incubation at 22°C. 60
- 3.8 Mycelial dry weight (g) and final pH in the basal medium with and without a range of possible precursors and/or inhibitors on shake culture after 8 days incubation at 22°C. 62
- 3.9 Mycelial dry weight (g) and final pH of *B. cinerea* grown in basal medium with a range of ACC concentrations in shake cultures after 8 days incubation at 22°C. 64
- 3.10 Mycelial dry weight of *B. cinerea* in basal medium supplemented with 35 mM methionine and a range of concentrations of Co⁺⁺ in shake culture after 4 days incubation at 22°C. 68
- 4.1 The efficiency of the conversion of ACC to C₂H₄ 92

LIST OF FIGURES

FIGURES	PAGE
1.1 Mid cross-section of mature 'Hayward' kiwifruit.	2
3.1 Ethylene production by the 'K3' kiwifruit isolate of <i>B. cinerea</i> on shake culture at 22°C with basal medium supplemented with 35 mM methionine.	35
3.2 Ethylene production by <i>B. cinerea</i> in shake culture, grown in media supplemented with 35mM methionine, over 7 days of incubation at 22°C.	37
3.3 Ethylene production, as area under the curve, by <i>B. cinerea</i> grown in a range of methionine concentrations over 7 days of incubation in shake culture at 22°C.	38
3.4 Peak ethylene production by <i>B. cinerea</i> in shake cultures at 22°C grown in media supplemented with 35 mM methionine, glutamate or α -ketoglutarate at a range of pH values.	40
3.5 Ethylene production by <i>B. cinerea</i> grown in shake culture at 22°C using basal medium supplemented with 35 mM methionine, glutamate or α -ketoglutarate.	41
3.6 Peak ethylene production by <i>B. cinerea</i> in shake culture at 22°C grown on media supplemented with 35 mM methionine, glutamate or α -ketoglutarate, at a range of pH values.	45
3.7 Ethylene production by <i>B. cinerea</i> grown in static culture at 22°C using basal medium supplemented with 35 mM methionine,	47

glutamate or α -ketoglutarate.

- | | | |
|------|--|----|
| 3.8 | Dry weight of <i>B. cinerea</i> mycelium grown in shake culture at 22°C in basal medium with or without 35 mM methionine. | 50 |
| 3.9 | Ethylene production by <i>B. cinerea</i> grown in shake culture in basal medium at 22°C with and without 35 mM methionine (Series a: non-destructive harvest). | 51 |
| 3.10 | Ethylene production by <i>B. cinerea</i> in shake culture, grown in basal medium supplemented with 35 mM methionine at 22°C (Series b: destructive harvest). | 53 |
| 3.11 | Ethylene production by <i>B. cinerea</i> in shake culture, grown in basal medium supplemented with 35 mM methionine at 22°C. (Series b:destructive harvest).
N.B, Use of logarithmic scale on x axis | 54 |
| 3.12 | pH changes in medium when <i>B. cinerea</i> was grown in shake culture at 22°C with and without 35 mM methionine. | 55 |
| 3.13 | Ethylene production by <i>B. cinerea</i> grown in shake culture medium supplemented with a range of precursors and/ or inhibitors (35mM) except Co ⁺⁺ [0.5mM] after 8 days of incubation at 22°C. | 58 |
| 3.14 | Ethylene production by <i>B. cinerea</i> grown in shake culture at 22°C supplemented with 35 mM precursors and/or inhibitor. | 61 |
| 3.15 | Ethylene production by <i>B. cinerea</i> in shake culture grown in media containing different ACC concentrations, after 8 days incubation at 22°C. | 63 |

3.16	Ethylene production by <i>B. cinerea</i> in shake culture grown in media supplemented with 35 mM ACC and/ or 0.02 mM Co ⁺⁺ .	66
3.17	Ethylene production by <i>B. cinerea</i> in shake culture, grown in media supplemented with 35 mM methionine and different concentrations of Co ⁺⁺ , after 4 days incubation at 22°C.	67
3.18a	Ethylene production by <i>B. cinerea</i> in shake culture, grown in medium supplemented with 35 mM methionine and/ or 0.5 mM Co ⁺⁺ at 22°C.	69
3.18b	Ethylene production by <i>B. cinerea</i> in shake culture, grown in medium supplemented with 35 mM methionine and/ or 0.5 mM Co ⁺⁺ at 22°C.	70
4.1	Flesh firmness of healthy fruits during the first 8 weeks at 0°C. Fruit was packed in a trays. Differential treatments were: 1 inoculated fruit packed in each tray; 4 inoculated fruits packed in each tray; no inoculated fruit packed in tray.	82
4.2	<i>B. cinerea</i> growth rate at a range of temperatures on kiwifruit of differing maturity (harvest 1, 2 & 3= TSS of 7.1, 8.6 & 10.8 % respectively).	96
4.3	<i>B. cinerea</i> growth on malt agar, at a range of temperatures.	97
4.4	Ethylene production by intact kiwifruit at 20°C inoculated with <i>B. cinerea</i> using different methods of inoculation in 1991.	99
4.5	Ethylene production by intact kiwifruit at 0°C inoculated with <i>B. cinerea</i> using different methods of inoculation in 1991.	100
4.6	Ethylene production by healthy kiwifruit at 20°C after 8 months storage at 0°C.	102

4.7	Lesion development on kiwifruit stored at 0°C inoculated with <i>B cinerea</i> using different methods of inoculation in 1991.	103
4.8	Percent infected kiwifruit, inoculated with <i>B cinerea</i> , using different methods of inoculation, after 4 months at 0°C in 1991.	104
4.9	Ethylene production from intact <i>B. cinerea</i> infected and control fruit and from zones of the same kiwifruit stored at 20°C in 1991.	105
4.10	Ethylene production from intact fruit and from zones of the same kiwifruit stored at 0°C in 1991.	107
4.11	Ethylene production by intact kiwifruit at 20°C inoculated with <i>B cinerea</i> in 1992.	108
4.12	Ethylene production by intact kiwifruit at 0°C inoculated with <i>B cinerea</i> in 1992.	109
4.13	Percent infected kiwifruit in different treatments after 4 months at 0°C in 1992.	110
4.14	Ethylene production from intact fruit and from slices of same kiwifruit stored at 20°C in 1992.	112
4.15	Ethylene production from intact kiwifruit and from slices of the same fruit stored at 0°C in 1992.	113
4.16	Normalized graph (Fig 4.9) of ethylene production from intact kiwifruit and from zones of fruit stored at 20°C in 1991.	115
4.17	Normalized graph (Fig 4.14) of ethylene production from intact	117

kiwifruit and from zones of fruit stored at 20°C in 1992.

- | | | |
|------|--|-----|
| 4.18 | ACC levels from zones of infected and noninfected kiwifruit after 2 months storage at 0°C. | 120 |
| 4.19 | Ethylene production from slices of uninfected kiwifruit start after 16 h slicing at 20°C without exogenous ACC. | 121 |
| 4.20 | Ethylene production from slices of uninfected kiwifruit at 20°C with or without 1 ml of [0.05 mM] exogenous ACC. | 122 |
| 4.21 | <i>In vivo</i> ACC oxidase activity from outer pericarp of different zones of infected and noninfected kiwifruit at 25°C. | 123 |
| 4.22 | <i>In vitro</i> ACC oxidase activity from outer pericarp of kiwifruit with the range of NaHCO ₃ levels and 10 mM ACC at 25°C. | 125 |
| 4.23 | <i>In vitro</i> ACC oxidase activity from outer pericarp of kiwifruit with the range of ACC levels and 600 mM NaHCO ₃ . | 126 |
| 4.24 | <i>In vitro</i> ACC oxidase activity from different zones of infected and noninfected kiwifruit. | 127 |
| 4.25 | TSS, sucrose, glucose and fructose concentrations in zones of infected kiwifruit stored after 2 months at 0°C. | 128 |
| 4.26 | Titrateable acidity and pH levels in zones of infected kiwifruit after 2 months at 0°C. | 129 |
| 4.27 | Quinic, malic and citric acid concentrations in the zones of infected kiwifruit after 2 months storage at 0°C. | 131 |

4.28	Oxalic and fumaric acids concentration in zones of infected kiwifruit after 2 months at 0°C.	132
------	--	-----

LIST OF PLATES

4.1.1	Model for zones of infected kiwifruit	89
4.1.2	<i>B. cinerea</i> infected kiwifruit stored at 0°C	89
4.1.3	Zones of infected kiwifruit stored at 0°C	89
4.2	<i>B. cinerea</i> infected kiwifruit immersed in tetrazolium for 4 hours at 25°C.	117
4.3	Hyphae of <i>B. cinerea</i> immersed in tetrazolium for 10h at 20°C.	117
4.4	Longitudinal section through infected fruit pericarp tissue showing hyphae of <i>B. cinerea</i> in both xylem vessels and parenchyma tissue.	118
4.5	Longitudinal section through infected fruit pericarp tissue. Hyphae of <i>B. cinerea</i> have grown both inter- and intra-cellularly.	118