PLEASE DO NOT BIND OR STAPLE THIS FORM OR ANY ATTACHMENTS. SECURE PAPERS TOGETHER WITH A BULLDOG CLIP.

COMPLETION OF THE CORE COMPONENT OF THE STRUCTURED PROGRAM

(*Delete where appropriate)

Student Name: LE Duc Thang  
Id No: 1200275

E-mail address: dule@adelaide.edu.au

Faculty: Science  
School/Discipline: Agriculture, Food and Wine

Program: PhD  
Candidature Commencement Date: 28/12/2010

Supervisors:

<table>
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<tr>
<th>Supervisor’s Name (in full)</th>
<th>Principal (P) / Co-(C) / External (E)</th>
<th>Discipline, School</th>
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</thead>
<tbody>
<tr>
<td>Amanda Pike</td>
<td>P</td>
<td>Agriculture, Food &amp; Wine</td>
</tr>
<tr>
<td>Eileen Scott</td>
<td>C</td>
<td>Agriculture, Food &amp; Wine</td>
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<tr>
<td>Glenn MacDonald</td>
<td>C</td>
<td>Agriculture, Food &amp; Wine</td>
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A. FINANCIAL IMPLICATIONS

It is possible that in addition to the basic infrastructure support for your research, your project may involve fieldwork or research outside the University (local/interstate, or overseas). You may require special facilities such as inter-library loans, or access to specific materials/facilities/data.

1. With this in mind, what are the estimated total costs of your research?

$4000 p.a.

2. To what extent, if any, will your School contribute to these costs?

$2000 p.a. plus a further $2000 p.a. upon application to the School postgraduate committee.

NOTE: It is your responsibility to arrange for funding for the research, or to bear any costs not covered by funding from your School.
### B. ACCESS TO RESTRICTED DATA AND/OR MATERIALS

1. Does this research require access to restricted materials/information?
   - YES
   - NO [✓]

   (If "NO", proceed to Section C.)

2. Has official permission been granted for the use of these materials/information?
   - YES
   - NO

3. Does the use of these materials/information restrict the publication of your thesis?
   - YES
   - NO

4. Is there an expectation of an embargo being placed on your thesis when it has been examined?
   - YES
   - NO

   (If "YES", you must lodge a written application with the Graduate Centre for an embargo on your thesis well in advance of your submission.)

### C. ETHICS AND SAFETY CLEARANCE

1. Does the proposed research involve use of animals, recombinant DNA or use of teratogens?
   - YES
   - NO [✓]

2. Does the proposed research involve use of human subjects?
   - YES [✓]
   - NO

   If "YES", ethics clearance is compulsory. Please attach copy of relevant documentation.

   Indicate below which of the following clearances have been approved and attach a copy of the clearances and/or documents.

   - Animal Ethics Committee
   - Biohazards Committee
   - Human Ethics Committee
   - Biosafety Committee

   If clearance has not been obtained, please enclose a statement giving the reasons why this is so and an indication of when the clearance can be obtained. Completion of the Core Component will not be approved until the required clearance(s) have been provided.

   Please note: Application for ethical clearance is to be made in the name of the principal supervisor with the student involved also specified.

   (i) Candidates whose research involves the use of animals must obtain approval in advance from the University of Adelaide Animal Ethics Committee. Candidates located within other institutions also require ethical clearance from the AEC at the place where research is to be conducted. Please refer to the AEC website for information on requirements: http://www.adelaide.edu.au/research/ethics/animal

   (ii) Candidates whose research involves human subjects must obtain ethical approval in advance from the University of Adelaide Human Research Ethics Committee unless the following applies. For a research proposal submitted by a member of its clinical staff (including any postgraduate research candidates) the University accepts protocol approval granted by the Ethics Committees of the Royal Adelaide Hospital, the Queen Elizabeth Hospital or the Women’s and Children’s Hospital. However, for candidates whose research is conducted (a) at these hospitals and is not supervised by a member of the University’s clinical staff, or (b) at any other institution, ethical clearance must be obtained in advance from the University of Adelaide Human Research Ethics Committee as well as from the committee at the place where the research is to be conducted. Please refer to the Committee’s web site for information on requirements: http://www.adelaide.edu.au/research/ethics/human/
You and your supervisor(s) are required to complete the Higher Degree by Research: Safety Management Plan. This may be found at: http://www.adelaide.edu.au/hr/ohs/docs/higher_degree_res_safety_mgmt_plan.doc

Have you and your supervisor discussed and arrived at a mutual agreement regarding authorship in the event that there are publications resulting from your Higher Degree Research work, in accordance with the policy and guidelines set out in the Research Student Handbook?

YES ☐ NO ☐

Comment: Where the student has done the bulk of the work/writing, they will be listed as first author. Principal supervisor will be listed as last author while co-supervisors will be listed according to their contribution, where others may contribute to the work, their listing will be negotiated.

Is this project likely to generate intellectual property, which has the potential for commercial development?

YES ☐ NO ☒

Does the University itself or an outside funding body have an interest under any legal contract or arrangement?

YES ☐ NO ☒

(Note to both questions, proceed to Section G. If "YES" to either questions, please answer Questions 3, 4, 5 & 6.)

- If "Yes" and you did not sign a Student Project Participation Agreement (SPPA) on your enrolment please forward signed SPPA (in triplicate) to the Graduate Centre with this form.
- Please note that the approval of the proposed research is dependent upon the signing of this Agreement (where applicable).

3a. Have attempts been made by your supervisor(s) to obtain external funding?

YES ☐ NO ☒

Comment: But in-kind contribution (capsicum) has been provided by seminis seeds.

3b. Are funds available for the establishment of a scholarship or supplementary scholarship to assist you in your research?

YES ☐ NO ☒

Comment: ________________________________

____________________________

____________________________

____________________________

____________________________
4. Are you in an employment relationship with an outside organisation?

YES ☐ NO ☒

4.1 If "YES", have the intellectual property implications of this relationship been appropriately dealt with by your supervisors and School?

YES ☐ NO ☒

5. Please indicate below if you, your supervisor(s), and/or School have signed a commercial and/or confidentiality agreement or contract with any organisation or funding body relating to this research project.

YES ☐ NO ☒

If "YES", you must enclose a copy of the agreement/contract with this form.

6. Please indicate below if your supervisors and other interested parties have signed and attached the relevant 'Lift Embargo' forms.

YES ☐ NO ☒

G. MINIMUM RESOURCES PROFORMA

This proforma indicates that the following minimum resources will be available to the above-mentioned student for use relating to his/her postgraduate research candidature. Resource entitlements will be reviewed at the time of the annual review of progress.

Minimum Resources (includes HDR students working at remote locations)

- Regular and planned access to quality panel supervision for the duration of candidature as defined in the Research Student Handbook
- Access to computing facilities appropriate to the needs of the candidate's research
- Unrestricted Internet access for research purposes at no cost to the student
- Full access to Library resources and services
- The right to input into a Discipline’s book purchases and journal subscriptions
- Access to shared Discipline office resources for research-related purposes including (i) a fax, (ii) a photocopier, (iii) a pigeon hole and (iv) mail
- 24-hour access to a shared postgraduate study area that meets the Occupational Health and Safety requirements for lighting, noise and ventilation
- Individual desk or study carrel with an ergonomically sound chair and two-drawer filing cabinet located in an area that meets the Occupational Health and Safety requirements
- Access to a shared telephone for research-related purposes
- Adequate laboratory space and bench space (if applicable)
- Access to a parking permit (if student is on a remote campus)
- Access to a Discipline vehicle where necessary and where the Discipline is satisfied that the student has an appropriate licence and sufficient experience - but no licence (learn only)
If it is not possible to guarantee the minimum resources listed above, please indicate below (a) what alternative arrangements have been made and (b) the likely effect on the student’s progress. Attach a separate sheet if necessary.

A fixed amount to be agreed between the student and Discipline at the commencement of candidature ($pa) (may include travel, conference and workshop attendances, photocopying, printing and binding of thesis, attending courses/training as part of Structured Program, inter-library loans, database searches etc)

<table>
<thead>
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<th>Access to Discipline Research Infrastructure*</th>
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<tr>
<td>Please indicate below the major items of equipment/facilities that the student will have access to in the host discipline and the level of access that can be expected:</td>
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<th>Equipment/Facility</th>
<th>Level of Access/Comment</th>
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<tr>
<td>In the event that access to specific equipment and facilities not available in the discipline has been arranged, please list the name and location of the equipment/facility and the level of access the student can expect:</td>
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<td>E.g. Electron Microscope</td>
<td>CEMMSA</td>
<td>Twice per week</td>
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* Please attach a separate sheet to the Minimum Resources Proforma section for any additional comments
We recommend

✓ APPROVAL OF COMPLETION OF THE CORE COMPONENT OF THE STRUCTURED PROGRAM

The student has:

✓ completed the Core Component of the Structured Program (including the Integrated Bridging Program, if applicable);
✓ completed and attached the research proposal;
✓ completed the Minimum Resources Proforma;
✓ attached the necessary ethics clearance;
✓ attached the completed Higher Degrees by Research: Safety Management Plan and,
✓ agreed to a program of professional development and skills training as part of the Development Component of the Structured Program and that details for the next twelve months are attached.

N.B. By approving Completion of the Core Component of the Structured Program, the Head of School is also certifying that s/he authorized the provision of the facilities listed in the Minimum Resources Proforma section on the understanding that the facilities listed will be sufficient to meet the resource needs of this candidature.

☐ CONVERSION TO THE APPROPRIATE MASTER - Specify the Master

In this instance, details of unsatisfactory progress should be attached and accompanied by a completed "Transfer from a PhD to a Masters Application". A copy of this form may be obtained from the web at: http://www.adelaide.edu.au/graduatecentre/policy or upon request from the Adelaide Graduate Centre.

☐ TERMINATION OF CANDIDATURE

Please attach a detailed report of unsatisfactory progress to support the recommendation:

ENDORSEMENT OF SCHOOL RECOMMENDATION

Principal Supervisor's signature

Date

Head of School's signature

Date

Postgraduate Coordinator's signature

Date

Deputy Head of School's signature†

Date

Senior School/Academic Staff Member's signature†

Date

STUDENT'S CERTIFICATION AND ASSESSMENT OF PROGRESS

✓ All material in the enclosed research material is my own work except where there is clear acknowledgement and reference to the work of others. I have read the University Policy and Guidelines on Plagiarism (http://www.adelaide.edu.au/policies/230/) and give permission for my work to be evaluated from plagiarism if required. I also acknowledge that plagiarism associated with RESEARCH will be dealt with under the Guidelines and Rules for Responsible Practice in Research which are available on the web at: http://www.adelaide.edu.au/policies/96

✓ I agree/disagree with the School's assessment of my progress.

In the case of disagreement with the School's recommendation, please attach details separately.

✓ I acknowledge that I am required to conduct my research according to the requirements of The Australian Code for the Responsible Conduct of Research and that it is a requirement that I lodge all the original data or primary research materials with the University or my Principal supervisor. I recognize that if I fail to do so, I will be in breach of The Code and my thesis will not be accepted for examination.

Student's signature

Date

Page 9 of 10
Student Name: L E Dae Mong
Student ID Number: 1208275

APPROVAL BY HIGHER DEGREES COMMITTEE CONVENOR († where applicable)

I approve/do not approve the recommendation:

- [ ] completion of the Core Component of the Structured Program be confirmed
- [ ] conversion to the appropriate Master
- [ ] candidature be terminated

I wish to make the following comments:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Signature Convenor †† Date

APPROVAL BY DEAN OF GRADUATE STUDIES (†† where conversion to Master/candidature termination recommended)

I approve/do not approve the recommendation.

________________________________________________________________________

Signature Dean of Graduate Studies†† Date
**HIGHER DEGREE BY RESEARCH : SAFETY MANAGEMENT PLAN**

**TO BE COMPLETED BY THE STUDENT & SUPERVISOR IN CONSULTATION**

**NOTE: ONLY THE FIRST PAGE MUST BE COMPLETED AND SUBMITTED TO THE ADELAIDE GRADUATE CENTRE**

If further information related to the Hazard Id Form is required please consult with your local Health and Safety Officer or HSW Manager.

<table>
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<th>LE Duc Thong</th>
<th>Date</th>
<th>6th August 2010</th>
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<tbody>
<tr>
<td>Student Number</td>
<td>1200275</td>
<td>Supervisor</td>
<td>Amanda Abke</td>
</tr>
<tr>
<td>School</td>
<td>Agriculture, Food and Wine</td>
<td>Room no</td>
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<tr>
<td>Contact Details</td>
<td>Email: <a href="mailto:duc-le@adelaide.edu.au">duc-le@adelaide.edu.au</a></td>
<td>Mobile/Phone</td>
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</tr>
</tbody>
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**HAZARD IDENTIFICATION FORM**

(In discussion with your supervisor identify any foreseeable hazards associated with your project by ticking the appropriate boxes. Hazards identified will need to be addressed on the Hazard Management Log - see page 2)

**Physical/Environmental Hazards**
- Remote areas/isolated locations
- Confined space (e.g. pit, tank, silo, entry through a hatch)
- Fall from a height (e.g. ladder, elevated platform, cliff)
- Noise > 85dB(A)
- Temperature or weather extremes (e.g. heat, cold, wind, rain)
- Working on a slippery or uneven surfaces
- Poor lighting/visibility
- Hit by flying, moving or falling object (including vehicles)
- Fire hazards/naked flame other than controlled lab work
- Vibrating objects or surface

**First Aid/Evacuation**
- Additional first aid requirements due to the nature of the research (e.g. field work or specific hazards)
- A rescue effort would be difficult in the event of an emergency (e.g. remote areas, difficult to access to work site)

**Communications**
- Potential for communication problems (e.g. by virtue of location)
- Research may disrupt/impact on adjacent areas of the University

**Electrical**
- Electrical risk from portable electrical appliances or tools
- Water and electricity in close proximity to each other

**Ergonomics/Manual Handling**
- Working for long periods in a sustained awkward posture
- Required to lift, push or pull heavy objects

**Stress/Duress**
- Personal security (e.g. physical harm by others)
- Stressful situations
- Working alone/home visits
- Working hours (e.g. long shifts required)

**Plant and Equipment**
- Mobile plant will be introduced (e.g. forklift, mobile platform)
- Scaffolding, raised platforms or ladders
- Pressurised vessels/systems (e.g. autoclave, boiler)
- Hazardous plant (e.g. lathes, lasers, microtomes, cryostats, etc)

**Radiation**
- Sealed sources
- Unsealed sources
- Other (e.g. UV)

**Biological**
- Contamination (i.e. virus, bacteria, disease)
- Animals (including fish, birds or insects that may introduce a hazard e.g. handling, bite, sting, venom)
- Human or animal body fluids/tissue
- Needles/matrix bands
- Surgical Instruments
- AQIS/OGTR

**Chemical**
- Explosive substances
- Flammable gas
- Toxic or asphyxiant gas (e.g. CO₂ including dry ice, liquid N₂)
- Highly flammable liquid (packing Group 1)
- Respiratory irritants (e.g. nanotech, asbestos, dust)
- Chemical spraying (e.g. agriculture, pest)
- Hazardous substances (not included above)

**Other**

- 
- 
- 

- No hazards identified with this research

**Student's signature**

**Supervisor's signature**

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S:\Services\Resources\Human_Resources\Health Safety and Wellbeing\Tools, Templates and Resources\Current Blank Templates and Forms\Higher Degree by Research Safety Management Plan (31 July 09)
RESEARCH PROPOSAL

EFFECT OF CALCIUM (Ca) AND BORON (B) NUTRITION ON GREY MOULD OF CAPSICUM (Capsicum annuum L.) AND FRUIT QUALITY

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<tr>
<td>Name</td>
<td>Le Duc Thong</td>
</tr>
<tr>
<td>Principal supervisor</td>
<td>Dr Amanda J. Able</td>
</tr>
<tr>
<td>Research group</td>
<td>Plant Protection</td>
</tr>
<tr>
<td>External advisor</td>
<td>James Stangoulis</td>
</tr>
</tbody>
</table>

School of Agriculture, Food and Wine
Faculty of Sciences
The University of Adelaide
August 2010
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1. Project title

EFFECT OF CALCIUM (Ca) AND BORON (B) NUTRITION ON GREY MOULD OF CAPSICUM (Capsicum anuum L.) AND FRUIT QUALITY

2. Project summary

The capsicum fruit (Capsicum annum L.), also called pepper, is a warm-season crop that is a member of the Solanaceae family. Capsicum fruit is the major source of red food colourant and pungency for spice production and can also have medicinal uses [Food and Agricultural Organization of United State (FAO), 2009]. The world capsicum production has steadily increased by 25% from 2000 to 2008 with over 27.87 million tons (Mt) and 1.8 million hectares of cultivation (FAO, 2009). Capsicum fruit are susceptible to several fruit rots including grey mould caused by the fungus Botrytis cinerea Pers. (teleomorph: Botryotinia fuckeliana (de Bary) Whetzel). B. cinerea also causes grey mould disease in a wide range of economically important plants, and it has traditionally been considered as a non-specialised necrotrophic fungus that multiplies on debris of a broad range of plant species. However, its main impact occurs postharvest, making fruit unmarketable (Utkhede and Mathur, 2003).

Botrytis cinerea causes serious loss in more than 200 crops worldwide (Williamson et al., 2007). B. cinerea is therefore a significant concern for growers and sellers in many countries. Fungicide application has been the most commonly used method to kill fungal pathogens in plants. However, fungicides may be toxic for health and may damage the environment. Alternative means of control such as nutrient application to plants are now considered to be a safer solution. This is not only to improve plant growth and fruit quality but also to control diseases (Fallahi et al., 1997; Hansch and Mendel, 2009).

Calcium (Ca) and Boron (B) are critical nutrients for plant growth, fruit quality and disease resistance. Adequate Ca in plants has been demonstrated to increase cell wall strength and thickness, while the role of boron in maintaining the structural integrity of plant membranes is well known (Hewett, 2006; Sams, 1999). There are some studies
describing the effects of these nutrients on disease development after harvest in plants and in fruits, such as bean and tomatoes (Elad and Volpin, 1993), table grape (Amiri et al., 2009) and strawberry (Singh et al., 2007; Wójcik and Lewandowski, 2003). However, there is a lack of understanding about the role of Ca and B in grey mould development in capsicum. Therefore, this research project will study the effect of Ca and B nutrition on grey mould development and fruit quality in capsicum.

3. Project details

3.1 Literature review

3.1.1 Capsicum

Capsicums (Capsicum annuum L.) belong to the Solanaceae family and originated from South and Central America (Burt, 2005). Capsicums are primarily classified as one of three cultivar types: capsicum, paprika and chilli. Capsicum is a sweet non-pungent fruit often called bell pepper and used for fresh consumption. Paprika is non-pungent and used to produce spice, while chillies are smaller and hotter than non-pungent capsicum (Bosland et al. 1996 in Klieber 2000) and are used in both spice and sauces (Rajput and Paruleke, 1998).

Capsicums are planted world-wide and the production has increased steadily from 24.31 Mt in 2003 to 27.87 Mt in 2008 with international trade being valued at approximately US$2.3 billion (FAO, 2009). Capsicums normally grow in warm conditions with 16°C to 21°C on average being best for fruit setting, high yields and good quality fruit (Burt, 2005). Asia produces two-thirds of the world’s production (18.57 Mt) (FAO, 2009). Capsicums can be grown on many different kinds of soil with a wide range of pH (5 - 9), but its optimum is well-drained soil at a pH of 5.5 to 6.5 (Burt, 2005).

Capsicum is self-pollinating and the capsicum fruit are normally mature (fully grown and light green in colour) approximately 30 to 35 days after anthesis, with fruit taking a further 20 to 25 days to turn red depending on season (Burt 2005; Rajput, 1998). Harvesting at the light green stage affects fruit quality because fruit colour does not change properly to red while fruit harvested at the “breaker” stage (40% to 50% red surface colouration) will normally ripen and develop colour sufficiently (Pham, 2007). Postharvest changes in fruit will be discussed in the next section.
3.1.2 Postharvest behaviour of capsicum

Based on their postharvest physiology and biochemical changes, fruit have been divided into two groups: climacteric and non-climacteric fruit (Biale and Young, 1981). In climacteric fruit, respiration peaks before fruit ripening and this is associated with significant changes in sugar content, colour and texture. Ethylene production also increases sharply to reach a peak and remains at a relatively high rate throughout the ripening period (Rhodes, 1980). The autocatalytic activity of ethylene plays an important role to coordinate and complete the ripening process of climacteric fruit (Giovannoni, 2001). In contrast, there is no peak in respiration or ethylene production in non-climacteric fruit and ripening processes are not sensitive to ethylene. Capsicum is difficult to classify as either climacteric or non-climacteric due to variation amongst cultivars and species. Saltveit (1997) did not consider capsicums to be climacteric because there was a lack of the typical increase in respiration and ethylene production during ripening. Pham (2007) also reported limited ethylene production in the paprika-type cultivar (cv.) Papri Queen and the bell pepper Aries. However, even though bell pepper from cv. Maor at the green and red stages had a significant increase in ethylene level, there was very low respiration during storage (Lurie et al., 1986). The chilli from cv. Changjiao did not respond to ethylene and fruit respiration production did not increase during ripening (Lu et al., 1990).

Changes in colour, firmness and sugar content during ripening of capsicum fruit on the plant have also been widely investigated. Chlorophyll content commonly decreases and is absent from tissues when the fruit turns fully red (Mendez and Mosquera, 2002). Associated with the colour changes, firmness declined due to modification of the fruit cell walls. These changes are caused by the increasing activity of hydrolase enzymes including polygalacturonase (PG) and pectinesterase (PE) (Priya Sethu et al., 1996). The hydrolase enzymes, especially PG, are reported to be absent or inactive when fruit are not ripe, but to have high activities when fruit ripen (Harpster et al., 1997). During ripening, the neutral sugars decreased dramatically from 25% initially (first day of harvest) to 15% (21st day of harvest) (Priya Sethu et al., 1996). The change in total soluble solid content (TSSC) in capsicum fruit also depends on ripening stages (Pham,
The TSSC of fruits which ripened on the plant increased greatly and reached a peak when fruit turned full red and was higher than for fruits harvested at deep and light green stage in Papri Queen, Aries and Caysan cultivars.

Postharvest loss in capsicum is related to shriveling (weight loss), nutrient reduction and rot caused by diseases. Although weight loss occurs, the extent of this is limited because peppers have a thick wax layer on their epidermis. Disease is therefore the main factor limiting the shelf life of capsicum (Sharma et al., 2009). Most postharvest diseases are caused by fungi such as white mould caused by Fusarium solani, black mould caused by Alternaria alternata and grey mould caused by Botrytis cinerea (Barkai-Golan, 2001). Several species of bacteria, such as Bacillus polymyxa and Erwinia carotovora ssp. carotovora, also cause rotting, which may occur in fruit from any region growing crops (Snowdon, 1991). Under suitable conditions, fungi germinate on the surface of the host to penetrate into the host tissues and to develop there (Barkai-Golan, 2001). Fallik et al., (1996) demonstrated that grey mould and black mould are the two most important fungal diseases of capsicum. Seed, placenta and pericarp can be infected by fungi, which causes an internal rot and symptoms are not observed until postharvest (Elad and Shtienberg, 1995). B. cinerea is considered to be the most important disease in many countries and efficacy of fungicides is being lost because of development of resistance. Development of alternative means to control fungi may therefore be essential to contribute to sustainable production.

3.1.3 Grey mould

Grey mould (also called Botrytis fruit rot or ash mould) caused by Botrytis cinerea Pers. (teleomorph: Botryotinia fuckeliana (de Bary) Whetzel), shows many different symptoms. B. cinerea causes soft rot associated with destruction and water-leaking of parenchyma tissues and then a grey mass of conidia appears rapidly. These are the most common symptoms on leaves and fruits with soft tissues (Williamson et al., 2007). Grey mould also might occur anywhere on the fruit, including the stem-end and blossom-end (Figure 3.1) (Snowdon, 1991). In capsicum fruit, infected tissue is water-soaked and brown-grey, and grey-brown conidia can develop accompanied by small black sclerotia (Snowdon, 1991).
3.1.3.1 *Botrytis cinerea* lifecycle

*Botrytis cinerea* infects tissues at many developmental stages, even seedlings, and remains latent for long periods before causing rot quickly when environmental conditions are optimal and the host physiology changes (Droby and Lichter, 2004). The life cycle for *B. cinerea* can be divided into two main modes depending on the weather and time of year (Figure 3.2). In the winter, the fungus can persist in dead organs or the soil as resting bodies (sclerotia and mycelia) or in infected plant debris (Snowdon, 1991). In early spring when the weather is warmer, the mycelia and sclerotia become active and germinate to produce conidiophores on the surfaces of the infected plant debris. The conidia are disseminated from conidiophores by wind, rain-splash, irrigation (Maas, 1998) and by human hands to fruit (Barkai-Golan, 2001; Plakidas, 1964). In the summer, the fungus does not enter a resting period, but produces conidiophores directly on infected tissue. In the presence of moisture, conidia in infected organs produce fresh mycelia which quickly spread in the tissue, causing cells to collapse and the tissue is destroyed. Conidia commonly infect susceptible tissue first by germination and form appressoria which allow penetration via wounds. Mycelia then spreads to other tissues when resistance of the fruit to the pathogen decreases (discussed in more detail in section 3.1.3.3). Consequently, these tissues become susceptible to soft rots. *B. cinerea* re-enters the reproductive stage by generating conidiophores again and releases fresh conidia. This process is constantly repeated during appropriate conditions in the summer and the fungus only enters the over-wintering cycle when cooler conditions occur (Williamson *et al*., 2007).
3.1.3.2 Development of grey mould disease

Conidia of *B. cinerea* are released from conidiophores by air currents in humid weather. They usually live and develop on dead plant organs and spread quickly into susceptible plant parts, especially wounded tissues. Moderate temperature (15 to 25°C) with high humidity conditions or a wet surface is optimal for grey mould to develop (Maas, 1998). However, a number of conditions have been shown to slow the disease development, such as cold temperature or hot and dry weather (Snowdon, 1991; Williamson *et al.*, 2007). Light also has effects on *B. cinerea* at different wavelengths. Elad and Shtienberg (1995) indicated that sporulation in *B. cinerea* was reduced by increasing the ratio of blue to UV light which passed through polyethylene film. In addition, fungal development decreases at low oxygen and high carbon dioxide concentration. Mycelial growth of *B. cinerea* and *Fusarium roseum* was inhibited by over 50% when the oxygen level was 4%, and germination of *B. cinerea* was inhibited by more than 90% in an atmosphere of 16% carbon dioxide (Wells and Uota, 1970). Because the carbon dioxide level is high inside capsicum fruit about 3 mL.L⁻¹.g⁻¹, *Botrytis* may prefer to develop in the outer tissues of capsicum fruit.

**Figure 3.2:** Lifecycle of *Botrytis cinerea* [modified from (Winedoctor, 2010)]
3.1.3.3 Infection pathways

Many experiments have confirmed that flowering is the most important time for *B. cinerea* to infect plants and cause subsequent infection of fruit such as strawberry (Jarvis and Borecka, 1968) and grapes (Keller et al., 2003). Conidia can germinate in drops of free water on the petal or any part of the flower, and then penetrate through the senescing parts, into the edge of the receptacle where they form mycelia that then become dormant as a latent infection (Barkai-Golan, 2001). During fruit ripening the cell wall becomes softer due to activity of the enzymes PG and PE. Mycelium of *B. cinerea* then invades and develops in the fruit, causing rot after harvest (Williamson et al., 2007). Grey mould development at the stem-end area in stored tomatoes is the result of infection of the young fruit by *B. cinerea* via the flower parts (Lavy-Meir et al., 1989). *Botrytis* also can infect fruit directly in the field via airborne conidia (Barkai-Golan, 2001), especially where fruit have been damaged during harvest, handling, and transport causing wounds and bruises. However, the infection pathway of *Botrytis* in capsicum is not well understood and it is not known if the flower stage is the most susceptible to *B. cinerea* infection. As such, the “behaviour” of *Botrytis* in capsicum will be studied in this project.

3.1.4 Disease control methods

Because capsicum is mostly grown in warmer regions in the field and glasshouses, both of which favour development of *B. cinerea*, grey mould needs to be controlled. Which methods are used is dependent upon cultural practice and the desired quality of products. However, three major methods of disease control commonly exist; physical or cultural, chemical and biological methods (Elad and Shtienberg, 1995).

3.1.4.1 Physical or cultural methods

As noted above, grey mould is stimulated by moderate temperature and high humidity. Thus in crop management, an open canopy is necessary to provide good air movement and high light interception so that free water dries as soon as possible. Avoiding rainfall during the blossom period by covering the crop with plastic can reduce disease in strawberry by 90% compared with open field plants (Williamson et al., 2007). In addition, removal of plant debris after each growing season can reduce the amount of inoculum (Elad and Shtienberg, 1995). Hence, removal of inoculum and making
conditions unsuitable for survival of the pathogen are the effective way to minimise growth of B. cinerea.

Physical methods after harvest such as heat treatment, ionising radiation and ultraviolet illumination (UV) are used to kill and inhibit fungi and bacteria on fruit. Heated fruit were often harder than non-heated fruits during storage (Klein and Lurie, 1991), helping fruit to resist pathogen infection. In a study by Fallik et al., (1996), the authors treated red sweet pepper by dipping these fruit at 50°C for 3 minutes. The treated fruit completely inhibited decay development caused by B. cinerea in both naturally infected fruit and artificially inoculated fruit. However, heat damage was shown in fruit when dipping at a higher temperature or for a longer time.

Ionising radiation may be harmful for living cells of pathogens and it may also prevent decay by delaying ripening and senescence (Barkai-Golan, 2001). Barkai-Golan et al. (1971) found that radiation of 2-kGy from CO\textsuperscript{60} extended the shelf-life of naturally infected strawberry by grey mould from 3 to 10 days at 15°C. Ultraviolet illumination at low doses has a germicidal activity that can reduce diseases in a wide range of fruits and vegetables (Wilson et al., 1994). Additionally, UV treatment has been found to delay the ripening process in some commodities, such as tomato and peach (Liu et al., 1993), leading to an indirect reduction in their susceptibility to infection. Inoculated berries treated with UV-C doses of 0.125-0.5 kJ/m\textsuperscript{2} had a significantly lower incidence of infection and a reduction of B. cinerea compared with control berries (Nigro et al., 1998).

3.1.4.2 Chemical methods

Although chemical methods of disease control include fungicides (compounds lethal to fungi) and fungistatins (compounds that inhibit fungal growth), fungicide application is more common on seed or plant surfaces either to kill fungal spores or prevent their germination (Persley, 1993). Pre-harvest fungicide application is an effective way to reduce infections initiated in the field (Barkai-Golan, 2001) and to prevent the formation of latent infections in the young fruit (Sommer et al., 1973). However, preharvest fungicides do not protect fruit if wounded during postharvest handling, so fungicide application is also needed in the postharvest period. Postharvest fungicide
application should be done as soon as possible after harvest to prevent mycelial growth in the host tissue (Barkai-Golan, 2001).

However, there are now some concerns about increased chemical use in agricultural systems because of the development of strains of fungi resistant to chemicals (Elad and Shtienberg, 1995; Sharma et al., 2009). This might be potentially harmful for consumers and damage the environment. Therefore, alternative and more environmentally friendly methods such as biological control are now necessary to reduce the amount of fungicides using in agricultural system.

3.1.4.3 Biological methods

Biological control (biocontrol) has become a focus for research to reduce the presence of fungicide residues in foods and to slow the development of pathogens resistant to major fungicides. Biological control has been used to antagonise preharvest and postharvest pathogens (Barkai-Golan, 2001) and occurs via four main modes of action: the secretion of an antibiotic compound by the antagonist; competition with pathogens for nutrients at wound sites on the plant tissue; secretion of enzymes to injure the pathogen; and induction of host defence mechanisms (Droby et al., 1992; Wilson et al., 1994). The effectiveness of a biological agent depends on factors such as time of application, presence of moisture in the wound, and the number of pathogen spores or antagonist concentration. Smilanick (1994) stated that biocontrol should be applied to the wound site prior to the arrival of the pathogen. The antagonist yeast *Candida oleophila* controlled *B. cinerea* more effectively when applied to a fresh wound than a dry wound (Mercier and Wilson, 1995), and the greatest antagonist activity of *Trichoderma* was observed at the highest concentration of the antagonist and the lowest inoculum levels of the pathogens (Mortuza and Ilag, 1999). Schena (1999) indicated that application of the yeast-like fungus, *Aureobasidium pullulans*, at $10^8$ and $10^7$ cells/mL controlled *B. cinerea*, *Rhizopus stolonifer* and *Aspergillus niger* on table grape, and *B. cinerea* and *R. stolonifer* on cherry and tomato. When wound sites in apple fruit were inoculated with *Candida oleophila* prior to inoculation with *B. cinerea*, the percentage of fruit with grey mould after 14 days storage was significantly reduced compared with the control (Mercier and Wilson, 1995).
In addition, host protection plays a significant role in reducing the incidence of disease. The physiological status of fruit significantly influences its susceptibility to infection by pathogens. Pathogens often penetrate into the fruit in the field and symptoms only develop as fruit ripen and the fruit cell wall structure becomes softer. Berries are generally more susceptible to pathogens than are stone fruit (Plakidas, 1964). During ripening, the texture of bell pepper fruit changes from firm to soft because of an increase in PG activity (Priya Sethu et al., 1996). All capsicum cultivars are susceptible to B. cinerea and there is no one group trying to breed for resistance. Instead, the normal method is by keeping the humidity low in the glasshouse (Conrad, L., Seminis Sales, Monsanto, Personal Communication, March 2010). However, the varieties that ripen quicker seem to have a greater incidence of disease.

An adequate supply of nutrients, especially micronutrients, is important for plant growth, fruit quality and disease resistance. The application of nutrients to plants to improve their resistance is therefore considered to be a biological method to reduce pathogen diseases. Ca and B have been demonstrated to improve fruit quality and reduce disease incidence in crops such as bean and tomato (Elad and Volpin, 1993), table grape (Amiri et al., 2009), and strawberry (Singh et al., 2007; Wójcik and Lewandowski, 2003). However, there is limited research on the effect of Ca and B on grey mould development and fruit quality in capsicum. The role of these elements will be discussed below.

3.1.5 Calcium and boron application to control disease

Both Ca and B are essential nutrients for plant growth, but they also have a role in protecting plants from disease. Calcium is an essential component of the plant cell wall and confers structural rigidity and firmness (Easterwood, 2002; Maas, 1998). Moreover, adequate calcium may increase the resistance of the plant to diseases because the Ca$^{2+}$ cation forms cross-bridges between adjacent pectic acids or between pectic acids and other polysaccharides which makes the cell wall more resistant to the action of the pathogen's pectolytic enzymes (Conway et al., 1994; Preston, 1979). The role of B is linked to cell wall synthesis by cross-linking of cell wall polysaccharides and the structural integrity of biomembranes as well (Hansch and Mendel, 2009; Maschner, 1995). Therefore, fruit becomes less susceptible to attack by the pathogen. Furthermore,
application of Ca and/or B significantly deceased the activity of the fruit softening enzymes PG and PC (Dong et al., 2009). As a result, the fruit displays better resistance to grey mould development.

However, Ca and B concentration in plant tissue may be less than optimal because of some main factors. Firstly, both Ca and B are immobile in plants amongst old and in actively growing tissues, leaves and fruits. Ca and B content was often higher in older tissues than growing tissues and higher in the leaves than in fruit (Gupta, 1979; Reuter and Robinson, 1997). Moreover, Ca is taken up less effectively than some other nutrients such as potassium (K), sodium (Na) and ammonium (NH₄) (Kirby and Pilbeam, 1984). This factor causes blossom-end rot and blackheart in celery, tomato and pepper (Geraldson, 1957). In addition, environmental factors also affect the uptake of Ca and B. Increasing soil pH and wet winters both cause B deficiency (Mengel and Kirkby, 1987). Salinity and high temperature are also reported to reduce Ca and B content in plant tissues (Mengel and Kirkby, 1987; Taylor and Locascio, 2004).

The interaction of Ca and B may affect plant growth and disease resistance. A high Ca level caused a B deficiency (Dong et al., 2005; Geraldson, 1957), while B sprays increased the mobility of Ca and the Ca concentration of apple fruit as well (Shear and Faust, 1971). Boron sprays during blossoming effectively reduced bitter bit in apple, which is caused by Ca deficiency (Dunlap and Thompson, 1959 in Mengel and Kirkby, 1987). In addition, B has a role in keeping Ca within the wall which is equally important for maintenance of cell wall integrity. The host would therefore have reduced susceptibility to pathogen (Stangoulis and Graham, 2007).

*B. cinerea* often infects fruit in the preharvest period as a latent pathogen (see section 3.1.3.3) and grey mould symptoms are evident only in the postharvest period. In order to prevent pathogen development, it is necessary to ensure Ca and B are present at a high enough level in both the preharvest and postharvest period.

### 3.1.5.1 Preharvest application

Ca and B deficiencies cause serious problems in the plant. Ca deficiency was related to poor germination in seed, reduction in tissue growth and absence of fruit (Taylor and Locascio, 2004). Ca deficiency also led to reduced firmness of tissue due to the cell
wall dissolution (Mengel and Kirkby, 1987), increased blackheart in celery and blossom-end rot in watermelon, pepper, eggplant and tomato (Taylor and Locascio, 2004). B deficient plants have abnormal development and restricted flower and fruit development (Jones, 1998). In some plants, inadequate B causes fruit to be very small and of poor quality (Mengel and Kirkby, 1987).

The literature suggests a wide range of optimal concentrations for Ca in plants due to requirement variation among plant species. Generally, Ca concentration in plants and in fruit were from 0.1 to 5% and 0.2 to 0.3% of the dry weight of the tissue, respectively (Taylor and Locascio, 2004). In bell pepper the concentrations of Ca in the youngest mature leaf for deficient, adequate and high range of Ca content are 1.00-1.29%, 1.30-2.80% and > 2.80%, respectively (Reuter and Robinson, 1997). In contrast, B has a very narrow range between deficiency and toxicity. Mengel and Kirkby (1987) indicated that at less than 1 ppm water soluble B in soils may not supply enough B to plant growth, while values above 5 ppm may be toxic.

Preharvest calcium and boron application have been demonstrated to reduce grey mould development and/or improve fruit quality in some crops (Table 3.1). Soil application of Ca has occurred at concentrations ranging from 1 to 8 mM and for boron was from 0.1 to 0.8 mM. However, B has been reported to be toxic in capsicum at concentrations higher than 1 ppm (Nabi et al., 2006). Calcium nitrate [Ca(NO₃)₂] is often used in industry or in research as a source of Ca because it is more soluble than calcium sulphate (CaSO₄) or calcium chloride (CaCl₂), and boric acid (H₃BO₃) is most commonly used as a boron-containing compound for soil application.

Foliar Ca and B application have been used widely as an effective method to increase Ca and B content in fruit due to immobility of these elements when applied to soil. CaCl₂ has most commonly been applied as a calcium-containing compound and Ca concentration of foliar spraying solutions have a range from 0.8 to 2 % CaCl₂ (Table 3.1). The boron-containing compound most commonly used in foliar sprays is H₃BO₃ at concentrations ranging from 0.025 to 0.1%. The first time of spraying is often at the fetal fall stage, with later applications at 7-days intervals in strawberry and 14-days intervals in pepper (El-Tohamy et al., 2006; Singh et al., 2007).
Table 3.1 The result of preharvest Ca and B application in some fruit crops

<table>
<thead>
<tr>
<th>Application methods</th>
<th>Crops</th>
<th>Treatments</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil application</td>
<td>Bean and tomato</td>
<td>1-3mM CaCl₂ or Ca(NO₃)₂</td>
<td>Decreased severity of grey mould. Reduced severity of fruit ghost spots.</td>
<td>Elad and Volpin, 1993</td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>27 mg B kg⁻¹ soil (H₃BO₃)</td>
<td>Increased fruit yield and higher fruit soluble solid content</td>
<td>Wojcik et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Sweet pepper (four cultivars)</td>
<td>Ca(NO₃)₂ at low, medium and high level (1.5, 4 and 8 mM)</td>
<td>Increased marketable yield and improved fruit appearance at 4 and 8 mM</td>
<td>Rubio et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B at rate of 0, 1, 2, 4, and 8 mg B kg⁻¹ soil (H₃BO₃)</td>
<td>Maximum crop biomass at ~1 mg B kg⁻¹. Toxic for plant at higher than 1 ppm.</td>
<td>Nabi et al., 2006</td>
</tr>
<tr>
<td>Foliar</td>
<td>‘Asgari’ grape</td>
<td>CaCl₂ at 0, 0.8, 1.2, 1.6 and 2% w/v</td>
<td>Significantly improved berry firmness, colour and appearance associated with increase of Ca concentration. Reduced Botrytis infection and berry drops. Leaf injury (lesions) was sometime observed at high Ca level (2% w/v) but not observed on cluster and fruit</td>
<td>Amiri et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Anna apple</td>
<td>0.0, 0.025, 0.05, and 0.1% H₃BO₃</td>
<td>Increase in fruit yield, fruit physical properties and decrease in severity of blossom-end rot</td>
<td>Khalifa et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Strawberry</td>
<td>CaCl₂ @ 2.0 kg ha⁻¹ spray⁻¹. 150g B (H₃BO₃) ha⁻¹ spray⁻¹ Ca + B in combination at same levels</td>
<td>Significantly reduced albinism and grey mould for Ca and Ca + B treatments Significantly decreased malformation in B-treated fruits Increased fruit firmness and reduction in disorder incidence compared to B alone and control.</td>
<td>Singh et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Pepper</td>
<td>CaCl₂ at 1 and 2 % w/v</td>
<td>Improved yield and fruit quality</td>
<td>El-Tohamy et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca(NO₃)₂ 1% + 0.5% Tween 20</td>
<td>Significantly increased Ca content in leaf, fruit and reduced blossom-end rot</td>
<td>Schon, 1993</td>
</tr>
</tbody>
</table>
Preharvest Ca and B application has been reported to increase marketable yield of fruit, improve quality, appearance and reduce blossom-end rot in capsicum (Keinan et al., 2000; Rubio et al., 2010; Schon, 1993). However, there has not been any report on the effect of Ca fertilisation on grey mould development. B fertilisation at 1 ppm boric acid was suitable for capsicum to produce biomass (Nabi et al., 2006), but the role of this element on grey mould development and fruit quality remains unclear. Capsicum fruit quality was reported to be better with greater foliar Ca application, but previous research only involved two concentrations (1 and 2 % w/v CaCl$_2$) (El-Tohamy et al., 2006), thus it is necessary to study the effect of more Ca concentrations on fruit quality in capsicum. Ca foliar application resulted in reduction of blossom-end rot in capsicum (Schon, 1993). However, the effect of foliar application of Ca and B on grey mould has not been reported. Therefore, further research in this study will focus on the effect of preharvest Ca and B application on grey mould development and fruit quality in capsicum.

3.1.5.2 Postharvest application

Both Ca and B have limited mobility in plant phloem and the lowest concentration is found in the fruit (Mengel and Kirkby, 1987). Postharvest application has been an effective way to increase the level of nutrients in fruit. Postharvest Ca application is a means of applying Ca directly to harvested fruits via the standard practice of dipping fruit in solution at ambient pressure (normal dipping) or active infiltration (pressure infiltration or vacuum infiltration) (Conway et al., 1994). However, B is not commonly applied postharvest due to toxicity of inorganic boron-containing compounds to human health if adults uptake more than 13 mg per day (Nielsen, 1997).

Normal dipping of fruit in a CaCl$_2$ solution increased the fresh calcium content in apples. However, an active infiltration procedure was more effective in increasing Ca content in apples (Conway et al., 1994). When comparing vacuum infiltration and normal dipping procedure, Scott and Wills (1977) found that when the active method was used in apples, there was a lower incidence of bitter pit and fruits had less senescence than when dipped. Fruit quality during storage was better when the active method was used because Ca content in these fruit was higher than in fruit that were dipped in CaCl$_2$. 

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Conway (1982) examined the effect of postharvest calcium application on decay of 'Delicious' apples. Fruits were treated with a range of CaCl₂ solutions (0, 2, 4, 6 and 8%) by dipping, vacuum infiltration (33.32 kPa) or pressure infiltration (103 kPa), all applied for 2 min. Following dipping of calcium chloride, there was no significant difference between treated fruit and controls in terms of both Ca content and decay caused by *P. expansum*. Pressure infiltration of 8% CaCl₂ resulted in the least decay and the highest concentration of calcium. Vacuum infiltration also increased calcium content in flesh, but did not reduce decay.

Calcium treatment delayed decay caused by pathogens due to either calcium increasing cell wall strength or calcium directly inhibiting the pathogen growth (Barkai-Golan, 2001). Grey mould development in the strawberry cultivar ‘Selva’ was reduced significantly by normal dipping in calcium lactate solution (1500 ppm Ca) or calcium chloride solution (4500 ppm Ca) (Naradisorn, 2008). This research also indicated that both calcium lactate and calcium chloride did not significantly affect the growth of *B. cinerea* in *vitro*. Therefore, calcium lactate and calcium chloride had a role on reduction of grey mould development by increasing calcium content in fruit tissue.

Postharvest calcium treatment has potential benefits in reducing grey mould development and extending fruit shelf life in some fruits as discussed above. However, to my knowledge the effect of this element on the storage life of capsicum is not well understood. Thus further research on its effect on shelf life and grey mould development in capsicum fruits needs to be done.

### 3.1.6 Literature summary

Capsicum plants are susceptible to *B. cinerea* which causes grey mould. Grey mould has been considered to be responsible for the main losses during the preharvest and postharvest period. The flowering stage and wound sites on fruit have been demonstrated to be the most susceptible to infection by *B. cinerea* in crops such as grape, strawberry and tomato. However, how *B. cinerea* infects the capsicum plant is unclear. Fungicide application has been used as a common method to control pathogens and diseases in both the preharvest and postharvest period. Using fungicide raises a risk to human health and the environment. The safer control method of Ca and B application has been shown to improve fruit quality and decrease grey mould development in a number of fruit crops. However, an
understanding of the effect of Ca and B in capsicum is limited. In particular, the effect of Ca and B on grey mould development in capsicum and fruit quality is unclear.

3.2 Research questions

In order to understand clearly how *Botrytis cinerea* infects capsicum and the effect of Ca and B on grey mould development and fruit quality, this research addresses the following questions:

- How does *B. cinerea* infect the capsicum at preharvest and during latent infection, and postharvest via the wound sites?

- Does preharvest Ca and B application affect grey mould development and fruit quality in capsicum? What is the effect of the different types of foliar application versus soil application?

- Does postharvest Ca application affect grey mould development and fruit quality in capsicum? What is the effect of the different sources of calcium and method of application?

3.3 Aims of the project

1. To conduct a comprehensive study of how *Botrytis cinerea* infects capsicum fruit in both preharvest and postharvest periods.

2. Establish the effect of preharvest calcium and boron applications on grey mould development, postharvest physiology and fruit quality.

3. Establish the effect of postharvest calcium treatment on grey mould development and fruit quality.

3.4 Theoretical framework and methods

3.4.1 Plant material and preparation

Two capsicum cultivars, cv. Aries and cv. Papri Queen, previously well characterised for their postharvest physiology and ripening behaviour (Pham, 2007) will be used for all studies.
Seeds supplied by Seminis® Seeds will be sown in 15-cm diameter plastic pots containing about approximately 2.5 kg “UC mix” soil. UC (University of California at Davis, Baker, 1957) mix consists two thirds washed Waikerie sand, one third of peat moss with the addition of fertiliser (2.25kg/m³ Osmocote plus 14N:4.8P:10.8K:1.2Mg), hydrated lime (0.7kg/m³) and Ag-lime (1kg/m³) (Pham, 2007).

Capsicum plants will be grown in a glasshouse at 25±5°C under a 12 h light/12 h dark cycle. The flower will be tagged for all experiments. Fruit at three different ripening stages will be harvested for postharvest experiments: Deep Green (DG) – completely green and intense colour, Breaker Red (BR) – 40 to 50% red surface colouration and Red (R) – 100% red with low intensity.

3.4.2 Infection pathway of B. cinerea in capsicum fruit

3.4.2.1 Botrytis cinerea growth and culture

Botrytis cinerea will be isolated from a naturally infected capsicum fruit and cultured on potato dextrose agar (PDA, Difco) amended with 100 mg L⁻¹ streptomycin sulphate (Naradisorn, 2008). Stock Botrytis will be stored long term by a swab technique. Using a plastic stem, the end of a swab will be rolled around a three-week-old culture of B. cinerea. The swabs will coat with Botrytis conidia and then these swabs will be stored at 4°C. Conidia will be prepared by placing a disc (5.0 mm in diameter) from the edge of cultures of the fungus onto V-8 juice agar at 21-24°C under a 12 h light/12 h dark cycle (El-Kazzaz et al., 1983; Fallik et al., 1993). Two-week-old cultures will be flooded with 20 mL Tween-20 solution (three drops of Tween-20 in 1 L sterile nanopure water) and the conidia suspension will then be filtered through two layers of sterile cheesecloth. Conidia will be washed two times by centrifugation and re-suspended in fresh Tween-20 solution. The concentration of conidia will be counted by haemacytometer and adjusted to the required concentration in each experiment.

3.4.2.2 Preharvest infection and growth

Inoculation of flowers by B. cinerea will occur at three different stages of anthesis (Halfon-Meiri and Rylski, 1983): one day before anthesis when petals are opened and stigma clearly visible; 5 days after anthesis and 20 days after anthesis. Flowers will be randomly selected and tagged for each experiment. Conidial suspension (100 µL) will be
applied directly onto the stigma in the flower and stem-end in young fruit by using a pipette (Utkhede and Mathur, 2005).

Three concentrations of *B. cinerea* suspension will be applied in this experiment: $10^4$, $10^5$ and $10^6$ spores mL$^{-1}$ (Elad and Volpin, 1993; Fallik *et al.*, 1996; Halfon-Meiri and Rylski, 1983). After inoculation, flowers and fruits will be covered by a plastic bag for at least 8 h to maintain moisture (Naradisorn, 2008). Controls will be treated with sterile nanopure water in the same way. Each treatment will have 15 flowers and the experiment will be repeated at least twice.

Treated fruits will be harvested at deep green and full red stage for assessment of grey mould development. Treated fruits will be surface-sterilised by dipping in 2 % sodium hypochlorite for 1 min. Surface-sterilised fruit will be air-dried and then placed on a sterile, moist cloth in plastic containers and monitored daily during storage at 10°C temperature, RH = 90% ± 5%. The numbers of fruit exhibiting rot will be recorded and presented as a percentage of fruit that are rotten. Rot development will be measured by using digital calipers (digiMax, Switzerland) and results expressed as the diameter (in mm) of the mean of the length and width of the decayed area. Microscopy will be used to observe the mycelium development in tissue. Re-isolation will be done to prove that *Botrytis cinerea* observed in treated fruit was that inoculated in the preharvest period. Fruit tissue will be cut into segments of 5 x 5 mm, then surface-sterilised by dipping in 2 % sodium hypochlorite for 1 min and placing on a PDA plate. The plate will be incubated under the laboratory conditions at ambient temperature (22 ± 2°C) and observed for growth of *B. cinerea* daily. Where grey sporulation develops on the PDA plate, conidia will be transferred to a drop of sterile water on a slide and covered with a coverslip and observed by microscopy. Conidiophores with a basal cell, irregularly branched and bearing grape-like clusters of conidia will be identified. The inoculum concentration and the flowering stage to give the highest percentage of disease will be chosen for subsequent experiments.

3.4.2.3 Postharvest inoculation and growth

Fruit at three ripe stages as described by Pham (2007), will be harvested for inoculation: deep green; breaker red and red. Postharvest inoculation will be done as described by Fallik *et al.* (1996). Fruit will be surface-sterilized by dipping in 2 % sodium hypochlorite...
for 1 min and then wounded on two sides to a depth of 1.5 mm by puncturing them with the point of a sterile 1.5 mm diameter nail. Each wound site will be inoculated with 40 μL of spore suspension (10⁴, 10⁵ and 10⁶ spores mL⁻¹). Treated fruit will be stored and assessed for grey mould development as described in section 3.4.2.2. Treatments will be arranged in a completely randomised design, with 10 fruit per treatment and conducted at least twice. The inoculum concentration and the ripening stage to give the highest percentage of disease will be chosen for subsequent experiments.

3.4.3 The effect of calcium application on grey mould development, postharvest physiology and fruit quality

3.4.3.1 Preharvest calcium application

To study the effect of preharvest calcium application on grey mould development and fruit quality, this experiment will use both calcium applied directly to the soil and calcium applied in foliar spray and compare them at different concentrations.

To look at the effect of soil application, three Ca²⁺ concentrations: 1.5, 4 and 8 mM will be trialed. These concentrations were determined to be the deficient, optimal and super optimal concentrations for capsicum plants in previous research (Rubio et al., 2010). Capsicum seeds will be sown in small pots (5 cm diameter) in UC mix soil to germinate before transplanting to the larger pots and treatment. Mount Compass sand will be used as the potting soil. The sand will be passed through a 2-mm stainless steel sieve to remove organic matter and other debris and washed with RO water and then air-dried in the glasshouse. Sieved Mount Compass sand (2.5 kg) will be weighed into larger individual pots (15 cm diameter). Young seedlings will then be transplanted one per pot into larger pots when seedlings have reached the four leaf stage by 6 to 7 weeks. Plants will be randomly allocated on a bench such that the distance between pots is never less than 20 cm. Treatments will be initiated immediately after transplanting seedlings. The basic nutrient solution will be modified from Hoagland solution (Hoagland and Amon, 1938) as described by Rubio et al. (2010) to obtain three Ca treatments (Table 3.2). Ca(NO₃)₂ is the main source of calcium-containing compound. The micronutrients will be applied similarly in all the treatments, in the following form: 0.025 mM H₂BO₃, 0.002 mM MnSO₄·H₂O, 0.002 mM ZnSO₄·7H₂O, 0.0005 mM CuSO₄·5H₂O, 0.0005 mM (NH₄)₆Mo₇O₂₄·4H₂O and 0.02 mM Fe²⁺-EDTA. The pH will be kept within the range of 5.5-6.0 by 1 μmol L⁻¹ or 0.5
µmol L⁻¹ KOH. The nutrient solution (100 mL) for treatment will be applied manually to each plant by pouring into soil every 2 days. Reserve osmosis (RO) water treatment will be used as control.

**Table 3.2** Concentration of nutrient (mM) in the nutrient solution of the calcium treatments to be applied to the soil preharvest

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th>Low calcium concentration 1.5 mM Ca</th>
<th>Medium calcium concentration 4 mM Ca</th>
<th>High calcium concentration 8 mM Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Ca(NO₃)₂.4H₂O</td>
<td>1.5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>CaCl₂.6H₂O</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

To look at foliar application, the literature suggests CaCl₂ has been used widely at preharvest in foliar spraying and based on the previous research by El-Tohamy *et al.*, (2006), four concentrations will be trialed: 1, 2 and 3 % w/v CaCl₂.

Plant materials and micronutrient will be prepared as per Section 3.4.3.1. Solution without Ca will be prepared as per Table 3.3. The nutrient solution (100 mL) will be applied manually to each plant by pouring into soil every 2 days. Capsicum plants will be sprayed with CaCl₂ by hand sprayer pump at flowering stage at a rate of 200 mL/m² to run-off at 2 week-intervals (El-Tohamy *et al.*, 2006). Spraying RO water will be served as control.
Table 3.3 Concentration of nutrient (mM) in the nutrient solution that will be applied to the soil of the capsicum plants used for foliar spraying treatments (Rubio et al., 2010)

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>6</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>3</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>1</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>1</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
</tr>
</tbody>
</table>

3.4.3.1.1 Assessment of nutrient status

Nutrient status will be analysed by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) at Waite Analytical Services. This analysis will focus on the main micronutrients in leaves and fruit. The youngest mature leaf (YML) will be collected for nutrient status of the plants at first bloom (Reuter and Robinson, 1997) and after harvest. Postharvest fruit samples will be collected after harvest. Three replicates of leaf and fruit samples will be prepared by washing and rinsing in RO water to remove soil and dust particles and pesticide residues. Individuals will then be placed in a paper bag and dried in an oven at not over 80°C for 24 h or until a constant dry weight is obtained. Samples will be ground to a fine powder by a high-speed grinder before providing them to Waite Analytical Services.

3.4.3.1.2 Assessment of grey mould development

In order to examine the effect of calcium in both the soil and foliar application trials, preharvest and postharvest inoculation will be performed.

Preharvest inoculation will be done on 20 flowers at the appropriate stage as determined earlier at the section 3.4.2.2. Postharvest inoculation will be done on 20 fruits at the
appropriate stage as determined earlier at the section 3.4.2.3. Inoculated fruit will be stored and assessed for grey mould development as per 3.4.2.2.

3.4.3.1.3 Assessment of fruit quality

A number of parameters will be used to assess fruit quality: shelf life, firmness, colour change, total soluble solid (TSS) and titratable acidity (TA). Fruit from treated plants will be harvested, placed in single layers in plastic trays covered with plastic wrap and stored at 10°C (considered to be supermarket temperature) and 90 ± 5% RH. At least ten fruits will be used for each replicate and each storage duration. Initial quality evaluation will be done at days 0, 2, 6, 9, 12 and 15 of storage (Lerdthanangkul and Krochta, 1996; Smith et al., 2006).

Shelf life will be assessed by using a 9-point general appearance (GA) scale (9 = the best condition and 1 = the worst condition) as described by Able et al. (2002). When fruit has reached a GA of 5.5, they will be considered to have reached the end of their storage life.

Fruit firmness will be determined by measuring penetration force in kilogram-force (kgf) by a Fruit Pressure Tester (FT 110, Italia) equipped with a 6-mm diameter plunger tip as described by Naradisorn (2008). A puncture test will be performed on two sides of each fruit by holding the fruit against a hard surface before forcing the plunger tip into the fruit at a uniform speed so that the depth of penetration is consistently to the line inscribed on the tip.

Colour change will be measured via extractable colour described as per Pham (Pham, 2007, p.26-27).

Total soluble solids (TSS) will be determined by using a hand refractometer (Stanley Limited, Switzerland). Fruit samples will be collected as described by Pham (2007). Sample will be collected from four fruit in each treatment to evaluate TSS. Fruit will be sliced, ground by a porcelain mortar and pestle prior to wrapping with cheesecloth and squeezing with a hand press. Juice samples will then be filtered through cheesecloth to make sure a clear juice is available for measurement. A drop of juice sample will be placed on the glass surface of the refractometer using a micropipette. The TSS content will be read and presented as °Brix.
Titratable acidity (TA) will be determined by taking 6 g of the juice sample as described above diluting to 50 mL with nanopure water and using 0.1N NaOH to titrate to pH 8.1. Three independent samples per treatment will be analysed and each sample will be titrated in triplicate. Results will be expressed as percent citric acid in the juice (Rubio et al., 2010) using the formula:

$$\text{TA} = \frac{\text{mL(NaOH)} \times N(\text{NaOH}) \times \text{acid milliequivalent factor} \times 100}{\text{mL juice titrated}}$$

### 3.4.3.2 Postharvest calcium application

To study the effect of postharvest calcium application on grey mould development and fruit quality, this experiment will use both calcium dipping and vacuum infiltration (33.32 kPa) at different concentrations. Plant material will be prepared as per Section 3.4.1. Postharvest calcium application will be trialed at three ripening stages of fruit as described by Pham (2007): deep green; breaker red and red. Before treatment fruit will be surface-sterilised by dipping in 2% sodium hypochlorite for 1 min. Each treatment will include 10 fruits and each experiment be repeated at least twice.

CaCl₂ will be chosen as the calcium-containing compound as suggested by the literature (Conway et al., 1994; Naradisorn, 2008). Fruits will be treated at four concentrations: 2, 4, 6, and 8%. Dipping will be done by placing fruit in the respective calcium solution for 2, 5 and 8 min. Vacuum infiltration will place fruit in respective calcium solution for 2, 5 and 8 min and then holding fruit in solution corresponding to time of vacuum infiltration for 2, 5 and 8 min before release, respectively (Conway, 1982). The fruit will then be air-dried at room temperature in a lamina flow before inoculation.

The fruit will be inoculated with conidial suspensions of *B. cinerea* prepared as per Section 3.4.2.3. Grey mould development will be assessed as per Section 3.4.2.2. Fruit quality will be assessed as per Section 3.4.3.1.3. Fruit will be peeled by knife about 1mm deep before collecting for nutrient analysis to ensure that calcium content is only measured in the tissue. Sample for ICP-OES will be prepared as per 3.4.3.1.1.
3.4.4 The effect of boron application on grey mould development, postharvest physiology and fruit quality

3.4.4.1 Preharvest boron application

To study the effect of preharvest B application on grey mould development and fruit quality, this experiment will use both boron applied directly to the soil and boron applied in foliar spray and compare them at different concentrations.

Plant materials and micronutrient solution without boron will be prepared as per Section 3.4.3.1. To look at the effect of soil application, three B applications will be trialed: 0.01, 0.05, and 0.1 mM. The basic nutrient solution will be modified from Hoagland solution (Hoagland and Arnon, 1938) as described by Rubio et al. (2010) to obtain three B treatments (Table 3.4). Boric acid (H$_3$BO$_3$) is the main source of boron-containing compound. The micronutrients without boron will be applied to all plants as per section 3.4.3.1. The nutrient solution (100 mL) for treatment will be applied manually to plants by pouring into soil every 2 days. Water treatment will be used as the control.

Table 3.4 Concentration of nutrients (mM) in the nutrient solution of the boron treatments for soil application

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th>Low boron concentration</th>
<th>Medium boron concentration</th>
<th>High boron concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01 mM B</td>
<td>0.05 mM B</td>
<td>0.1 mM B</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>K$_2$SO$_4$</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MgSO$_4$.7H$_2$O</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$.2H$_2$O</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>
H$_3$BO$_3$ has been used widely preharvest for foliar spraying and based on the previous research by Khalifa et al. (2009), four concentrations will be trialed: 0, 0.025, 0.05, and 0.1 % w/v H$_3$BO$_3$.

Plant materials and micronutrients will be prepared as per Section 3.4.3.1. Solution without Ca will be prepared as per Table 3.3. The nutrient solution (100 mL) will be applied manually to each plant by pouring into the soil every 2 days. Capsicum plants will be sprayed by B solution at from the flowering stage at 2 week-intervals by hand sprayer pump at rate of 200 mL/m$^2$ to run-off (Keinan et al., 2000). Spraying RO water will be used as control.

Nutrient status will be assessed as per section 3.4.3.1.1. Grey mould development will be assessed as per section 3.4.3.1.2. Fruit quality will be assessed as per section 3.4.3.1.3.

3.4.5 Effect of preharvest Ca and B in combination on grey mould development, postharvest physiology and fruit quality

To study the effect of preharvest calcium and boron application in combination on grey mould development and fruit quality, Ca and B will be applied in combination either directly to the soil or via foliar spray and compare them with single calcium or boron application.

To look at the effect of soil application, the concentration of Ca or B that had the most effect when applied to the soil in previous experiments (section 3.3.3 and 3.3.4) will be trialed (Table 3.5). Plant materials and micronutrient solutions will be prepared the same as per Section 3.4.3.1.
Table 3.5 Concentration of nutrients (mM) in the nutrient solution where the Ca and B will be in combination for soil application

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂BO₃</td>
<td>The best concentration</td>
</tr>
<tr>
<td>Ca(NO₃)₂.4H₂O</td>
<td>The best concentration</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>6</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>3</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>1</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>1</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
</tr>
</tbody>
</table>

To look at the effect of foliar application, the best concentration of foliar preharvest calcium or boron application in earlier works will be trialed. Plant materials and micronutrient will be prepared as per Section 3.4.3.1. Solution without Ca will be prepared as per Table 3.3. The nutrient solution (100 mL) will be applied manually to each plant by pouring into the soil every 2 days. Capsicum plants will be sprayed by calcium chloride and boric acid solution at flowering stage and then 2 week-intervals by hand sprayer pump and each treatment will be sprayed with 200 mL/m² to run-off.

Nutrient status will be assessed as per section 3.4.3.1.1. Grey mould development will be assessed as per section 3.4.3.1.2. Fruit quality will be assessed as per section 3.4.3.1.3.

3.5 Significance

*Botrytis cinerea* is considered to be the main cause of capsicum fruit rot. However, how *B. cinerea* infects the capsicum plant is unclear. Therefore, this research will provide an understanding of how or when *B. cinerea* infects the capsicum plant via the latent infection
pathway and at which stage of development fruit is most susceptible to infection by *B. cinerea*.

Ca and B are critical nutrients for plant growth, fruit quality and disease resistance. On the other hand, both Ca and B may be toxic to the plant especially B which has a narrow range between deficiency and toxicity. However, there is a lack of research about the effect of calcium and boron on grey mould development and fruit quality in capsicum. The results of this research will indicate the role of preharvest Ca and B application on grey mould development and fruit quality in capsicum. Moreover, this study will result in knowledge of the optimal concentrations for Ca and B in the soil and by foliar spray. In preharvest application, the most suitable application method to increase Ca content will be investigated for capsicum in reducing disease and improving fruit quality. In addition, Ca has been demonstrated to have the lowest concentration in fruit. This research will therefore provide insight as to whether Ca postharvest application will increase Ca content in fruit including optimal concentration of Ca and the most suitable method.
4. Budget

<table>
<thead>
<tr>
<th>Items</th>
<th>2010 (AUD)</th>
<th>2011 (AUD)</th>
<th>2012 (AUD)</th>
</tr>
</thead>
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<tr>
<td>Statistics course</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glasshouse cost</td>
<td>360</td>
<td>800</td>
<td>800</td>
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<tr>
<td>Agar, Potato Dextrose Agar, V-8 juice</td>
<td>516</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Petri dishes, sodium hypochlorite</td>
<td>120</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chemicals for Hoagland's Solution including boric acid, manganese sulphate, zinc sulphate, copper sulphate, ammonium molybdite, iron, potassium nitrate, calcium nitrate, sodium nitrate, potassium sulphate, magnesium sulphate, potassium chloride, calcium chloride, sodium dihydrogen orthophosphate, potassium dihydrogen orthophosphate</td>
<td>1989</td>
<td>2000</td>
<td>1900</td>
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<tr>
<td>ICP-OES analysis by Waite Analytical Service (approx. 15 sample at ~ $20 ea)</td>
<td>300</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Consumables for digest for ICP-OES</td>
<td>150</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Antibiotics for fungal culture</td>
<td>59</td>
<td>120</td>
<td>60</td>
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<tr>
<td>Others consumables (gloves, tips, tags) and freight</td>
<td>150</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3739</strong></td>
<td><strong>4120</strong></td>
<td><strong>4060</strong></td>
</tr>
</tbody>
</table>

NB – Some consumables are shared by other students using the same code

4.1 Will you submit an application to a funding agency for support for your project?
   Yes □  No √

4.2 Is this project contingent on funding support from elsewhere?
   Yes □  No √
5. Research Plan

<table>
<thead>
<tr>
<th>Tasks/Milestones</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature review</td>
<td></td>
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<tr>
<td>Research proposal</td>
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<td></td>
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<tr>
<td>Completion of the core component</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To conduct a comprehensive study of how *Botrytis cinerea* infects capsicum fruit in both pre and postharvest periods

| Plant material and preparation                         |      |      |      |      |
| Infection pathway of *B. cinerea*                     |      |      |      |      |
| Data analysis                                          |      |      |      |      |
| Preparation of journal paper                           |      |      |      |      |

Establish the effect of preharvest calcium and boron applications on grey mould development, postharvest physiology and fruit quality

| Plant material and preparation                         |      |      |      |      |
| Preharvest calcium application                         |      |      |      |      |
| Plant material and preparation                         |      |      |      |      |
| Postharvest calcium application                        |      |      |      |      |
| Data analysis                                          |      |      |      |      |
| Preparation of journal paper                           |      |      |      |      |

Australasian Postharvest
<table>
<thead>
<tr>
<th>Conference</th>
<th>Establish the effect of postharvest calcium treatment on grey mould development and fruit quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant material and preparation</td>
<td>Preharvest boron application</td>
</tr>
<tr>
<td>Effect of preharvest Ca and B application in combination</td>
<td>Data analysis</td>
</tr>
<tr>
<td>Thesis writing/editing</td>
<td>Thesis review by supervisors</td>
</tr>
<tr>
<td>Final seminar (research group)</td>
<td>Annual review</td>
</tr>
<tr>
<td>Submission thesis</td>
<td></td>
</tr>
</tbody>
</table>
6. Skills Training and Professional Development

Integrated Bridging Program workshops on research communication

Annual School postgraduate symposium

Attend School Writing Workshops

Attend the Adelaide Graduate Centre workshop for Higher Education Students

Attend regular Plant Protection Group meetings

The Postgraduate Short Course Research Methodology and Experimentation

The combined Australian Society of Horticultural Science (AUSH) Conference and the Australasian Postharvest Conference in 2011
7. References

Able, A.J., Wong, L.S., Prasad, A. and O'Hare, T.J. (2002) 1-MCP is more effective on a floral brassica (Brassica oleracea var. italica L.) than a leafy brassica (Brassica rapa var. chinensis). Postharvest Biology and Technology, 26, 147-155.


