

Bangor University

DOCTOR OF PHILOSOPHY

Improved procedures for the transport and storage of fruit and vegetables

Harper, Karen

Award date:
2014

Awarding institution:
Bangor University

[Link to publication](#)

General rights

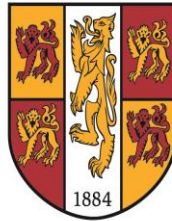
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Improved Procedures for the Transport and Storage of Fruit and Vegetables



PRIFYSGOL
BANGOR
UNIVERSITY

A thesis submitted to Bangor University by Karen Harper in candidature for the degree of Doctor of Philosophy

School of Environment, Natural Resources and Geography
Bangor University
Bangor
Gwynedd
LL57 2UW

May 2014

Abstract

Natural methods (biocontrol) for the preservation of harvested fruits and vegetables were investigated. This was in order to potentially help the industry overcome the environmental problems and pathogen resistance being encountered with the use of synthetic fungicides.

The main focus of research was the use of volatile compounds found in citrus fruit. These were utilised in *in vivo* studies with citrus fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, as well as strawberries inoculated with *Botrytis cinerea*. The extent of pathogen growth was measured after exposure to the volatiles for a number of days (7 for citrus fruit and 3 for strawberries) in environmentally-controlled containers. The 'freshness' of the fruit was also ascertained via measurements of water/weight loss. The essential oil of orange itself was highly effective at reducing *Penicillium* growth (> 30% inhibition) and water/weight loss in the fruit (> 20% reduction), as were some of its individual components - (E)-2-hexenal, neryl acetate and linalool. The aldehyde (E)-2-hexenal also had high efficacy against *B. cinerea* and reduced water/weight loss in strawberries, although in these investigations the ketone, (R)-carvone was the most effective overall (> 30% mean pathogen inhibition and > 35% mean reduction in water/weight loss).

Organic and non-organic fruit were utilised in the *in vivo* experiments and discrepancies were observed in the results for each type. *In vitro* studies were therefore conducted with the volatiles, both alone and in combination with commercial fungicides (imazalil, thiabendazole and fludioxonil) in amended-agar investigations. Aldehydes (including (E)-2-hexenal) displayed the highest efficacy against *Penicillium* species and *B. cinerea* (> 90% inhibition), followed by carvone and methyl salicylate (> 40% inhibition). These observations were repeated in the fungicide-amended-agar work, where it was shown that these volatiles could be utilised to supplement the activity of synthetic fungicides and therefore reduce their overall use within the industry.

Chitosan was also investigated as a biological coating for harvested fruit. At 2% concentrations it reduced water/weight loss in oranges compared to uncoated fruit, whilst at 0.1% it reduced *Penicillium* infection in inoculated fruit by almost 50%. Acid and enzyme hydrolysis techniques were performed to obtain chitooligosaccharides with a degree of polymerisation (DP) of 3-10, reported to possess superior antifungal properties than commercially-available chitosan. The hydrolysates were analysed via MALDI-TOF-MS. Chitooligosaccharides produced by one of the acid hydrolysis methods and one obtained using the enzyme Laminarinase were found to be of the desired DP.

Overall, the work revealed the potential of some alternative methods for maintaining the freshness and extending the storage-life of fresh fruits. A number of citrus volatiles were found to be effective at reducing pathogen growth as well as protecting the fruit from water/weight loss. They were also found to operate in parallel with synthetic fungicides, thus implying that the domination of these chemicals in the post-harvest industry could be diminished by combined applications. Chitosan as an edible coating for fresh produce has also been shown to have potential, although additional work is required to elucidate this further.

TABLE OF CONTENTS

Chapter One

Introduction

1.1: Overview	1
1.2: The Physiology of Fruits and Vegetables	2
1.2.1: Structure and Composition	2
1.2.2: Life after Harvest	3
1.3: Physical Means to Prevent Deterioration	4
1.3.1: Controlling Temperature and Relative Humidity	4
1.3.2: High Temperature Treatments	5
1.3.3: Modified Atmosphere Treatments	6
1.3.4: Ultraviolet Light	6
1.4: Chemical Means to Prevent Deterioration	8
1.5: Biological Means to Prevent Deterioration	10
1.5.1: Antagonistic Microorganisms	10
1.5.2: Natural Plant Products	12
1.5.3: Inducement of Plant Defence Mechanisms	13
1.6: Research Objectives	14

Chapter Two

Water Loss in Selected Fresh Produce and Technique Development

2.1: Introduction	17
2.2: Materials and Methods	21
2.2.1: Fresh Produce	21
2.2.2: Experimental Conditions	21
2.2.2.1: Containers	21
2.2.2.2: Relative Humidity	21
a. Dilute NaCl Solutions	22
b. Saturated Salt Solutions	22
2.2.3: Air Circulation	24
2.2.4: Data Collection and Analysis	25
2.3: Results	27
2.3.1: Weight Loss (RH regulated by dilute NaCl solutions) Days 1-28	27
2.3.2: Weight Loss of Infected Fruit (RH regulated by dilute NaCl solutions)	28
2.3.3: Weight Loss (RH regulated by saturated salt solutions in beakers) Days 29-42	29
2.3.4: Weight Loss (RH regulated by saturated salt solutions covering bases of boxes)	30
2.3.5: Weight Loss (RH regulated via different methods) Days 1-45	31
2.3.6: Weight Loss in Strawberries and Lettuce Compared to Oranges	33
2.4 Discussion	34

Chapter Three

The Effect of Volatile Compounds on Water Loss and *Penicillium* Infection in Citrus Fruits

3.1: Introduction	37
3.2 Materials and methods	40
3.2.1: Fruit material	40
3.2.2: Pathogens	40
3.2.2.1: Initial Isolation and Identification	40
3.2.2.2: Maintenance of Cultures	41
3.2.2.3: Fungal Suspensions	41
3.2.3: Chemicals	41
3.2.4: Incubation Conditions	43
3.2.5: Inoculation of Fruit	43
3.2.6: Volatile Delivery Method	43
3.2.7: Data Collection and Analysis	43
3.3 Results	45
3.3.1: Organic Oranges and Volatiles	45
3.3.2: Non-Organic Oranges and Volatiles	45
3.4 Discussion	50

Chapter Four

Effect of Citrus Volatiles on the Growth of *Penicillium digitatum* and *P. italicum* in vitro

4.1: Introduction	55
4.2: Materials and Methods	59
4.2.1: Media	59
4.2.1.1: Standard Agar	59
4.2.1.2: Amended Agar – ascertaining minimum inhibitory concentrations (MICs)	59
4.2.1.3: Amended Agar – ascertaining minimum concentrations required to inhibit 50% growth of the organisms (MIC50s)	59
4.2.2: Pathogens	60
4.2.2.1: Initial Isolation and Identification	60
4.2.2.2: Maintenance of Cultures	60
4.2.2.3: Fungal Suspensions	61
4.2.3: Chemicals	61
4.2.4: Inoculation of Media and Volatile Delivery Method	61
4.2.5: Data Collection and Analysis	62
4.3: Results	64
4.3.1: <i>Penicillium digitatum</i> and <i>P. italicum</i> and Volatiles	64
4.3.2: <i>Penicillium digitatum</i> and <i>P. italicum</i> and Amended Agar	65
4.3.3: <i>Penicillium digitatum</i> and <i>P. italicum</i> , Amended Agar and Volatiles	66
4.4: Discussion	69

Chapter Five

The Effect of Volatile Compounds on *Botrytis cinerea* Infection and Water Loss in Strawberry Fruits

5.1: Introduction	77
5.2: Materials and Methods	79
5.2.1: Fruit Material	79
5.2.2: Pathogens	79
5.2.3: Chemicals	79
5.2.4: Incubation Conditions	80
5.2.5: Inoculation of Fruit	80
5.2.6: Volatile Delivery Method	80
5.2.7: Data Collection and Analysis	80
5.3: Results	82
5.3.1: Organic Strawberries and Volatiles	82
5.3.2: Non-organic Strawberries and Volatiles	85
5.4: Discussion	88

Chapter Six

Effect of Citrus Volatiles on the Growth of *Botrytis cinerea* *in vitro*

6.1: Introduction	93
6.2: Materials and Methods	95
6.2.1: Media	95
6.2.1.1: Standard agar	95
6.2.1.2: Amended Agar – ascertaining MIC	95
6.2.1.3: Amended Agar - MIC50	95
6.2.2: Pathogens	96
6.2.2.1: Initial Isolation and Identification	96
6.2.2.2: Maintenance of Cultures	96
6.2.2.3: Fungal Suspensions	96
6.2.3: Chemicals	97
6.2.4: Inoculation of Media and Volatile Delivery Method	97
6.2.5: Data Collection and Analysis	98
6.3: Results	99
6.3.1: <i>Botrytis cinerea</i> and Volatiles	99
6.3.2: <i>Botrytis cinerea</i> and Amended Agar – MIC establishment	100
6.3.3: <i>Botrytis cinerea</i> , Amended Agar and Volatiles	101
6.4: Discussion	103

Chapter Seven

The Effects of Chitosan and/or Pectin Coatings on Orange fruit, Plus Chitosan Hydrolysis and Analysis

7.1: Introduction	107
7.2: Materials and Methods	111
7.2.1: Chemicals	111
7.2.2: Fruit material	111
7.2.3: Pathogens	111
7.2.4: Organic Oranges with Chitosan and/or Pectin Coatings	112

7.2.4.1: Preparation of Coatings	112
7.2.4.2: Incubation Conditions	112
7.2.4.3: Inoculation of Fruit	112
7.2.4.4: Volatile Delivery Method	112
7.2.4.5: Date Collection and Analysis	112
7.2.5: Chitosan Hydrolysis and Analysis	113
7.2.5.1 Mass Spectrometry	113
7.2.5.2: Acid Hydrolysis of Chitosan with Hydrogen Chloride	114
Method a.	114
Method b.	114
7.2.5.3: Enzyme Hydrolysis of Chitosan with Pepsin	115
7.2.5.4: Enzyme Hydrolysis of Chitosan with Amano Lipase A	115
7.2.5.5: Enzyme Hydrolysis of Chitosan with Laminarinase	116
7.3: Results	117
7.3.1 The Effects of Chitosan Coatings on Weight/Water Loss in Orange Fruit	117
7.3.2: The Effects of Chitosan Coatings on Weight/Water Loss and <i>Penicillium</i> Infection in Orange Fruit	118
7.3.3: The Effects of Chitosan or Pectin Coatings Plus Volatile Compounds on Weight/Water Loss and <i>Penicillium</i> Infection in Orange Fruit	119
7.3.4: MALDI-TOF-MS Analyses	123
7.3.4.1: Inulin and Commercial Chitosan	123
7.3.4.2: Chitosan Oligosaccharides Produced by Acid Hydrolysis	126
7.3.4.3: Chitosan Oligosaccharides Produced by Enzyme Hydrolysis	133
7.4: Discussion	141
Chapter Eight	
General Discussion and Conclusions	147
Chapter Nine	
References	157
Appendices	177

List of Figures

Figure 2.01: Container used to analyse water loss from fresh produce (oranges illustrated)	26
Figure 2.02: Weight loss of oranges incubated for 9 days at 20°C and 94% RH (unstirred)	28
Figure 2.03: Rates of weight/water loss of oranges incubated at 20°C and various RH conditions maintained by saturated salt solutions poured directly into the experimental boxes (stirred)	30
Figure 2.04: Weight/water loss of oranges over the course of 45 days, illustrating how the introduction of saturated salt solutions (for RH regulation) for all treatments (after 28 days) and the introduction of an air circulation system for treatments at 20°C (after 35 days) affected the rates of weight/water loss in the fruit	32
Figure 2.5: Weight/water losses of three different fresh commodities after three days of incubation at the same temperature (20°C) and various RH levels	33
Figure 2.06: Containers set up in a (dark) controlled-temperature room	34
Figure 3.01: Effects of volatile compounds on the infection of <i>P. digitatum</i> and <i>P. italicum</i> on organic oranges compared to untreated fruit	46
Figure 3.02: Effects of volatile compounds on the weight/water loss of organic oranges inoculated with <i>P. digitatum</i> and <i>P. italicum</i> compared to untreated fruit	47
Figure 3.03: Effects of volatile compounds on the infection of <i>P. digitatum</i> and <i>P. italicum</i> on non-organic oranges compared to untreated fruit	48
Figure 3.04: Effects of volatile compounds on the weight/water loss of organic oranges inoculated with <i>P. digitatum</i> and <i>P. italicum</i> compared to untreated fruit	49
Figure 4.01: Petri dishes containing samples from <i>in vitro Penicillium</i> and volatiles assay showing inoculated PDA plates with slow-release packets	63
Figure 4.02: Effects of volatile compounds on the growth of <i>P. digitatum</i> and <i>P. italicum in vitro</i>	64
Figure 4.03: Effects of different concentrations (0.001, 0.01, 0.1, 1.0, 10 and 100 µg/ml; converted to log10 values) of PacRite® Fungaflor 500EC (IMZ 50), PacRite® Fungaflor 75WSG (IMZ 75) and Shield-Brite® TBZ on the growth of <i>P. digitatum</i> and <i>P. italicum in vitro</i>	65
Figure 4.04: Effects of volatile compounds on the growth of <i>P. digitatum</i> and <i>P. italicum</i> on PDA amended with Shield-Brite® TBZ fungicide at MIC50	66
Figure 4.05: Effects of volatile compounds on the growth of <i>P. digitatum</i> and <i>P. italicum</i> on PDA amended with PacRite® Fungaflor 500EC (IMZ 50) fungicide at MIC50	67
Figure 4.06: Effects of volatile compounds on the growth of <i>P. digitatum</i> and <i>P. italicum</i> on PDA amended with PacRite® Fungaflor 75WSG (IMZ 75) fungicide at MIC50	68
Figure 5.01: Effects of different volatile compounds on the infection of <i>B. cinerea</i> on organic strawberries compared to untreated fruit	82
Figure 5.02: Effects of volatile compounds on the weight/water loss of organic strawberries inoculated with <i>B. cinerea</i> compared to untreated fruit	83
Figure 5.03: Effects of volatile compounds on the infection of <i>B. cinerea</i> on non-organic strawberries compared to untreated fruit	86

Figure 5.04: Effects of volatile compounds on the weight/water loss of non-organic strawberries inoculated with <i>B. cinerea</i> compared to untreated fruit	87
Figure 6.01: Effects of volatile compounds on the growth of <i>B. cinerea</i> <i>in vitro</i>	99
Figure 6.02: Effects of different concentrations (0.01, 0.1, 1.0, 10 and 100 µg/ml; converted to log10 values) of Geox 50 WG fungicide (FLU) on the growth of <i>B. cinerea</i> <i>in vitro</i>	100
Figure 6.03: The effects of volatile compounds on the growth of <i>B. cinerea</i> on PDA amended with Geox 50 WG fungicide (FLU) at MIC50	102
Figure 7.01: Weight loss of oranges over the course of 14 days when coated in different concentrations of chitosan and stored at 5 and 20°C	117
Figure 7.02: Weight loss in oranges treated with different concentrations of chitosan and subsequently infected (or not) with <i>P. digitatum</i> and <i>P. italicum</i>	118
Figure 7.03: Effects of chitosan and pectin coatings of different concentrations plus volatile compounds on <i>Penicillium</i> infection in organic oranges compared to untreated fruit	121
Figure 7.04: Effects of chitosan and pectin coatings of different concentrations plus volatile compounds on the weight/water loss of organic oranges inoculated with <i>P. digitatum</i> and <i>P. italicum</i> compared to untreated fruit	122
Figure 7.05: Mass spectrum of inulin produced via MALDI-TOF-MS	123
Figure 7.06: Mass spectrum of inulin trimer produced by MALDI-TOF-MS.	124
Figure 7.07: Mass spectrum of inulin tetramer produced by MALDI-TOF-MS.	124
Figure 7.08: Mass spectrum of commercial chitosan obtained from Sigma (U.K) not subjected to hydrolysis	125
Figure 7.09: Mass spectrum of OT52 sample produced by acid hydrolysis method a., illustrating oligomers of DP3 to DP7.	127
Figure 7.10: Mass spectrum of OT53 1st fraction sample illustrating oligomers of DP3 to DP7. All assigned peaks are M + Na+	128
Figure 7.11: Mass spectrum of OT53 2nd fraction sample illustrating oligomers of DP3 to DP8. All assigned peaks are M + Na+	128
Figure 7.12: Mass spectrum of OT52 produced by acid hydrolysis method a., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer	129
Figure 7.13: Mass spectrum of OT53 1st fraction produced by acid hydrolysis method b., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer	130
Figure 7.14: Mass spectrum of OT53 2nd fraction produced by acid hydrolysis method b., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer	130
Figure 7.15: Mass spectrum of OT52 produced by acid hydrolysis method b., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer	132

- Figure 7.16:** Mass spectrum of OT53 1st fraction produced by acid hydrolysis method b., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer 132
- Figure 7.17:** Mass spectrum of OT53 2nd fraction produced by acid hydrolysis method b., highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer 133
- Figure 7.18:** Mass spectrum of OT54 produced by enzyme hydrolysis with Pepsin, illustrating oligomers of DP1 to DP3 134
- Figure 7.19:** Mass spectrum of OT55 produced by enzyme hydrolysis with Amano Lipase A, illustrating oligomers of DP2 to DP8 134
- Figure 7.20:** Mass spectrum of OT56 1st fraction produced by enzyme hydrolysis with Laminarinase, illustrating oligomers of DP3 to DP8 135
- Figure 7.21:** Mass spectrum of OT56 2nd fraction produced by enzyme hydrolysis with Laminarinase, illustrating oligomers of DP3 to DP9. 135
- Figure 7.22:** Mass spectrum of OT55 produced by enzyme hydrolysis with Amano Lipase A, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer. 137
- Figure 7.23:** Mass spectrum of OT56 1st fraction produced by enzyme hydrolysis with Laminarinase, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer. 138
- Figure 7.24:** Mass spectrum of OT56 2nd fraction produced by enzyme hydrolysis with Laminarinase, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan tetramer. 138
- Figure 7.25:** Mass spectrum of OT55 produced by enzyme hydrolysis with Amano Lipase A, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer 139
- Figure 7.26:** Mass spectrum of OT56 1st fraction produced by enzyme hydrolysis with Laminarinase, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer 140
- Figure 7.27:** Mass spectrum of OT56 2nd fraction produced by enzyme hydrolysis with Laminarinase, highlighting the different combination of glucosamine (GlcN) and acetylated glucosamine (GlcNAc) units of the chitosan trimer 140

Appendix

Figure 7.1.1: Mass List of Inulin	177
Figure 7.1.2: Mass List of Commercial Chitosan	179
Figure 7.1.3: Mass List of OT52	181
Figure 7.1.4: Mass List of OT53 First Fraction	182
Figure 7.1.5: Mass List of OT53 Second Fraction	183
Figure 7.1.6: Mass List of OT54	184
Figure 7.1.7: Mass List of OT55	186
Figure 7.1.8: Mass List of OT56 First Fraction	187
Figure 7.1.9: Mass List of OT56 Second Fraction	189

List of tables

Table 2.01: Treatments and RH values obtained with dilute NaCl solutions	22
Table 2.02: Saturated salt solutions – expected RH values	22
Table 2.03: Saturated salt solutions in beakers – expected RH values (Greenspan, 1977) versus measured Values	23
Table 2.04: Saturated salt solutions in beakers combined with the air circulation system – measured RH values	24
Table 2.05: Treatments used on one batch of oranges, showing the development of the techniques to regulate RH	25
Table 2.06: Saturated salt solutions covering base of boxes – measured RH values	25
Table 2.07: Cumulative weekly mean percentage weight loss of oranges incubated at 5 & 20°C with RH maintained by NaCl solutions (unstirred)	27
Table 2.08: Cumulative weekly mean percentage weight/water loss of oranges at 5 & 20°C with RH maintained by the saturated salt solutions	29
Table 3.01: List of volatile chemical compounds used throughout research	42
Table 8.01: Summary of the effects of volatile compounds on <i>Penicillium sp.</i> <i>in vitro</i> and <i>in vivo</i> , plus water/weight loss in oranges and mandarins.	148
Table 8.02: Summary of the effects of volatile compounds on <i>B. cinerea</i> <i>in vitro</i> and <i>in vivo</i> , plus water/weight loss in strawberries.	152

Acknowledgements

I would like to like to acknowledge the following people:

My supervisors, Professor Deri Tomos, Dr Mike Hale and Dr Radek Braganca, as well as my company supervisor Dr Owen Jones. Thank you for all your support and guidance, all the time that you have spent with me, and all your encouragement and expertise. This work would not have been possible without you.

KESS for funding the Ph.D. project and the BioComposites Centre for allowing me to use their facilities. I would particularly like to thank Dr Olga Tverezovskaya and Dr Viacheslav Tverezovskaya for their help with the chitosan hydrolyses.

My good friend and fellow Ph.D. student, (the now Dr) Rosie Anthony for being there with support, laughs and cake. Also my amazing friend Emma Fenwick Newcomb, who believed in me and kept me going through thick and thin, mostly with coffee and calamitous adventures.

My wonderful partner, Andy Maudsley and all my family, particularly my children Claire, John, Donna, Tara and Warren, my brother Kevin, and my Mum. You've always believed I could succeed, even when I didn't, and for that I shall always be grateful.

Lastly, I would like to dedicate this to my late father, Alexander Harper, whom I miss so very much. I know you'd be proud, Dad.

Declaration and Consent

Details of the Work

I hereby agree to deposit the following item in the digital repository maintained by Bangor University and/or in any other repository authorized for use by Bangor University.

Author Name:

.....

Title:

.....

.....

Supervisor/Department:

.....

Funding body (if any):

.....

Qualification/Degree obtained:

.....

This item is a product of my own research endeavours and is covered by the agreement below in which the item is referred to as “the Work”. It is identical in content to that deposited in the Library, subject to point 4 below.

Non-exclusive Rights

Rights granted to the digital repository through this agreement are entirely non-exclusive. I am free to publish the Work in its present version or future versions elsewhere.

I agree that Bangor University may electronically store, copy or translate the Work to any approved medium or format for the purpose of future preservation and accessibility. Bangor University is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Bangor University Digital Repository

I understand that work deposited in the digital repository will be accessible to a wide variety of people and institutions, including automated agents and search engines via the World Wide Web.

I understand that once the Work is deposited, the item and its metadata may be incorporated into public access catalogues or services, national databases of electronic theses and dissertations such as the British Library’s EThOS or any service provided by the National Library of Wales.

I understand that the Work may be made available via the National Library of Wales Online Electronic Theses Service under the declared terms and conditions of use (<http://www.llgc.org.uk/index.php?id=4676>). I agree that as part of this service the National Library of Wales may electronically store, copy or convert the Work to any approved medium or format for the purpose of future preservation and accessibility. The National Library of Wales is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Statement 1:

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless as agreed by the University for approved dual awards.

Signed (candidate)

Date

Statement 2:

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

All other sources are acknowledged by footnotes and/or a bibliography.

Signed (candidate)

Date

Statement 3:

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loan and for electronic storage (subject to any constraints as defined in statement 4), and for the title and summary to be made available to outside organisations.

Signed (candidate)

Date

NB: Candidates on whose behalf a bar on access has been approved by the Academic Registry should use the following version of **Statement 3:**

Statement 3 (bar):

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loans and for electronic storage (subject to any constraints as defined in statement 4), after expiry of a bar on access.

Signed (candidate)

Date

Statement 4:

Choose **one** of the following options

a) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University and where necessary have gained the required permissions for the use of third party material.	
b) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University when the approved bar on access has been lifted.	
c) I agree to submit my thesis (the Work) electronically via Bangor University's e-submission system, however I opt-out of the electronic deposit to the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University, due to lack of permissions for use of third party material.	

Options B should only be used if a bar on access has been approved by the University.

In addition to the above I also agree to the following:

1. That I am the author or have the authority of the author(s) to make this agreement and do hereby give Bangor University the right to make available the Work in the way described above.
2. That the electronic copy of the Work deposited in the digital repository and covered by this agreement, is identical in content to the paper copy of the Work deposited in the Bangor University Library, subject to point 4 below.
3. That I have exercised reasonable care to ensure that the Work is original and, to the best of my knowledge, does not breach any laws – including those relating to defamation, libel and copyright.
4. That I have, in instances where the intellectual property of other authors or copyright holders is included in the Work, and where appropriate, gained explicit permission for the inclusion of that material in the Work, and in the electronic form of the Work as accessed through the open access digital repository, *or* that I have identified and removed that material for which adequate and appropriate permission has not been obtained and which will be inaccessible via the digital repository.
5. That Bangor University does not hold any obligation to take legal action on behalf of the Depositor, or other rights holders, in the event of a breach of intellectual property rights, or any other right, in the material deposited.

6. That I will indemnify and keep indemnified Bangor University and the National Library of Wales from and against any loss, liability, claim or damage, including without limitation any related legal fees and court costs (on a full indemnity bases), related to any breach by myself of any term of this agreement.

Signature:

Date :

Abbreviations

ANOVA	Analysis of variance
C	Carbon
CI	Chilling injury
CO ₂	Carbon dioxide
COS	Chitooligosaccharides
DA	Degree of acetylation
DP	Degree of polymerization
FLU	Fludioxonil
FSA	Food Standards Agency
GlcN	Glucosamine
GlcNAC	N-acetylglucosamine
H ₂ O	Water
HCl	Hydrogen chloride
HMW	High molecular weight
IMZ	Imazalil
K	Potassium
K ₂ SO ₄	Potassium sulfate
KC ₂ H ₃ O ₂	Potassium acetate
KC	Potassium chloride
LMW	Low molecular weight
MeJ	Methyl jasmonate
MeS	Methyl salicylate
MgCl ₂	Magnesium chloride
MIC	Minimum inhibitory concentration
m/z	Mass-to-charge ratio
NaCl	Sodium chloride
NaOH	Sodium hydroxide
(NH ₄) ₂ SO ₄	Ammonium sulfate
O ₂	Oxygen
P	Phosphorous
<i>P. italicum/digitatum</i>	Penicillium.....
PD	Potato dextrose agar
PP	Polypropylene
RH	Relative humidity
TBZ	Thiabendazole
VPD	Vapour pressure deficit

