Disinfestation of apple leaf-curling midge, *Dasineura mali* (Diptera: Cecidomyiidae) on post-harvest apple fruits by ultraviolet-C radiation

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ABSTRACT

Apple leaf-curling midge (*Dasineura mali* Kieffer) (ALCM) is considered as an important quarantine pest of apple due to fresh fruit contamination by pupal cocoons. To meet the quarantine regulations of export markets and the expectations of customers, a series of non-chemical methods have been investigated for the potential to be applied to control insect pests. One approach, ultraviolet-C (UV-C) radiation, offers potential as a new disinfestation technique. However, the disinfestation effects of UV-C radiation in the control of ALCM have not been investigated previously.

To investigate the disinfestation effect of UV-C radiation, two individual experiments were conducted. Apple fruit-attached and non-fruit attached cocoons of ALCM were treated with a series of UV-C radiation doses, and then maintained in temperature-controlled (daily mean temperature around 20 ^{o}C) dark conditions. For non-fruit attached cocoons, the groups treated with UV-C radiation had significantly higher mortality rates than that of the control groups. For fruit attached cocoons, although the sample size was small, results indicated that cocoons treated with 20 mins, which was the most prolonged UV-C radiation treatment, exhibited the highest mortality rate. The insignificant mortality rate of cocooned larvae when comparing those groups treated with lower UV-C doses and control groups suggests that attachment of cocoons to the apple calyx may be a significant factor in limiting the effect of UV-C for the control of ALCM. To obtain understanding of the effects of UV-C radiation on the potential fecundity of female ALCM, a third experiment was conducted, where adult insects were reared following UV-C treatment of cocoons as before, and egg numbers carried by newly emerged adult females were assessed. Interestingly, although the difference in the egg quantity of female adult ALCM between treatments was not significant, it revealed that the UV-C treated group had a potential higher egg capacity with increased body size than the control group. It is possible that the short duration of UV-C radiation treatments might lead to increased egg capacity of female ALCM, and these effects are worthy of future investigation. Equally, the possibilities to provide greatly increased UV-C doses to potentially kill ALCM cocooned larvae during apple processing should be explored further.

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CHAPTER 1: INTRODUCTION

1.1 New Zealand apple industry

New Zealand has grown and exported apples for over 100 years since the missionary Samuel Marsden introduced the first apple tree in 1819 at the time when Europeans first settled in the country. In 1948, New Zealand Apple and Pear Marketing Board (NZAPMB) was established to market both export and locally sold pipfruit. In 2001, Pipfruit NZ Inc (PNZI) was established to market and support the NZ pipfruit industry via a grower levy payment system. Fresh apples exported from New Zealand supplied approximately up to 300,000 tonnes or almost 2% of the world's apples to more than 50 countries by 1997 (Batchelor et al., 1997). From 2006 to 2011, New Zealand annual apple export volumes settled into a range between 260,000 and 320,000 tonnes and approximately 5% of the global apple trade (Fresh Facts New Zealand Horticulture, 2012).

In 2012, the New Zealand Plant and Food Research Institute estimated that the national planted area for apples was 8,324 hectares. Specifically, Hawke's Bay (52%) and Nelson (29%) are the main growing regions with an estimated 7,000 hectares of planted area following by Central Otago (5%), Canterbury (3%) and Waikato (3%). The main cultivars of apple grown in New Zealand are moving away from well-known commodity cultivars of 'Braeburn' and 'Royal Gala' to newly developed and more marketable cultivars such as 'Fuji', 'Jazz™', 'Pacific Rose™', and various smaller cultivars. New Zealand apple exports by cultivars as percentage in weight is shown in Figure 1.1. To compete with overseas exporters, the production of high quality apple fruit which requires precise and guaranteed control of pests and diseases to prevent storage disorders and to meet the quarantine standards of importing countries is critically needed.

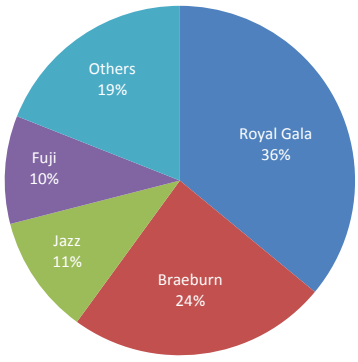

Figure 1.1: New Zealand apple exports by cultivar as percentage in weight by 2011.

In conventionally-managed apple orchards in New Zealand, common insect pests causing significant damage to apple trees or fruit consist of primary and secondary insect pests which include: leaf-roller species (Lepidoptera: Tortricidae), codling moth *Cydia pomonella* (L.), apple leaf-curling midge (ALCM) *Dasineura mali* Kieffer (Diptera: Cecidomyiidae), spider mite species (Acari: Tetranychidae), and so on (Penman, 1978). In terms of ALCM, it is normally not regarded as a pest in commercial orchards due to the insignificant leaf damage it caused. However, the tremendous concern regarding ALCM in apple is that fruit contamination by ALCM cocoons can cause quarantine concerns with countries that do not have ALCM.

During the last several decades, synthetic chemical insecticides have been applied for the control of insect pests (Harris, 1972; Pimentel, 1981; East and Holland, 1991). With the intensive use of insecticides, problems such as insecticide resistance, environmental contamination, and negative influence of natural beneficial insects have been induced (Zadocks, 1993; Dent, 1995). Furthermore, with the recognition of the risk of chemical residues to human health by consumers, the food industry realized the importance of the application of non-chemical methods or safer alternatives to chemical insecticides to control insect pests which changes the traditional pest management into a sustainable approach (Batchelor et al., 1997; Manktelow et al., 2001).

In response to meeting consumers' demand and maintaining overseas markets, the New Zealand horticultural industry realized that the way orchards managed would be positively changed. A new approach aimed to address continued challenges is introduced into New Zealand. The Integrated Fruit Production (IFP) programme which evolved from the Integrated Pest Management (IPM) was firstly introduced to New Zealand in 1991 by the former New Zealand Kiwifruit Marketing Board which is now known as Zespri International Ltd (Wiltshire, 2003). Five years later in 1996, IFP was introduced in the New Zealand pipfruit industry. By 2001, 100% of export pipfruit was managed by the IFP system in New Zealand (Wiltshire, 2003). But IFP is more than just IPM; IFP is a comprehensive pest management which takes all aspects of tree fruit production management including pest control into consideration. IFP is an effective and environmentally friendly orchard management system emphasizing the production of high quality fruit with minimized pesticide residues that relies on a combination of various practices (Walker et al., 1997, 1998). With the use of biological control through reduced use of broad-spectrum pesticides, adoption of more selective and

environmentally friendly chemicals and threshold-based application, the implementation of IFP system has greatly reduced the use of insecticides, and significantly benefited consumers, growers and environment (Walker et al., 1998; Wiltshire, 2003).

1.2 Apple leaf-curling midge

Cecidomyiidae is a midge family containing more than 4,600 species in which over 3,000 species are found worldwide (Gagne, 1989). These midges are also known as gall midges as the larvae of most midges feed within plant tissues, causing abnormal plant growth, called galls. Gall midges are usually 2 – 3 mm in length and are fragile insects with long antennae and hairy wings (Hill, 1987).

Many midge species cannot be easily distinguished due to the very similar morphological characteristics of both adults and larvae of different species. Normally, midge species are named after the specific host plant on which they induce a significant injury. For instance, one species of gall midges which attacks apples in many parts of the world has been identified as apple leaf-curling midge (ALCM) (Barnes, 1948). Similarly, another species which attacks pears is recognized as pear leaf-curling midge (PLCM), *Dasineura pyri* Bouche. Both ALCM and PLCM had been among the most poorly studied species in the gall midge family until quite recently. ALCM is closely related to PLCM by the similar injury to their particular hosts and the similar life cycles (Barnes, 1948; Kolbe, 1982).

ALCM has four life stages: egg, larva, pupa and adult (Figure 1.2). Larva of ALCM is a legless maggot up to 3 mm long. Larva starts as white but becomes orange to red as it develops. The pupa is brown inside a white silk cocoon. Adult is a mosquito-like small fly and is about 1.5 – 2.5 mm long. The female adult has a red abdomen which distinguishes it from the male adult (LaGasa, 2007).

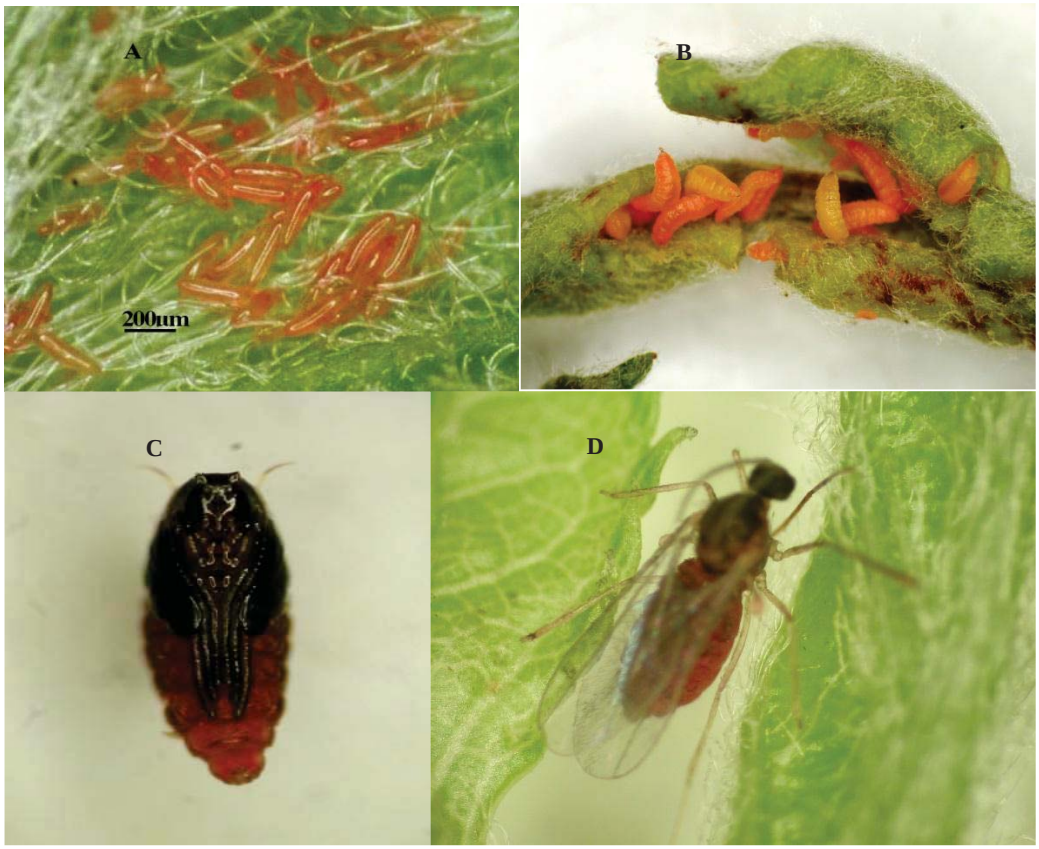

Figure 1.2: Life cycle of ALCM: eggs (A), larvae (B), pupa (C) and female adult (D) (Courtesy of X.Z. He).

ALCM is distributed throughout Europe, North America and New Zealand. Dating back to the 1950's in New Zealand, the first report revealed the occurrence of ALCM when ALCM was found to infest the apple stocks planted in an Auckland nursery (Morrison, 1953; Todd, 1956). Four years later in 1954, ALCM had spread to numerous orchards in the North Island of New Zealand (Todd, 1956). ALCM is an important pest of apple and a potential fresh fruit contaminant by pupal cocoon, causing quarantine concerns especially in New Zealand as a fruit export country. Specifically, it was found that the pupal cocoon of fourth generation of ALCM attaching to fruit in the calyx or the stem is the main fruit contaminant rather than that of other generations (Figure 1.3) (He and Wang, 2007). Furthermore, the damage to young leaf and shoot by the ALCM larvae is also considerable causing leaf curling which can result in the poor development of young shoot (Allison et al., 1995). Early-leafing cultivars of apples were observed being infested by the first generation ALCM adults (Todd, 1959). However, the infestation at this stage is usually insignificant. A heavy infestation by ALCM occurs only when the

terminal growth of apple trees reaches maximum, and midge populations increase rapidly during the second generation (Todd, 1959; Shaw et al., 2005).

Figure 1.3: Apple fruit contamination by ALCM cocoon in the calyx (Courtesy of X.Z. He).

In the middle 1900's in New Zealand, there was relatively little knowledge regarding the biology and life cycle of ALCM apart from the study undertaken by Morrison (1953) and Todd (1956, 1959). In the recent decade, numerous studies had been undertaken to investigate the comprehensive biology and life cycle of ALCM. For example, the phenology of ALCM had been studied by Tomkins (2000), followed by Shaw's study (2005) and He and Wang's research (2011). In addition, the phenology and aestivation of *Platygaster demades* Walker, an egg parasitoid of ALCM, had also been investigated (Tomkins et al., 2000; He and Wang, 2007). Following mating, the female ALCM adults lay eggs along the buds and unfolding young leaves. The newly laid eggs are oval in shape and white to orange in colour. Larvae start to feed on leaves after 3 to 6 days of incubation period (Barnes, 1948). Larval feeding will cause the formation of rolling leaves. Larvae turn white to orange as they mature (Barnes, 1948; Todd, 1956). After reaching maturity, larvae start to pupate after falling to the ground. Some mature larvae also pupate in the curled leaves, under bark, and at other sites on the tree like in the calyx end or the stem of fruit at some period of time (Barnes, 1948; Todd, 1956). Adults emerge from cocoons after about 2 to 3 weeks. While the others stay as pupae over winter and emerge as adults in the following spring.

The number of generations varies depending on the locations and seasons. Based on Todd's latter study (1959), it was found that there were five generations of ALCM each year during 1955-1958 seasons in Palmerston North. On the contrary, according to a

recent study, there were four generations of ALCM each year during 2005-2007 seasons in Palmerston North (He and Wang, 2007). According to the observation by He in 2005-2007, the oviposition by ALCM adults of the first, second, third and fourth generation was in late-November to early-January, mid-January to early-March, early March-early April, and late September-late October, respectively.

1.3 Impact of apple leaf-curling midge

The impact of ALCM on apple trees contributes to the damage caused by the larval stage of the insect. ALCM can stunt the growth of apple trees particularly on young trees and scions by attacking leaves, resulting in the curled leaves (Figure 1.2). Apple trees with little or no growth and expanded leaves are rarely being invaded (Barnes, 1948; Todd, 1956). Larval feeding can cause leaves to roll or curl resulting in the formation of gall, and leading to the thicken leaf tissues in red or purplish color. The infested leaves become red and rolled at the early stage of larval feeding. With the completion of larval feeding, those leaves become fragile and hard. Ultimately, they become broken when the mature larvae break out of them in search of the pupation sites.

Figure 1.4: Curled growing apple terminal leaves infected by ALCM larvae.

Due to the overlapping period of larval feeding, the severity of damage caused by larval feeding would be significant resulting in the defoliation of the apple trees (Todd, 1959). Furthermore, the damage caused by larval feeding can lead to further infection by plant pathogens like fire blight, *Erwinia amylovora* (Burrill) (Gouk and Boyd, 1999).

Though the ALCM larval feeding adversely affects the development of apple tree terminal leaves resulting in the reduction of leaf area, and leading to the reduction of photosynthetic product (Smith and Chapman, 1995a, b), the negative effect of the loss of photosynthetic product to the fruit yield on a mature tree is still unknown. However, some scientific debate still exits regarding such consequences of ALCM larval feeding. Based on what Allison et al. (1995) found, photosynthetic rate of apple trees was not reduced by the loss of leaf area. Furthermore, it is believed the reproductive organs like fruit had the priority for the use of photosynthetic product over the vegetative organs. Therefore, in the short term, fruit yield is unlikely reduced by the limited accumulation of photosynthetic product (Allison et al., 1995).

Regardless of if fruit yield is reduced by leaf damage induced by ALCM on apple trees, the impact of larval feeding is much more destructive to young and newly grafted trees (Smith and Chapman, 1995a, b). More important, being a fruit contaminant, the principal risk posed by ALCM in the horticultural industry is that the ALCM larvae and pupae can trigger quarantine concerns when they are found on apple fruit. This occurs when mature larvae evacuating them in search of the pupation sites are accidentally dropped in the calyx end or the stalk of fruit (Todd, 1959; Lowe, 1993; Smith and Chapman, 1995b). Maximum Pest Limits (MPL) for ALCM is usually in the order of 0.5% for most exports markets. In other markets where no tolerance has been set, Ministry for Primary Industries (MPI) will not issue an Export Phytosanitary Certificate if the total infestation (sum of all organisms found) exceeds 2% MPL (usually based on a 600 fruit samples) (pers. comm. Dr. Jim Walker, Plant and Food Research, 2014). However, although the high rate of fruit contamination by ALCM is uncommon, zero tolerance quarantine regulations for ALCM contamination in some markets particularly where no ALCM introduced exists. To those markets, the presence of ALCM cocoons or larvae on fruits is considered non-acceptable. For instance, Japan will decline fruit importation if a single ALCM is found during quarantine inspection (He and Wang, 2011). In many orchards in New Zealand, the rate of fruit contamination by ALCM is relatively high. To meet the quarantine regulations of importing countries, the cost of postharvest fruit quality and pest control cannot be ignored to growers.

1.4 Apple leaf-curling midge management

The control of ALCM is very important to the New Zealand pipfruit industry, primarily because of fruit contamination at the point of export. In conventionally- managed orchards in New Zealand, synthetic insecticides had been frequently and intensively applied to control primary and secondary insect pests including ALCM. Amongst them, chlorpyrifos and azinphos-methyl were the two most widely used broad-spectrum organophosphate insecticides which were also toxic to a wide range of beneficial insects (O'Conner, 1998). With the dramatically increased incidence of ALCM in commercial apple orchards in New Zealand, Diazinon which is a broad spectrum organophosphate insecticide specifically applied to control ALCM has been used (Tomkins et al., 1994; Smith and Chapman, 1995a, b). However, ALCM is difficult to control with insecticides due to larvae typically hiding in the tightly rolled leaves (Batchelor et al., 1997; Walker et al., 1997).

In order to meet supermarket requirement, Integrated Fruit Production (IFP) programme was introduced in the New Zealand pipfruit orchards by NZAPMB which is now known as PNZI in 1996 (Wiltshire, 2003). The principles of IFP are based on European guidelines (Avilla, 1995), with emphases on the use of biological control agents and the adoption of environmentally friendly products (Walker et al., 1997). With the introduction of IFP programme, the use of synthetic insecticides has been greatly reduced leading to an ecologically safer and economical production system (Wiltshire, 2003). Under IFP programme, special monitoring of pest levels and life stages which depended on the sticky traps is applied to determine the most effective and efficient methods to control ALCM (Smith and Chapman, 1996; Tomkins et al., 2000; Shaw et al., 2005). For instance, young and newly grafted apple trees provide suitable environment on where the female adults lay eggs and on which larvae feed. Synthetic pesticides are only applied if more than half of the apple trees are infested with the ALCM eggs. Furthermore, the use of pheromones for mating disruption can also provide great suppression of the ALCM population (Suckling, 2000; Suckling et al., 2007; Suckling et al., 2008).

In addition, one biological control agent, *Platygaster demades* Walker (Hymenoptera: Platygastridae), was originally introduced to New Zealand to control the population of PLCM in 1925, and was first observed parasitizing ALCM in 1954 (Todd, 1956; He and

Wang, 2007). *P. demades* lay eggs into the ALCM eggs, and the eggs of *P. demades* start to hatch after the mature ALCM larvae commence pupation. The *P. demades* larvae feed and complete their development in the hosts and eventually destroy them (Todd, 1956; He et al., 2010).

In addition to *P. demades*, other biological control agents feeding on the eggs of ALCM were also introduced in New Zealand, including the mirid bug *Sejanus albisignata* (Knight) and bdellid mite (Shaw et al., 2003; Wearing et al., 2013). The study of Shaw et al. (2003) presented that great number of *S. albisignata* is associated with low ALCM damage. Furthermore, Wearing et al. (2013) observed its greater number on ALCM damaged apple shoots compared with undamaged shoots, and its positive relationship with the distribution of ALCM population on the different cultivars of apple. All evidences show that *S. albisignata* and ALCM synchronize which indicates the role of *S. albisignata* as a predator of ALCM. Shaw et al. (2003) found that the early season insecticides application has tremendous impact on the biological control of ALCM in orchards. According to their investigations, the orchards, where the selective insecticides are applied, have lower survival rate of ALCM larvae and less shoot damage, comparing to the orchard applied the non-selective insecticides at early season (Shaw et al., 2003).

1.5 Ultraviolet-C radiation as a non-chemical method

The growth of the New Zealand horticultural exports relies on the strict quarantine procedures which prevent the introduction of exotic pests and diseases into imported countries. Postharvest insect control is one of the critical aspects of this process (Jamieson et al., 2009). To meet the quarantine regulations of export markets and customers' expectations of low chemical residues on horticultural products, postharvest pest management needs to be adapted to not only control insect pests, but lead to no undesirable side-effects on product itself. However, current disinfestation treatment is heavily dependent upon the use of the fumigant methyl bromide. Being an ozone-depleting substance which is also toxic to humans, the use of methyl bromide is restricted worldwide by the Montreal Protocol (Bell et al., 1996; Fields and White, 2002; Hallman, 2004; Jamieson et al., 2009). Other postharvest treatments as safe alternatives have been identified with the increased restrictions of chemical residues on food, and the accumulated public food safety concerns. There are a wide range of non-chemical

postharvest disinfestation methods available to minimize the use of synthetic pesticides. Based on the characteristics of these techniques, they can be classified into two categories; one group stands for physical control methods, including heat and water treatments, modified atmosphere (MA), controlled atmosphere (CA) and radiation treatment, by killing or restricting the growth of organisms or strengthening the natural defence mechanisms of horticultural crops. The other approach is biological control which uses the natural enemies of diseases like microbial antagonists by either inhibiting the growth of pathogens or killing them. In these approaches, ultraviolet (UV) radiation is a promising and effective technique for the disinfestation of insect pests at the postharvest (pack house) stage (Tatiana K., 2008; Ribeiro et al., 2012). In addition, UV radiation can prolong the shelf life of fresh produce by disinfecting undesirable organisms (Artes et al., 2009).

UV radiation (10-400 nm) is a promising alternative to chemical treatments with the accumulated negative public reactions over the use of aggressive chemicals in the food chain. The use of UV radiation is not limited but well established in the areas of water treatment, air disinfection and surface decontamination (Tatiana K., 2008; Ribeiro et al., 2012). UV radiation has been studied and is now applied intensively over the last few years in the areas of food production. The ability of UV radiation to sanitize and retard microbial growth on the surface of fruits and vegetables without causing undesirable quality changes has recently been recognized. The wave band from 100 to 280 nm also known as UV-C is often called the germicidal range as it is effective to inactivate bacteria and viruses (Koutchma et al., 2009). The effect of UV-C radiation on the destruction of microorganisms occurs as the result of the significant damage of DNA after the penetration of UV-C radiation into the outer membranes of the cells, leading to cell death. Moreover, the DNA transcription and replication of microorganisms can be prevented via the formation of thymine dimers triggered by UV-C radiation (Bintsis et al., 2000; Miller et al., 1999; Bank et al., 1990).

Many studies have investigated the effect of UV-C radiation on the control of pests. One study investigates the influence of UV-C radiation on stored-product pests, and reveals the potential pest disinfestation effect of UV-C radiation (Collins and Kitchingman, 2010). For instance, pest species including the storage beetles *Oryzaephilus surinamensis* L. and *Tribolium castaneum* (Herbst), and the mites, *Acarus siro* L. and *Tyrophagus putrescentiae* (Schrank) are treated with UV-C radiation at

different doses (Collins and Kitchingman, 2010). The results of the experiment demonstrate the effect of UV-C radiation on the inhibited development of storage pests. In addition, the experiment also reveals the effective doses (ED) of UV-C radiation varied according to species, which the mites are more sensitive than the storage beetles (Collins and Kitchingman, 2010). In addition, Ernieenor et al. (2012) found the germicidal effect of UV-C radiation on the eggs and adult of dust mites *Dermatophagoides pteronyssinus* (Trouessart) and *D. farina* (Trouessart).

It has been also found that the low dose of UV-C radiation ranging from 0.25 to 8.0 kJ m^{-2} could drive regulatory effects on the DNA of microorganisms (Terry and Joyce, 2004). These regulatory effects are known as hormesis which is defined as the stimulation of beneficial reactions in biological organs by low doses of many potentially harmful agents (Luckey, 1980; Shama, 2007). A number of studies confirm that the resistance of horticultural commodities to postharvest decay is induced by the UV-C radiation treatment (Wilson et al., 1997; Stevens et al., 2005; Pombo et al., 2011). For instance, exposure to UV-C radiation is reported to reduce the postharvest decay such as bitter rot, *Colletotrichum gloeosporioides* in apple, brown rot, *Monilinia fructicola* (G. Winter) in peaches, and green mold, *Penicillium digitatum* (Pers.) in tangerines by enhancing the resistance of horticultural crops themselves, and suppressing the growth of microorganisms (Stevens et al., 2005). Pombo et al. (2011) investigated the impact of UV-C radiation treatment on strawberry fruit. The resistance to *Botrytis cinerea* (De Bary) is induced after exposure to UV-C (254 nm). The pathogenesis-related genes including phenylalanine ammonia lyase (PAL), peroxidase, β-1, 3-glucanase (GNS) and chitinase (CHI) of strawberry fruit are stimulated and activated by UV-C treatment (Pombo et al., 2011).

1.6 Project aims and objectives

The overall aim of this project was to evaluate the disinfestation effect of UV-C radiation treatment, as a promising and innovative physical method on the control of ALCM, which is a quarantine pest on postharvest apple fruit. The doses of UV-C radiation applied were devised and adjusted to reach maximum disinfestation effectiveness. UV-C radiation is a promising non-chemical treatment for the disinfestation of insect pests, which may lead to market barriers for the New Zealand's billion-dollar horticultural industry. The application of UV-C radiation for the control

of ALCM has not been previously studied, and this project could lead to a new tool in the control of unwanted organisms into the produce value chain.

The objectives of this project were to investigate:

1. Mortality of non-fruit attached ALCM in response to UV-C radiation treatment at a series of UV-C doses.
2. Mortality of fruit attached ALCM in response to UV-C radiation treatment at a series of UV-C doses.
3. Egg load of fecundity of ALCM female after being treated with UV-C radiation at the cocoon stage.
4. What are the critical limits for the process?

CHAPTER 2: UV-C TREATMENT OF NON-FRUIT ATTACHED APPLE LEAF-CURLING MIDGE

2.1 Introduction

ALCM has been observed to affect the foliage development of young and newly grafted apple trees, and more important, play an important role in causing significant quarantine concerns as an apple fruits contaminant for the New Zealand apple exporters (Barnes, 1948; Todd, 1959; Smith and Chapman, 1995a, b; He and Wang, 2007). Synthetic pesticides have been applied to control ALCM for decades in the New Zealand orchards, but the current protection is inadequate as the ALCM larvae hide in the curled leaves, allowing for larval deposition into the fruit calyx or the stem influenced by heavy rainfall, temperature and other factors (Walker et al., 1995; Shaw et al., 2005). Besides chemical approaches, non-chemical methods also known as safer alternatives to synthetic pesticides had been introduced to control pests and diseases in the last decade via Integrated Fruit Production (IFP) practice. Admittedly, the performance of these non-chemical methods cannot be expected to have the equal efficacy and consistency as synthetic pesticides to control pests and diseases. However, these methods play a significant role as environmentally friendly alternatives in controlling pests and diseases, and reducing the losses of horticultural crops.

But very little is known about how ALCM can be controlled by the non-chemical methods particularly by radiation in New Zealand. Radiation has the ability to kill bacteria and pathogens on the surface of horticultural crops once they pass through the radioactive sources. Radiation can be classified as either ionizing or non-ionizing sources. Gamma and X-ray are ionizing radiation, and UV is non-ionizing radiation. Radiation such as UV, gamma and X-ray, is a new technique which can reduce postharvest decay and kill insect pests. For instance, besides the direct disinfestation effect of UV radiation, it can also induce the resistance mechanisms of horticultural crops themselves (Pombo, 2011). UV radiation has been widely studied and applied to control pests and diseases of many horticultural crops (Wilson et al., 1997; Stevens et al., 2005; Collins and Kitchingman, 2010; Ribeiro et al., 2012).

Typically, the wavelength of UV ranging from 200 to 280 nm is also known as UV-C radiation which has germicidal or disinfestation effect as it is effective to inactivate bacteria and viruses by damaging their DNA (Koutchma et al., 2009).

The aim of this experiment was to investigate the disinfestation effect of UV-C radiation on non-fruit attached ALCM in terms of the mortality, parasitism and developmental duration of ALCM. Results obtained of which are vital to the understanding of the impact of UV-C radiation on the ALCM control.

2.2 Materials and methods

2.2.1 Site description

This experiment was conducted in a mature apple orchard at Massey Fruit Crops Unit and Entomology Laboratory in the Agriculture and Horticulture Building, Massey University, Palmerston North (40°4' S, 175°6' E).

2.2.2 Materials preparation

The UV-C radiation source applied in this experiment was 4x market-sourced fluorescent lamps (PHILIPS, TUV 30W/G30 T8, Made in Holland). TUV T8 lamps were double-ended UV-C lamps offering constant short-wave UV output. They were mounted in a modified array. The Optronics OL-756 Spectroradiometer (Optronics Laboratories, Florida, USA) was used to confirm the UV-C radiation. The dosage 100 mm under UV-C lamps was measured in three different positions which were 45 cm from end, 15 cm from near end, and 15 cm from far end of UV box, respectively. The average dosage across tube was equivalent to 28.4579 W $m^{-2}s^{-1}$. For UV-filtered treatment, the average dosage was 0.00064 W m^{-2} s^{-1}. The dose of each treatment is calculated and presented in Table 2.1.

Table 2.1: The dose of each UV-C radiation treatment in relation to the exposure time applied in the experiment.

Treatment	Irradiance in KJ m^{-2}	No. of dishes
10 mins-UV	17.0747	5
5 mins-UV	8.5274	5
2 mins-UV	3.4149	5
10 minsUV-Filtered	0.000382	4
10 Foil-Sheltered	0	4

ALCM larvae infested apple leaves were collected from a mature apple orchard at the Massey Fruit Crops Unit on March 18th and 21st 2013, and maintained in temperature-controlled storage room in Entomology Laboratory. The ALCM larvae were collected in the middle of March indicating these larvae belonged to the fourth generation which were also known as overwinter generation. After collection, the curled apple leaves were broken to free larvae in the lab. The mature larvae were placed into Petri dishes (5.5 cm diameter × 1.3 cm height) and were buried with river sand. Then, Petri dishes were maintained in a storage room at 20°C with a day length of 16 hours light. After a week's storage, Petri dishes were removed from the controlled environment to collect cocoons. Cocoons were extracted from river sand in a mesh under running water (20°C). Extracted cocoons were mixed, and then were randomly chosen and placed into 23 Petri dishes in total with 100 cocoons each.

For UV-C treatment, Petri dishes labelled from 1 to 15 were treated with UV-C radiation on a series of UV doses, respectively. These Petri dishes with cocoons were placed 100 mm below the fluorescent tubes. Specifically, Petri dishes labelled from one to five were treated with UV-C radiation for 2 mins; Petri dishes labelled from 6 to 10 were treated with UV-C radiation for 5 mins; Petri dishes labelled from 11 to 15 were treated with UV-C radiation for 10 mins. The left Petri dishes which labelled from 16 to 19 were wrapped in film excluding all wavelengths below 320 nm, and labelled from 20 to 23 were shielded from UV radiation, were treated as the control groups, respectively.

After the UV-C radiation treatment, all Petri dishes were maintained in a completely dark environment at 20°C with 50% humidity.

2.2.3 Data collection and analysis

To record the mortality of both ALCM and *P. demades*, 20 cocoons from each Petri dish were randomly chosen and then dissected in a drop of ringer's solution under a microscopy (Olympus Japan) 5 and 10 days after the UV-C radiation treatment, respectively. The parasitism of ALCM by *P. demades* was determined at the same time when the ALCM larvae were dissected.

The emergence of both ALCM and *P. demades* were monitored and recorded daily since the first day after the UV-C radiation treatment. Finally when no adults emerged

42 days after the UV-C radiation treatment, the left cocoons were dissected to detect the mortality and parasitism of both ALCM and *P. demades*.

The developmental duration of both ALCM and *P. demades* were also calculated depending on the emergence of both ALCM and *P. demades* according to time, respectively.

A goodness of fit test (Kolmogorov-Smirnov test) was used to test the distribution of data before analysis. The data on the mortality of unparasitized ALCM larvae amount those died individuals detected in the second dissection, and on the developmental duration of ALCM required to emerge after being buried in sand were not normally distributed even after transformation, and thus analyzed by using the non-parametric Kruskal-Wallis test followed by the Dunn's procedure for multiple comparisons. The other data were normally distributed and analyzed by using ANOVA followed by the multiple Turkey test. The mortality data were arcsine square-root transformed before ANOVA.

2.3 Results

Based on He and Wang's work in 2007, four generations of ALCM were detected during 2005-2007 seasons in Palmerston North (He and Wang, 2007). Previous work shows the parasitism and super parasitism rate of ALCM by *P. demades* significantly increase from the first to fourth generation as season progressed, and therefore the number of parasitoid eggs per parasitized ALCM larva is significantly greater in the third and fourth generations than that in the first and second generations (He and Wang, 2011).

In this experiment, given to the suitable storage conditions for the ALCM cocoons both before and after the UV-C radiation treatment, it was considered that the majority of the mortality of ALCM larvae was caused either by the parasitism by *P. demades* or by the UV-C radiation treatment. Nevertheless, even stored under the suitable conditions, a certain percentage of ALCM larvae died by the natural causes which was proved by the results collected in this experiment.

The mortality of ALCM was determined by the cocoons dissections 5, 10 and 42 days later following the UV-C radiation treatment. During dissections, the average number of

cocoons displaying the signs of parasitism was relatively high. In the first dissection (460 cocoons dissected regardless of treatments), 56.7% of cocoons were found being parasitized by *P. demades.* In the second dissection, it was found that up to 76.7% of cocoons out of 460 cocoons were parasitized by *P. demades*. The results obtained via dissections showed the high parasitism rate in the fourth generation of ALCM. However, as the parasitism of ALCM by *P. demades* could eventually cause the death of ALCM, in order to distinguish those two mortal factors, the mortality of parasitized ALCM which was caused by the parasitism by *P. demades*, and the mortality of unparasitized ALCM which was caused by UV-C radiation rather than parasitism were calculated separately. For each dissection, the total mortality, and the mortality of parasitized and unparasitized ALCM larvae were calculated separately. After the completion of three individual dissections, the total mortality of ALCM was calculated as well.

2.3.1 Mortality of ALCM in the first dissection

The mortality of non-fruit attached ALCM, and the total mortality of parasitized and unparasitized ALCM larvae were shown in Figure 2.1. There was no significant difference in the mortality of ALCM between treatments (ANOVA: $F = 1.99$, $df = 4, 18$, $P = 0.1385$). Obviously, the UV-C filtered group had the highest mortality rate followed by the group treated with 5 mins UV-C radiation. And the groups treated with 2 mins UV-C and UV-C shielded had the least mortality rate.

The mortality of parasitized ALCM caused by parasitism was significantly different between treatments (ANOVA: $F = 5.97$, $df = 4, 18$, $P = 0.0031$). It was quite evident that the UV-C filtered group had the highest mortality rate than the rest of groups. The group treated with 2 mins UV-C had the least mortality rate. Amongst the UV-C treated groups, there was no significant difference.

The mortality of unparasitized ALCM caused by UV-C radiation largely was also significantly different between treatments (ANOVA: $F = 5.31$, $df = 4, 18$, $P = 0.0053$). It was significantly higher in UV-C treated groups than in the control groups. There was no significant difference amongst the UV-C treated groups and the same in the control groups.

Based on the results of the first dissection, the mortality of ALCM caused by UV-C radiation rather than by parasitism in the UV-C treated groups was significantly higher than that of the control groups.

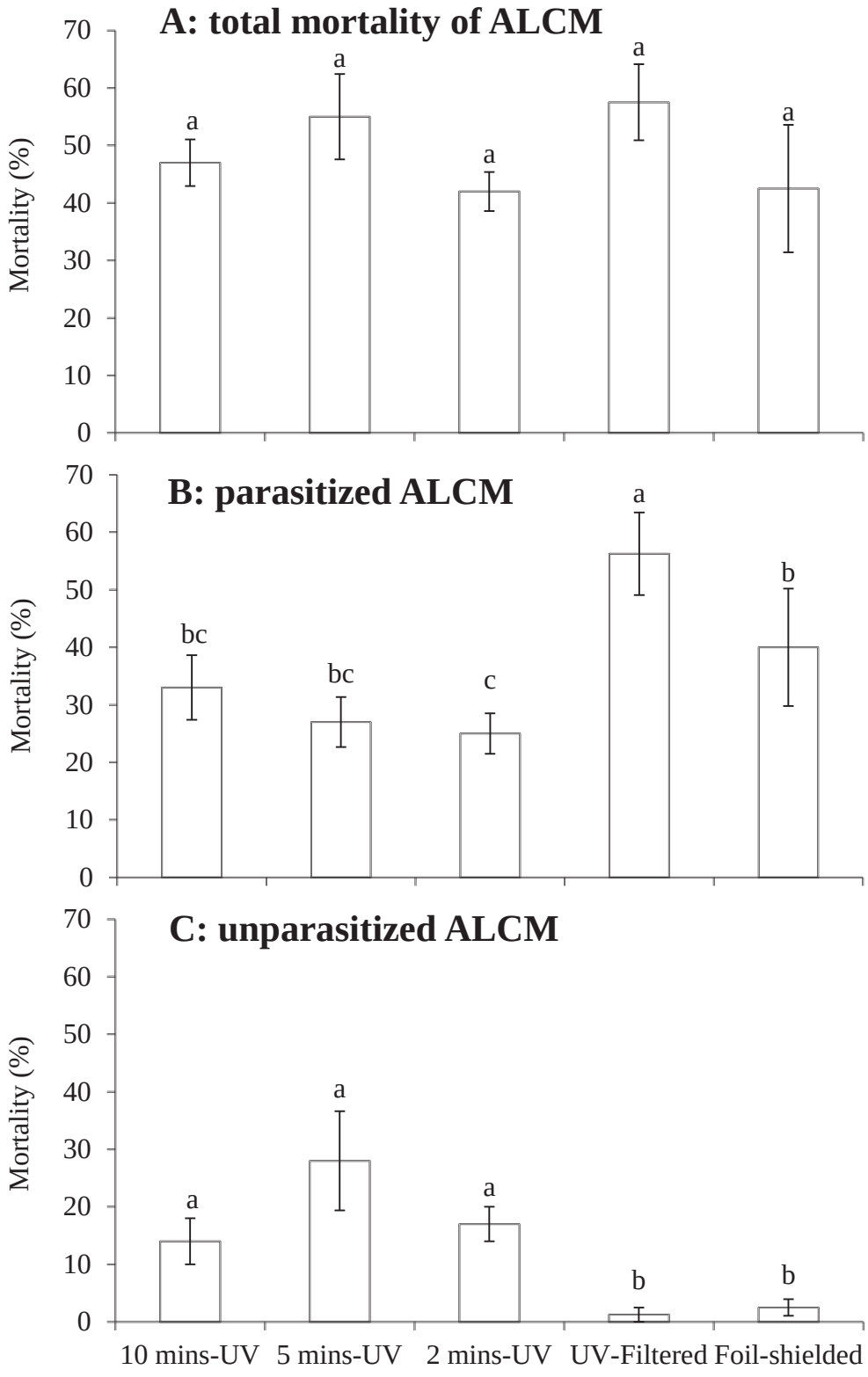

Figure 2.1: The mean (±S.E.) total mortality of ALCM larvae (A), parasitized ALCM larvae (B), and unparasitized ALCM larvae (C) in the first dissection, respectively. Columns with the same letters are not significantly different (P > 0.05).

2.3.2 Mortality of ALCM in the second dissection

As shown in Figure 2.3, the total mortality of ALCM was significantly different between treatments (ANOVA: $F = 3.83$, $df = 4, 18$, $P = 0.0202$). The two control groups had significantly higher mortality rate than that of the UV-C treated groups. The UV-C filtered group had the highest mortality rate, and the group treated with 5 mins UV-C had the least mortality rate. Amongst the control groups or the UV-C treated groups, there was no significant difference.

The mortality rate of parasitized ALCM had the similar pattern as that of the mortality of ALCM. The two control groups had significantly higher mortality rate than that of the UV-C treated groups. The group treated with 10 mins UV-C had the least mortality rate (ANOVA: $F = 4.86$, $df = 4, 18$, $P = 0.0078$).

Due to no unparasitized ALCM was found dead in the control groups during the second dissection, there was significant difference in the mortality of unparasitized ALCM caused by UV-C radiation between the UV-C treated groups and control groups (ANOVA: $F = 4.44$, $df = 4, 18$, $P = 0.0114$), even though the mortality rate of unparasitized ALCM in the UV-C treated groups was quite low.

Based on the results of the second dissection, it was found that the majority of the mortality of ALCM was contributed by the parasitism. Moreover, it was also observed that the UV-C radiation treatment had limited influence on the mortality of ALCM according to this dissection.

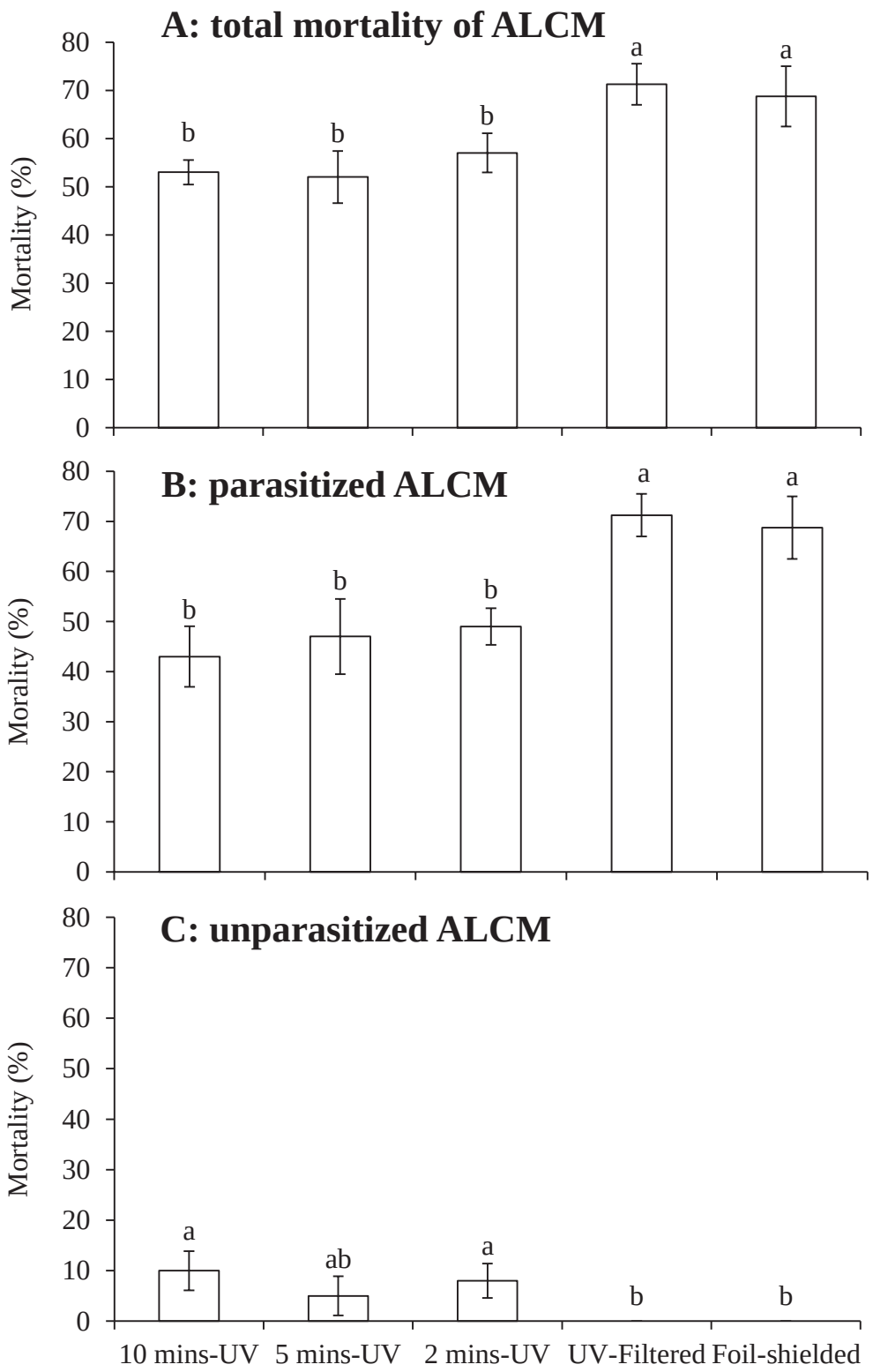

Figure 2.2: The mean (±S.E.) total mortality of ALCM larvae (A), parasitized ALCM larvae (B), and unparasitized ALCM larvae (C) in the second dissection, respectively. Columns with the same letters are not significantly different (P > 0.05).

2.3.3 Mortality of ALCM in the final dissection

At the final dissection, all of the left cocoons were dissected to determine the parasitism and mortality rate. Due to the large sample size comparing to the previous two dissections, the results obtained from this dissection were more persuasive and indicative regarding the influence of each treatment on the mortality of ALCM. The mortality of non-fruit attached ALCM, and the mortality of parasitized and unparasitized ALCM larvae were shown in Figure 2.4. The mean total mortality of ALCM was significantly higher in treatment of 10 mins UV-C than that detected in control treatments (ANOVA: $F = 4.03$, $df = 4, 18$, $P = 0.0166$). It also shows that the mortality rate of ALCM decreased with the diminution of UV-C dose which were represented by the exposure time.

The mortality of parasitized ALCM caused by parasitism was significantly higher in the 10 mins UV-C treatment than that in 2 mins UV-C and UV-shielded treatment (ANOVA: $F = 5.34$, $df = 4, 18$, $P = 0.0051$).

The mortality of unparasitized ALCM caused by UV-C radiation largely was significantly higher than that in UV-filtered treatment (ANOVA: $F = 3.06$, $df = 4, 18$, $P = 0.0435$) with no significant difference detected between the UV-C treatments or between control treatments. The same as the previous two dissections, the mortality of unparasitized ALCM in the UV-C treated groups was significantly higher than that of the control groups. The UV-C treated groups had the highest and nearly same mortality rate of unparasitized ALCM. In the control groups, although they were not treated with UV-C radiation, they had a comparably higher mortality rate comparing to that of the control groups of previous two dissections.

Based on the results of the final dissection, the majority of the mortality of ALCM was caused by the UV-C radiation treatment rather than the parasitism. Therefore, in this dissection which had large sample size, to some degree, it shows that the UV-C radiation treatments had a notable influence on the mortality of ALCM which was significantly different to the UV filtered group.

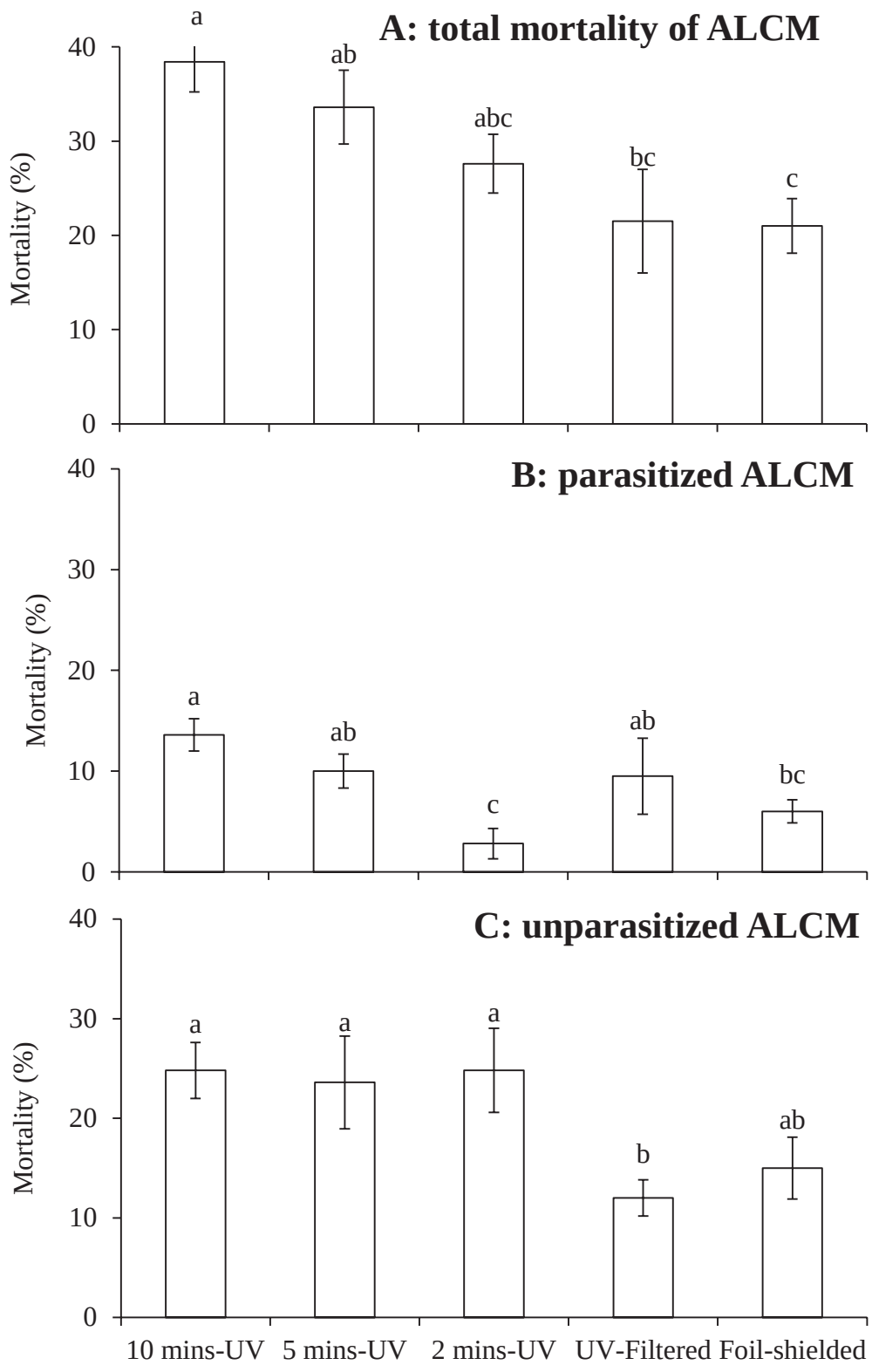

Figure 2.3: The mean (±S.E.) total mortality of ALCM larvae (A), parasitized ALCM larvae (B), and unparasitized ALCM larvae (C) in the final dissection, respectively. Columns with the same letters are not significantly different (P > 0.05).

2.3.4 Total mortality of non-fruit attached ALCM

The total mortality of non-fruit attached ALCM, and the total mortality of parasitized and unparasitized ALCM larvae were shown in Figure 2.1. There was no significant difference in the total mortality of ALCM between treatments (ANOVA: $F = 2.04$, $df = 4, 18$, $P = 0.1315$). Nevertheless, the group treated with 10 mins UV-C radiation had the highest mortality rate followed by the group treated with 5 mins UV-C radiation, as compared to the rest of groups. The UV-C shielded group had the lowest mortality rate. It shows that the UV-C radiation treatment had the potential benefit in the control of ALCM, although results obtained from this experiment were not that remarkable.

In terms of the total mortality of parasitized ALCM caused by parasitism, there was significant difference between treatments (ANOVA: $F = 6.97$, $df = 4, 18$, $P = 0.0014$). In total, the UV-C filtered group had the highest mortality rate of parasitized ALCM followed by the UV-C shielded group. The group treated with 2 mins UV-C radiation had the least mortality rate. The mortality rate of UV-C filtered group was significantly different from the UV-C treated groups. There was no significant difference between the control groups which were UV-C filtered and shielded groups. Amongst the UV-C treated groups, the group treated with 10 mins UV-C radiation had the highest mortality rate. There was no significant difference between the groups treated with 10 and 5 mins UV-C radiation.

The total mortality of unparasitized ALCM caused by UV-C radiation was significantly different between treatments (ANOVA: $F = 8.17$, $df = 4, 18$, $P = 0.0006$). The UV-C treated groups had a significantly higher mortality rate of unparasitized ALCM than that of the control groups. There was no significant difference amongst the UV-C treated groups or the control groups.

Based on the overall results of three individual dissections, the mortality of ALCM caused by UV-C radiation rather than by parasitism in the UV-C treated groups was significantly higher than that of the control groups. However, the mortality of ALCM caused by parasitism in the control groups was higher than that of the UV-C treated groups. Hence, there was no significant difference between treatments in terms of the total mortality of ALCM.

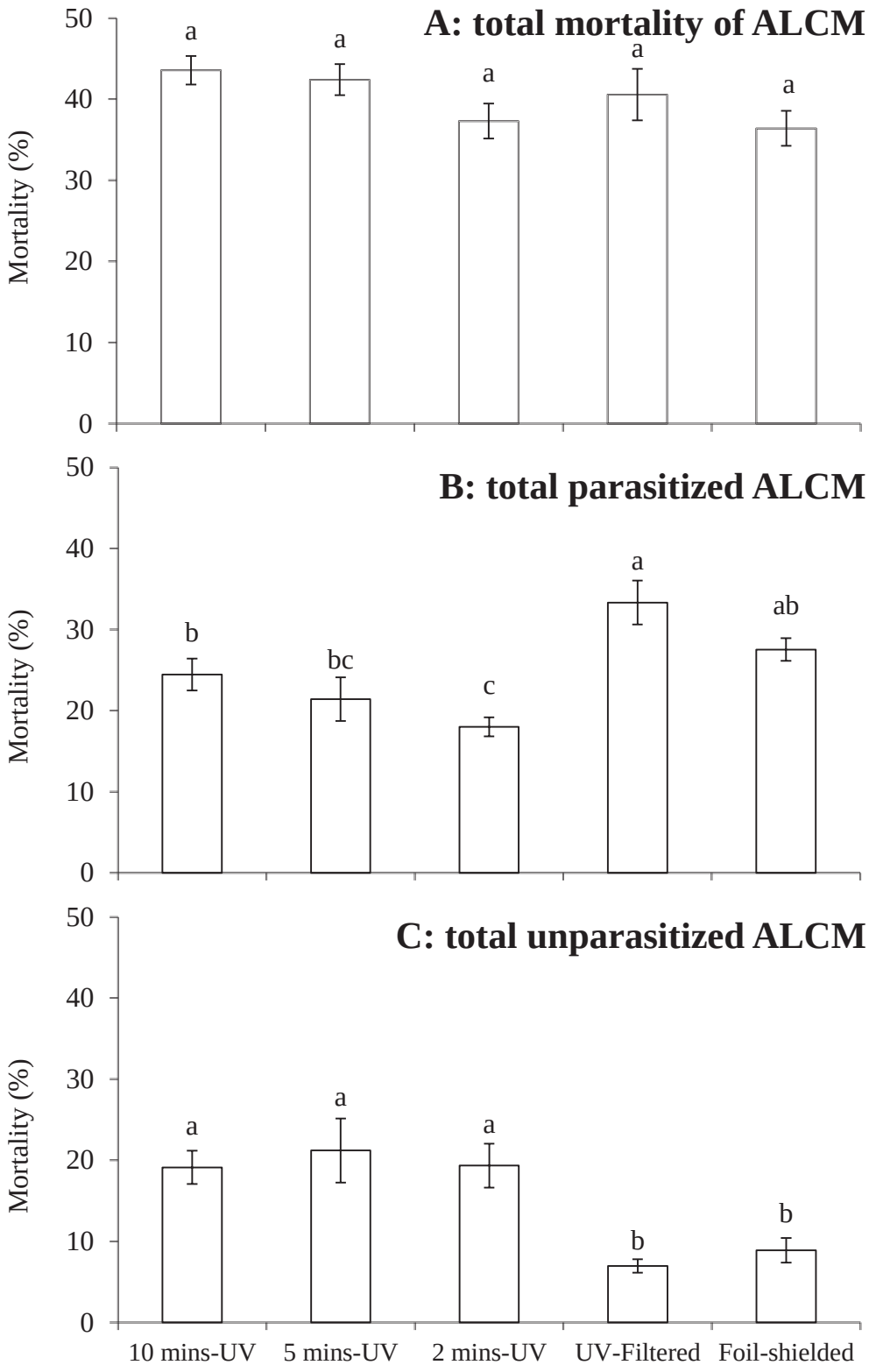

Figure 2.4: The mean (±S.E.) total mortality of ALCM larvae (A), total parasitized ALCM larvae (B) and total unparasitized ALCM larvae (C) in the whole experiment, respectively. Columns with the same letters are not significantly different (P > 0.05).

2.3.5 Emergence patterns of ALCM and *Platygaster demades*

The total number of ALCM and *P. demades* emerged was shown in Table 2.2. The emergence patterns of both species since the first adult emerged were shown in Figure 2.5.

Table 2.2: The total number of ALCM adults and *P. demades* adults emerged and the emergence rates.

	ALCM		*P. demades*	
Treatment	**emerged**	**% emerged**	**emerged**	**% emerged**
10 mins UV	14	2.80%	13	2.60%
5 mins UV	26	5.20%	10	2.00%
2 mins UV	33	6.60%	19	3.80%
UV-Filtered	68	17.00%	23	5.75%
Foil-shielded	75	18.75%	11	2.75%

For ALCM, the emerged adults were firstly found in the 2 mins UV-C, UV-C filtered, and UV-C shielded groups 8 days later since the UV-C treatment. At that date, in total, one, eleven, and thirteen adults were found emerged in those groups, respectively. The first adult of the group treated with 5 and 10 mins UV-C emerged 13 and 14 days after the UV-C treatments, respectively. Moreover, the majority of the emergence of ALCM in the control groups occurred in the first three or four days. And the maximal emergence of ALCM in the groups treated with 5 and 10 mins UV-C was occurred about 9 days later after the first adult emerged. For ALCM from the group treated with 2 mins UV-C, they started to emerge as early as that from the control groups, and they reached the maximal emergence rate at the time when the ALCM adults from the groups treated with 5 and 10 mins UV-C started to emerge. The emergence period from the first to the last emerged adult of ALCM in the groups treated 2 and 5 mins UV-C lasted approximately 17 days which was longer than that of the other groups lasted approximately 9 days.

For *P. demades*, the first adult was found 21 days after the UV-C treatment. At that date, one and three adults were found to emerge in the 5 mins UV-C and the UV-C filtered groups, respectively. During the first five days since the first *P. demades* adult emerged, no *P. demades* adults were found to emerge in the UV-C shielded group. There was no maximal emergence rate found in all five groups.

Based on the observation in this experiment, total number of ALCM adults emerged was higher in two control groups than in the UV-C treated groups. The group treated with 10 mins UV-C had the least number of ALCM adult emerged.

For the total number of *P. demades* emerged, there was no difference between treatments. Nevertheless, the group treated with 2 mins UV-C and the UV-C filtered group had the highest number of adults emerged.

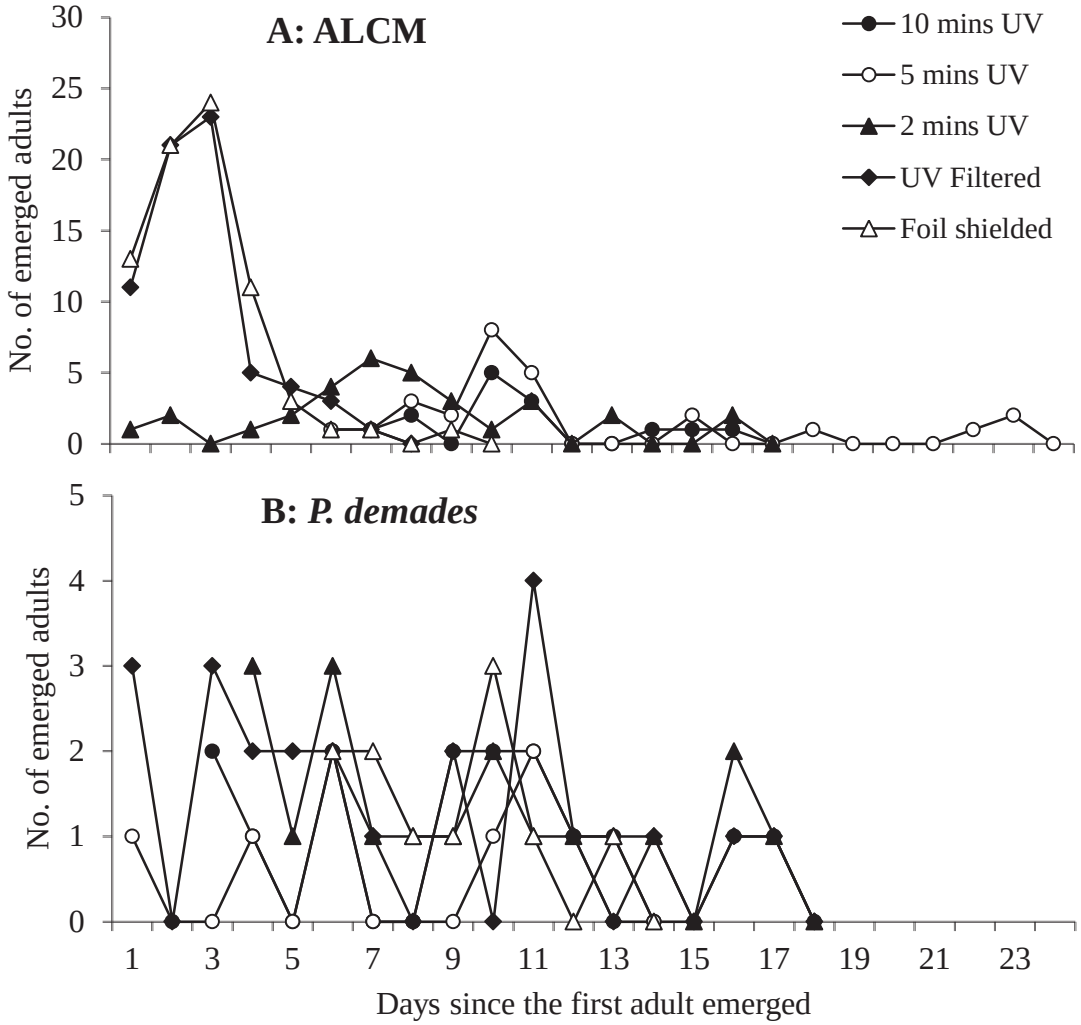

Figure 2.5: The emergence patterns of ALCM (A) and *P. demades* (B).

2.3.6 Developmental duration of ALCM and *Platygaster demades*

For the developmental duration of both ALCM and *P. demades*, they were calculated depending on the total emerged number of ALCM and *P. demades* according to time and were shown in Figure 2.6.

The developmental duration of ALCM and *P. demades* was shown in Figure 2.6. ALCM larvae took significant longer time to develop to adults when treated with UV-C radiation than that in the control groups (ANOVA: $F = 94.45$, $df = 4, 211$, $P < 0.0001$).

There was no significant difference between in developmental duration of *P. demades* between each treatment (ANOVA: $F = 0.83$, $df = 4, 70$, $P = 0.5117$). *P. demades* from each group had the similar developmental duration.

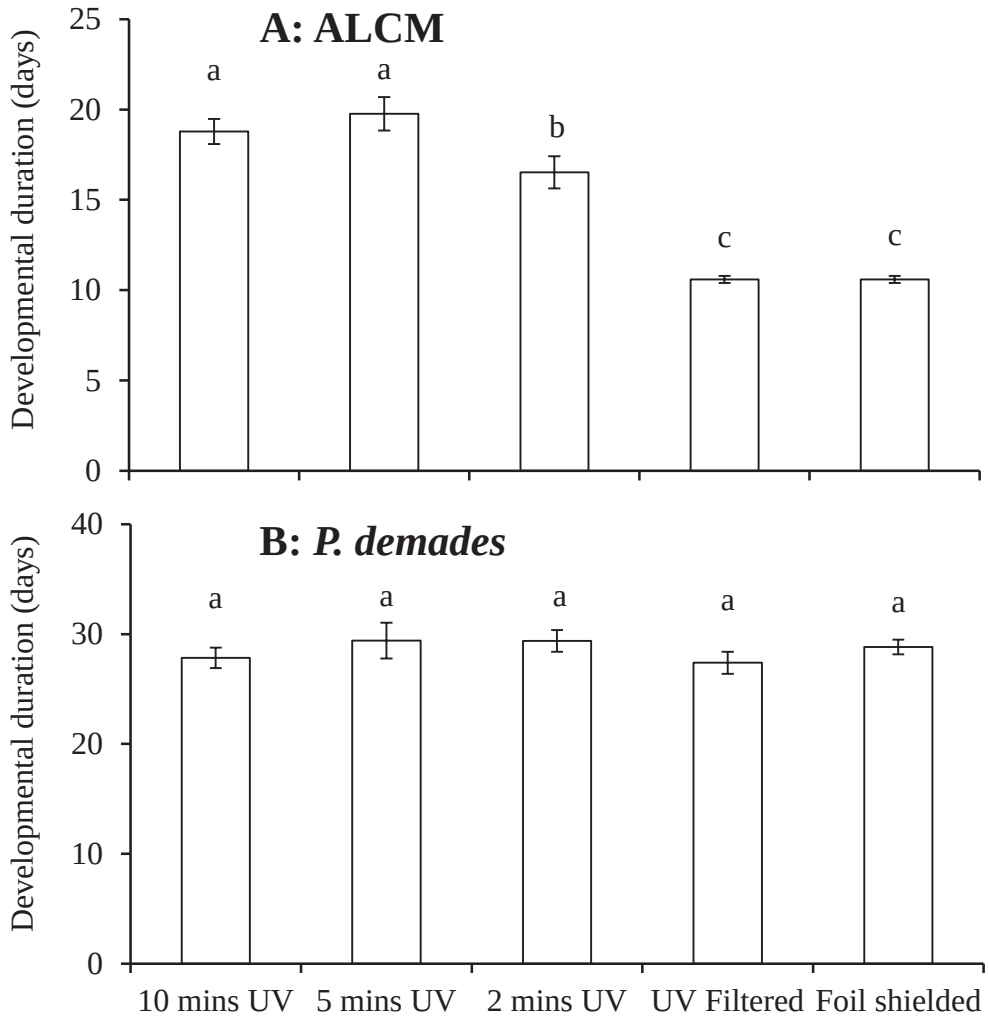

Figure 2.6: The mean (±S.E.) developmental duration of ALCM (A) and *P. demades* (B) from being buried in the sand after treatments to emergence. Columns with the same letters are not significantly different ($P > 0.05$).

2.4 Discussion

The mature ALCM larvae were collected in late March which belonged to the fourth generation of ALCM and was the major source of apple fruit contamination (He and

Wang, 2007). Generally, the disinfestation effect of UV-C radiation on the destruction of microorganisms or insect pests is triggered after the penetration of UV-C radiation into the outer membranes of cells, leading to cell death (Bintsis et al., 2000; Miller et al., 1999; Bank et al., 1990). However, the silk cocoon is the natural protective system of ALCM pupa against possible attacks from the outside during pupation, and it was observed that a thin layer of river sand was attached on the outer layer of cocoons. It made the study of the ability of UV-C radiation penetrating through both the outer layer of river sand and the silk cocoon essential, as the results obtained from the experiment are possibly compromised.

Many studies have been undertaken to investigate the microstructures and mechanical properties of silk cocoons in many aspects (Zhao et al., 2005; Zhao et al., 2007; Teshome et al., 2012). The roles of the cocoons of many insects have been studied as well. It is found that the cocoon structure of some insect species vary seasonally (Danks, 2004). Silk cocoon represents a distinctive and vital role in nature providing a range of protective and ecological functions with the optimum microstructures and mechanical properties (Zhao et al., 2005; Teshome et al., 2012). For instance, a silkworm cocoon was composed of three parts, the outermost floss, middle compact layers and innermost pelade. These three parts have different microstructures, and play different functions (Zhao et al., 2007). A silk cocoon contains two proteins, the fibroin and sericin (Teshome et al., 2012). Peigler (1993) found that the cocoons of *G. postica* and *E. bauhiniae* were packed with calcium oxalate crystals. Calcium oxalate, which was a chemical compound, played a significant role in heat absorption and light transmission (Peigler, 1993).

Based on other studies and the finding of this experiment, the ALCM larvae collected in this experiment was the overwinter generation which was believed the main fruit contaminant rather than the other generations, the roles of cocoon of this generation needed to be considered. Despite of the significant mortality of unparasitized ALCM observed, it would be interesting to investigate the characteristics of cocoons of the larval stage of ALCM in response to seasonal change and UV-C radiation as no such research had been done yet.

There is no significant difference in the total mortality of ALCM between treatments (Figure 2.4 A). However, the groups treated with 10 and 5 mins UV-C appeared to have

higher mortality rates than the rest of groups. Furthermore, the mortality of unparasitized ALCM which was largely caused by UV-C radiation in three individual dissections with all three UV-C treated groups having significantly higher mortality rates than those of the control groups (Figure 2.4 C). These results have three implications. Firstly, excluding the marked influence of parasitism on the mortality of ALCM, it is clear that UV-C radiation has a small to moderate influence on the mortality of ALCM. Thus UV-C radiation may be a potential physical disinfestation method applied in the postharvest apple packing house. Admittedly, the highest mortality rate of ALCM was observed in the groups treated with longer period and thus higher doses of UV-C radiation, but extending treatment time was not feasible and applicable in the packing house. However, the dosage of UV-C sources applied in this experiment was not the maximum. The UV-C sources definitely can be strengthened to reach the optimal and ideal disinfestation effect at the packing house stage. Thus, further studies are needed to investigate the optimum dose of UV-C radiation demanded to reach the highest mortality rate of ALCM leading to the maximal disinfestation effect.

It has been found that the low doses of UV-C radiation ranging from 0.25 to 8.0 KJ m^{-2} could drive regulatory effect on the DNA of microorganisms (Terry and Joyce, 2004). Numerous studies confirm that the resistance of horticultural products to postharvest decay can be induced by the UV-C radiation treatment (Wilson et al., 1997; Stevens et al., 2005; Pombo et al., 2011). However, the range of doses of UV-C radiation used in this experiment was from 3.4 to 34.14 KJ m^{-2}. The optimal disinfestation dose of UV-C radiation could exceed the beneficial hormesis doses which are from 0.25 to 8.0 kJ m^{-2} (Terry and Joyce, 2004). Hence, further investigations regarding the effects of the high doses of UV-C radiation on the postharvest quality of apple fruits would be highly recommended.

The results apparently indicate the significant effect of UV-C radiation on the delayed emergence of ALCM adults from cocoons. Moreover, the total number of ALCM adults emerged in the control groups outweighs that in the groups treated with UV-C radiation. By contrast, there is no influence of UV-C radiation on the emergence of *P. demades* adults. With the delayed emergence of ALCM, some external and/or internal factors

which could negatively affect the survival rate of ALCM larvae/pupae might be triggered.

Previous study shows that the parasitism and super parasitism rate of ALCM by *P. demades* significantly increase from the first to the fourth generation as season progresses, and the number of parasitoid eggs per parasitized ALCM larva is significantly greater in the third and fourth generations than that in the first and second generations (He and Wang, 2011). In this experiment, results of dissections indicate the parasitism and super parasitism of the fourth generation of ALCM by *P. demades* is relatively high. Thus the role of *P. demades* in the control of ALCM cannot be ignored.

To be conclusive, the higher dose of UV-C radiation had considerably greater disinfestation effect than the lower dose of UV-C radiation on the mortality of ALCM. However, the low dose of UV-C radiation is sufficient to drive regulatory effect on the DNA of microorganisms, leading to the improved postharvest quality of vegetables and fruits (Terry and Joyce, 2004; Wilson et al., 1997; Stevens et al., 2005; Pombo et al., 2011). Thus, the further investigations would be merited regarding the effects of the high dose of UV-C radiation on the postharvest quality of apple fruits. Besides, for the management of ALCM in orchards, the introduction of natural enemies of ALCM has reduced the need of chemical insecticides intervention. The as-yet untested benefits of combining plant resistance, natural predators with UV-C radiation deserve further investigations for better and comprehensive ALCM management.

CHAPTER 3: UV-C TREATMENT OF FRUIT ATTACHED APPLE LEAF-CURLING MIDGE

3.1 Introduction

ALCM is a small fly with four life stages: egg, larva, pupa and adult. The ALCM larvae and pupae have considerable influence on the young and newly grafted apple trees. For instance, the larval feeding can damage the terminal leaves of apple tree shoots, leading to the delayed or stunted structural development of nursery or young trees. More important, being a quarantine pest, the mature larvae and pupae can be caught on apple fruits causing quarantine concerns, leading to the economic losses (Barnes, 1948; Todd, 1959; Smith and Chapman, 1995a, b; He and Wang, 2007). Although the ALCM larval feeding could reduce leaf area, it is unlikely to decrease the yield of mature apple trees (Allison et al., 1995). In fact, there is more foliage than enough demanded for the fruit production in commercial apple orchards. By contrast, the impact of ALCM injury induced on the nursery and young trees is significant. The reduction in leaf area delays or stunts the foliage development resulting in the failure of extension or success (Smith and Chapman, 1995a, b).

In terms of the apple fruit contamination by the ALCM larval/pupal cocoons, it occurs when the mature larvae trying to move from trees to the ground for pupation are caught in the stalk or the calyx of fruits (Smith and Chapman, 1995). Based on He and Wang's work, the main period of activity resulting in fruit contamination is the third and fourth generation of ALCM (He and Wang, 2007). Fruit contamination by ALCM cocoons has no harmful effect to fruit itself, but it can cause quarantine problems with countries that have no ALCM. The apple fruits exported to Japan will be rejected if ALCM is found (Lowe, 1994). Apples exported to Australia from New Zealand are required to be assessed for fruit contamination, and the MPL should less than 0.5%. Furthermore, treatment is required if ALCM is detected on fruit exported to China, Taiwan, India, and California (New Zealand Government, 2008).

It is known that UV radiation can the prolong shelf life of fresh produce by disinfecting inimical organisms (Artes et al., 2009). With the recognition of the ability of UV-C radiation in sanitizing and retarding the microbial growth, the application of UV-C

radiation has been well established for water treatment, air disinfection and surface decontamination (Tatiana, 2008; Ribeiro et al., 2012).

Our previous experiment indicated that the UV-C radiation treatment has a notable influence on the mortality of non-fruit attached ALCM cocoons, and it significantly delays the emergence of ALCM as well. I carried out this experiment to further investigate the disinfestation effect of UV-C radiation in the control of fruit attached ALCM cocoons in relation to exposure time, and to determine the preferable treatment time. In addition, the effect of UV-C radiation in the postharvest quality of apple fruit was estimated at the same time.

3.2 Materials and methods

3.2.1 Site description

This experiment was conducted in Entomology Laboratory in the Agriculture and Horticulture Building, Massey University, Palmerston North ($40^{o}4$' S, $175^{o}6$' E).

Apple samples used in this experiment were collected in an orchard in Waikato area, which were supplied by Pipfruit NZ Inc and PickMee Fruit Company in Hamilton.

3.2.2 Materials preparation

'Braeburn' apples were collected at an orchard in Hamilton in 26th, April. Afterwards, they were washed under high pressure water and stored in a cold storage room at PickMee Fruit Company packing house. On the 16th of June 2013, 100 'Braeburn' apples contaminated with ALCM cocoons were selected and sent to Massey University by PickMee Fruit Company. Upon arrival of those apples, they were immediately stored in a cold storage room at 3 ^{o}C. Before the UV-C radiation treatment, each apple supposed to be contaminated by the ALCM cocoon(s), was undergone visual inspection to check the status of contamination and the number of cocoons.

The UV-C radiation source applied in this experiment was 4x market-sourced fluorescent lamps (PHILIPS, TUV 30W/G30 T8, Made in Holland). TUV T8 lamps were double-ended UV-C lamps offering constant short-wave UV output. They were mounted in a modified array. The Optronics OL-756 Spectroradiometer (Optronics Laboratories, Florida, USA) was used to confirm the UV-C radiation dosage. The

dosage 100 mm under UV-C lamps was measured in three different positions which were 45 cm from end, 15 cm from near end, and 15 cm from far end of UV box, respectively. The average dosage across tube was equivalent to 28.4579 W $m^{-2}s^{-1}$. For UV-filtered treatment, the average dosage was 0.00064 W m^{-2} s^{-1}. The dose of each treatment was calculated and presented in Table 3.1.

Table 3.1: The dose of each UV-C radiation treatment in relation to the exposure time applied in the experiment.

Treatment	Irradiance in KJ m^{-2}	No. of apples
20 mins-UV	34.1494	15
10 mins-UV	17.0747	10
5 mins-UV	8.5274	10
2 mins-UV	3.4149	10
10 mins Foil-Sheltered	0	10
20 mins Foil-Sheltered	0	15

In this experiment, two individual trials were carried out to assess the effect of UV-C radiation treatment on the mortality of apple fruit-attached ALCM cocoons, and to assess the effect of UV-C radiation on the postharvest quality of apple fruits. All apple samples were facing the UV-C lamps on the calyx side in where the ALCM cocoons were attached.

For the first trial, 40 apples were randomly chosen to undergo different treatments. Amongst them, apples labelled from 1 to 30 were treated with UV-C radiation on a series of UV doses. These apples attached with cocoons were placed 100 mm under the fluorescent tubes. Specifically, apples labelled from 1 to 10 were treated with 2 mins UV-C radiation; apples labelled from 11 to 20 were treated with 5 mins UV-C radiation, and apples labelled from 21 to 30 were treated with 10 mins UV-C radiation. The left 10 apples labelled from 31 to 40 were shielded from UV-C radiation as the control group for 10 mins. After the UV-C radiation treatment, all apples were placed in separate containers and maintained in a completely dark incubator at 20°C.

For the second trial, 30 apples were randomly chosen from the unused samples to undergo different treatments. Thirty apple samples were randomly divided into two groups with 15 apples in each group. These apples attached with cocoons were placed 100 mm under the fluorescent tubes. One group was treated with 20 mins UV-C

radiation. The other group was shielded from UV-C radiation as the control group for 20 mins as well. After the UV-C radiation treatment, all apples were placed in separate containers and maintained in a completely dark incubator at 20°C. The aim of the second trial was to investigate the effect of prolonged UV-C radiation on the mortality of ALCM comparing to that of the first trial.

The postharvest quality was evaluated during the second trial. The skin alteration of each apple fruit was recorded periodically. Skin alteration in this trial could be induced by decay, rot or other factors. At the end of this experiment, fruit firmness of all apple were measured by using Fruit Pressure Tester (FT 327).

3.2.3 Data collection and analysis

For both trials, the emergence of both ALCM and *P. demades* adults were monitored and recorded daily since the first day after the UV-C radiation treatment. Parasitism of ALCM by *P. demades* was determined at the same time when the ALCM larvae were dissected.

The apple fruit firmness was measured 25 days later after treatment. As each fruit was not of uniform firmness, generally, the blush side was firmer than the green side. Thus each apple was tested at both the blush and green sides, and the average readings were calculated as well. Because skin would distort a pressure test on an apple, the surface of apple fruits tested was peeled by using stainless steel peeler.

Data were analyzed by using Chi-square (multiple-test) followed by the Marasculio procedure (Daniel, 1990) for multiple comparisons.

3.3 Results

In the period of 25 days storage after the UV-C radiation treatment, for the first trial, two ALCM adults were found to have emerged from both the 5 and 10 mins UV-C treated apples. For the second trial, two ALCM adults and one parasitoid wasp adult were found emerged. Unlike the first trial, all adults emerged from the control groups. Based on the observations of emergence of both ALCM and parasitoid adults, results indicated that the high dose of UV-C radiation showed a better disinfestation effect compared to the lower dose of UV-C radiation. Admittedly, as no adult emerged from

the control group in the first trial, and due to small sample size in this experiment, further study is needed to confirm the greater disinfestation effect of high UV-C radiation dose comparing to that of low UV-C radiation dose.

3.3.1 Total mortality of fruit attached ALCM

Figure 3.1 showed the total mortality of fruit attached ALCM, and the mortality of parasitized and unparasitized ALCM, respectively. There was no significant difference in total mortality of ALCM between treatments (df = 5, P = 0.8017) (Figure 3.1 A). Nevertheless, the group treated with 20 mins UV-C radiation had the highest mortality rate followed by the control group, which was treated with 10 mins UV-C filtered radiation. The group treated with 2 mins UV-C radiation had the least mortality rate. Results indicated that the high dose of UV-C radiation had potential benefit in the control of ALCM, although outcome was not that remarkable comparing with the control groups. Of course, 20 mins of UV treatment was a significant treatment time, the small sample size and too many treatments undertaken in this experiment might account for the non-significant difference between treatments.

The total mortality of ALCM caused by parasitism was not significantly different between treatments (df = 5, P = 0.1094) (Figure 3.1 B). However, 20 mins and 10 mins UV-C radiation treated groups had the highest mortality rate of parasitized ALCM followed by the control group treated with 10 mins UV-C filtered radiation. The groups treated with 5 mins UV-C radiation and 20 mins filtered UV-C radiation had the least mortality rate. It was interesting that the parasitized insects would appear somewhat more vulnerable to UV here comparing to the non-fruit attached trials.

The total mortality of unparasitized ALCM caused by UV-C radiation was also not significantly different between treatments (df = 5, P = 0.8937) (Figure 3.1 C). The group treated with 20 mins filtered UV-C had the highest mortality rate of unparasitized ALCM followed by the group treated with 5 mins UV-C. Interestingly, the mortality rate of the group treated with 20 mins UV-C radiation was neither high nor low. Moreover, the control groups had notable high mortality rate comparing to the UV-C treated groups. These findings were possibly influenced by low sample size and too many treatments, thus further investigation is required to assess the observation in this trial.

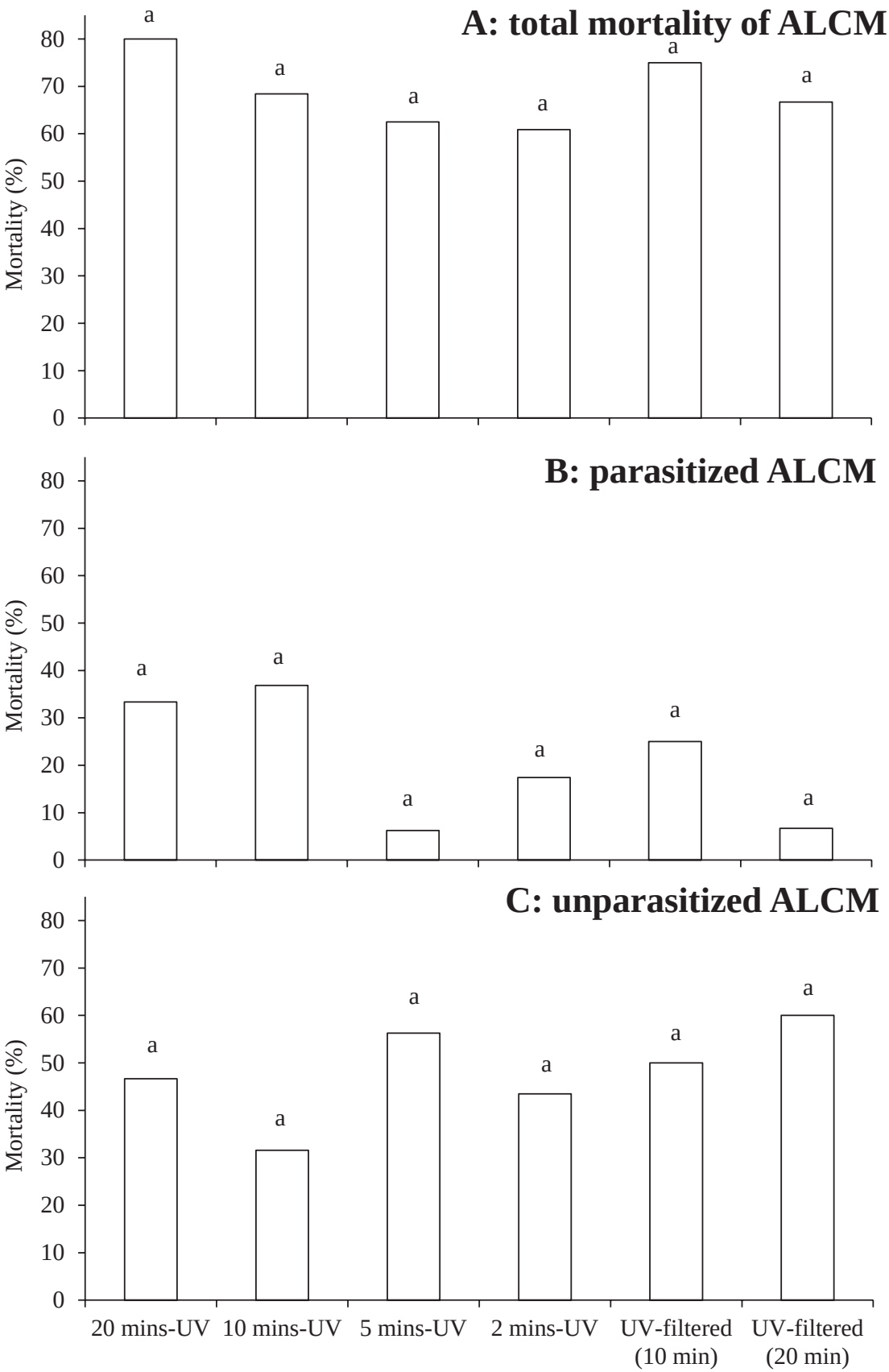

Figure 3.1: The total mortality of fruit attached ALCM larvae (A), parasitized ALCM larvae (B) and unparasitized ALCM larvae (C) in the whole experiment, respectively.

When considering the overall results, due to the high parasitism rate in the ALCM cocoons, the small sample size, and high number of treatments used in this experiment, results of the total mortality of unparasitized ALCM couldn't show any disinfestation effect of UV-C radiation in the control of ALCM. The UV treatment method can be amended to further investigate this. For instance, the application of higher UV dose treatment, and/or with the use of an adjuvant/co-disinfectant method would be recommended.

3.3.2 Proportion of ALCM larvae

Excluding the mortality of fruit attached ALCM caused by *P. demades*, Figure 3.2 which was the proportion of dead ALCM larvae amount those ALCM detected in dissection was established to illustrate the disinfestation effect of UV-C radiation in the control of ALCM. There was significant difference in the proportion of dead ALCM larvae between treatments (df = 5, P = 0.0192). The group treated with 20 mins UV-C radiation had the highest mortality rate than the rest of groups. Interestingly, the mortality rate of ALCM from the control groups was considerably higher than that of groups treated with 2 mins, 5 mins, and 10 mins UV-C radiation. Amongst groups treated with UV-C radiation, there was a trend that the mortality of ALCM gradually increased with the increased UV-C exposure time, regardless of the mortality rate of the group treated with 10 mins U-C.

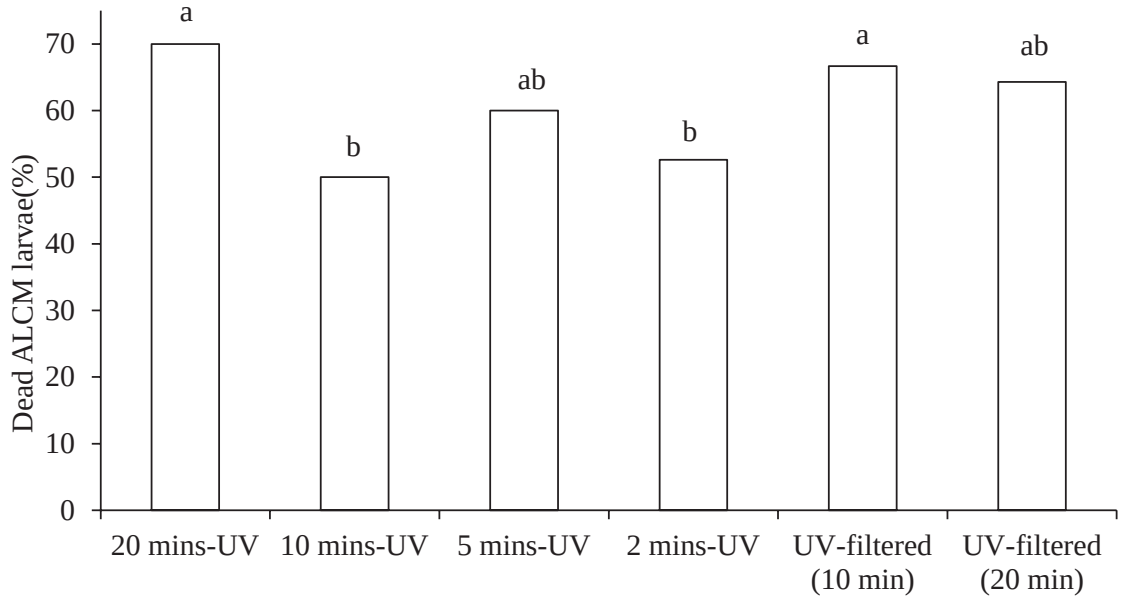

Figure 3.2: The proportion of dead ALCM cocooned larvae detected in dissection.

3.3.3 Postharvest quality of apple fruit

There were two apple fruits found rotted in the group treated with 20 mins UV-C radiation. In the control group of 20 mins UV-filtered, six apple fruits were rotted.

The average readings and the standard error of both blush and green sides were calculated as shown in Table 3.1, respectively. There was no significant difference in fruit firmness between treatments either in blush side or green side.

Table 3.2: Average apple fruit firmness tested by Fruit Pressure Tester (kg).

Treatment	Average blush side	Average green side
UV-C	7.15±0.35	5.53±0.35
UV-filtered	6.17±0.32	5.19±0.38
$F_{1,19}$	0.81	0.44
P	0.3798	0.5156

3.4 Discussion

Sandanayaka and Charles (2006) thought that the main cause of apple fruit contamination by ALCM could be the fourth generation of ALCM as the first three generations emerged normally during the season (Sandanayaka and Charles, 2006). 'Braeburn' apple samples used in this experiment were picked at an orchard in Waikato in early April. He and Wang (2007) also observed that the ALCM cocoons attached in the calyx of apple fruits in late March to April are belonging to the fourth generation of ALCM. Apple samples were washed under high pressure water, and then stored in a cold storage room. Two month later, apple samples, which were contaminated by ALCM cocoons, were delivered to Massey University Palmerston North, and stored in a temperature controlled room at 3 ^{o}C before experiment. The time and conditions from pick to store, through transit and experimental trial, were likely to have affected apple fruits and ALCM cocoons conditions considerably.

In this experiment, the first trial was carried out to further assess the disinfestation effect of UV-C radiation on the mortality of fruit attached ALCM. The total mortality of attached ALCM was not significantly different between treatments. However, after excluding the mortality caused by parasitism, there was difference between treatments (Figure 3.2). Both results show that the group treated with 20 mins UV-C radiation had

the highest mortality rate than other groups. These results are somewhat questionable as the sample size was very small once parasitism was excluded. In addition, the results of total mortality of fruit attached ALCM did not show the disinfestation effect of UV-C radiation. Admittedly, despite the small sample size, our data show a potential trend of the mortality of ALCM in relation to the doses of UV-C radiation, which the mortality of ALCM increased with the prolonged UV-C exposure time. In addition, it is found that the mortality of ALCM in the groups treated with the low doses of UV-C radiation was even less than that of ALCM in the control groups. Reasons for this finding were not clear. But the small sample size was likely to be the cause.

The groups treated with 20 mins and 10 mins of UV-C radiation had the highest mortality caused by parasitism comparing to other groups. Interestingly, the parasitized insects would appear somewhat more vulnerable to UV-C radiation comparing to the non-fruit attached trials. For the mortality of unparasitized ALCM, two control groups, particularly the 20 mins UV-filtered group, had notable higher mortality rate than the UV-C treated groups. This finding was probably influenced by dark storage of apples, and moderately high continuous storage temperature. Our findings also support the previous study that the parasitism and super parasitism rate of the fourth generation of ALCM by *P. demades* is the highest comparing to that of the other generations (He and Wang, 2011).

The second trial was to investigate the effect of UV-C radiation on the postharvest quality of apple fruits. Fruit firmness is one of postharvest quality parameters to determine fruit maturity and ripeness. There were fewer rotted fruits in the UV-C treated group than that in the control group. Furthermore, results of apple fruit firmness test indicate that average pressure applied to puncture the UV-C radiation treated apples was higher than that to puncture apples from the control group, indicating the side-benefits or responses to UV-C treatment. Although the effect of UV-C radiation on the shelf life of intact apple fruit is promising, the 20 mins UV-C radiation is equivalent to 34.1494 KJ, a significant UV-C dose. On the other hand, previous studies demonstrate the low dose of UV-C radiation ranging from 0.25 to 8.0 KJ m^{-2} could trigger the hormesis effect (Luckey, 1980; Terry and Joyce, 2004; Shama, 2007). Hence, further investigations would be merited to confirm the effects of high dose of UV-C radiation

on the postharvest quality of apple fruit, as the apple sample size used in the second trial was quite small.

Besides the UV-C radiation treatment used as a disinfestation approach at the postharvest packing stage, previous studies have assessed the effects of another quarantine treatment, the heat treatment, on insect control and postharvest quality in apples (Spotts et al., 2006; Bai et al., 2006; Hansen et al., 2006; Shellie and Mangan, 2000; Smith and Lay-Yee, 2000). These studies have already presented the promising results of heat treatment on the postharvest quality of apple fruits. The development of postharvest quarantine treatments has to consider the species and stage of the pest most tolerant to those treatments (Neven, 2008). The control of quarantine insects such as leafroller species, lightbrown apple moth (Smith and Lay-Yee, 2000), fruit fly (Shellie and Mangan, 2000) and codling moth (Hansen et al., 2006) by heat treatment has been studied, and the results obtained are promising.

In addition to heat treatment, many other postharvest treatment approaches are currently applied to control postharvest diseases and insects of horticultural crops (Al-ati and Hotchkiss, 2002). Although no studies have been undertaken to investigate the effect of commonly applied approaches on the mortality of ALCM larvae, previous related studies would merit the future investigations to evaluate the influence of these methods on ALCM control in apples. With the combination of the UV-C radiation treatment and other approaches, a better and efficient ALCM control in postharvest apple fruits will be achieved.

CHAPTER 4: THE EFFECT OF UV-C RADIATION ON THE EGG LOAD OF APPLE LEAF-CURLING MIDGE

4.1 Introduction

ALCM is widespread throughout New Zealand. The overwintered generation of ALCM adults emerges from pupae in the soil in spring (Todd, 1956; Gagne, 1989; He and Wang, 2007). The peaking hour for the emergence of the ALCM adults is between 6.00 and 10.00 a.m. in the field (Harris et al., 1999). After emergence, the female adults exhibit a calling behaviour to expose the pheromone gland, and then release sex pheromone to attract the male adults (Harris et al., 1996). Approximately 20 - 90 mins after mating, the female adults start to find host plants to lay eggs (Galanihe, 1996). Similar to many other plant-feeding cecidomyiids which are typically specialised in attacking one single host plant, ALCM exclusively attack apple trees (Barnes, 1948; Gagne, 1989). The selection of suitable host apple plants by the female adults would be critical to their offspring survival as larvae have limited mobility and cannot move from plant to plant. The female adults oviposit between the folds of immature leaves on new shoots (Shaw et al., 2005; Sandanayaka and Charles, 2006). Considering the limited amount of terminal growth at the start of the season, only a small percentage of shoots are infested, and the number of ALCM eggs per shoot is quite low (Todd, 1956). Later in the season, with the abundant growth of terminal shoots, plentiful oviposition sites are available for the female adults laying eggs, leading to the serious infestation (Todd, 1959). In addition, apple cultivars also play a significant role in the timing of egg laying due to the diverse phenology of apple cultivars (Smith and Chapman, 1997). It is found that the peak egg laying period on ‘Royal Gala’ is 7 - 14 days earlier than that on ‘Braeburn’. However, in terms of severity of apple shoot infestation by the ALCM eggs, no remarkable difference occurs between cultivars (Smith and Chapman, 1997).

Although ALCM is an important quarantine pest, the fecundity of female adults is still not well known. Whitcomb (1941) found an average of 155.5 eggs per female ALCM after dissecting 12 gravid females. Some studies investigating the phenological dynamics of ALCM indicate that the egg density per shoot is the highest in the second generation of ALCM (Todd, 1956; Shaw et al., 2005; He and Wang, 2011). The ALCM

egg density per infested shoot varies in different generations from 10 eggs in the fourth generation to 200 eggs in the second generation (He and Wang, 2011).

ALCM is not an orchard pest. Instead, it can cause quarantine concerns when fresh apple fruits are contaminated by the ALCM cocoons (He and Wang, 2011). This problem makes the ALCM control particularly in the postharvest control essential to the New Zealand pipfruit sector. In addition, the results from previous experiments showed the potential disinfestation effect of UV-C radiation on the control of ALCM. In this experiment, a further investigation was carried out to obtain information on the potential fecundity of female ALCM after the UV-C radiation treatment.

4.2 Materials and methods

4.2.1 Site description

This experiment was conducted in a mature apple orchard at Massey Fruit Crops Unit and Entomology Laboratory in the Agriculture and Horticulture Building, Massey University, Palmerston North ($40^{o}4'$ S, $175^{o}6'$ E).

4.2.2 Materials preparation

The UV-C radiation source applied in this experiment was 4x market-sourced fluorescent lamps (PHILIPS, TUV 30W/G30 T8, Made in Holland). TUV T8 lamps were double-ended UV-C lamps offering constant short-wave UV output. They were mounted in a modified array. The Optronics OL-756 Spectroradiometer (Optronics Laboratories, Florida, USA) was used to confirm the UV-C radiation dosage. The dosage 100 mm under UV-C lamps was measured in three different positions which were 45 cm from end, 15 cm from near end, and 15 cm from far end of UV box, respectively. The average dosage across tube was equivalent to 28.4579 W $m^{-2}s^{-1}$. The dose of each treatment was calculated and presented in Table 4.1.

Table 4.1: The dose of each UV-C radiation treatment in relation to the exposure time applied in the experiment.

Treatment	Irradiance in KJ m^{-2}	No. of dishes
2 mins-UV	3.4149	10
2 Foil-Sheltered	0	10

ALCM larvae infested apple leaves were collected from a mature apple orchard at the Massey Fruit Crops Unit in early November 2013, and maintained in temperature-controlled storage room at Massey University Entomology Laboratory. After collection, these curled apple leaves were broken to free larvae in the lab. The mature larvae were placed into Petri dishes (5.5 cm diameter × 1.3 cm height) which were buried in river sand. Then, Petri dishes were maintained in a storage room at 20 °C with a day length of 16 hours light. After a week's storage, Petri dishes were removed from the controlled environment to collect cocoons. Cocoons were extracted from river sand in a mesh under running water (20 °C). Extracted cocoons were mixed, randomly chosen, and then placed into 20 Petri dishes in total with 50 cocoons each. These Petri dishes with cocoons were placed 100 mm below the fluorescent tubes. For the UV-C treatment, Petri dishes labelled from 1 to 10 were treated with 2 mins UV-C radiation. The left Petri dishes labelled from 11 to 20 were shielded from UV radiation as the control group.

After the UV-C radiation treatment, all Petri dishes were maintained in a completely dark environment at 20 °C with 50% humidity.

4.2.3 Data collection and analysis

One week later after treatment, the ALCM adults began to emerge. ALCM adults emerged from 2 mins UV-C treatment were kept apart from those emerged from the control group. For each treatment, to determine the potential fecundity of ALCM, emerged female adults were captured and dissected in a drop of ringer's solution under a microscopy (Olympus Japan) to count the egg load. Before dissecting, their body size, the body length and width, was measured.

A goodness of fit test (Kolmogorov-Smirnov test) was used to test the distribution of data before analysis. The data were normally distributed and analyzed by using linear regression. Linear regression was applied to analyze the relationship between female

body size and egg load. Analysis of co-variance (ANCOVA) was used to compare the slopes of regression lines between treatments.

4.3 Results

4.3.1 Body size of female ALCM

There were 45 and 40 female ALCM adults emerged in the UV-C treated group and the control group, respectively. As shown in Table 4.2, there was no significant difference in body length (ANOVA: F = 3.16, df = 1, 83, P = 0.079), and body width (ANOVA: F = 0.04, df = 1, 83, P = 0.8342) between two groups.

Table 4.2: The body size of female ALCM emerged in UV-C treated and control groups. Average body length and width values are ± S.E.

body size (mm)	UV-C group	Control group
Max body length	1.774	1.625
Min body length	0.783	1.109
Max body width	0.953	0.852
Min body width	0.577	0.565
Ave body length	1.295±0.0292	1.361±0.0227
Ave body width	0.752±0.0124	0.749±0.0099

4.3.2 Egg load

The average egg load of female ALCM was shown in Figure 4.1. Based on dissection, the egg capacity of each female ALCM adults varied in the range between 51 and 281 in the UV-C treated group, and between 90 and 235 in the control group. The average egg was 195 in the UV-C treated group, and 189 in the control group, which was not significantly different (ANOVA: F = 0.43, df = 1, 83, P = 0.5158).

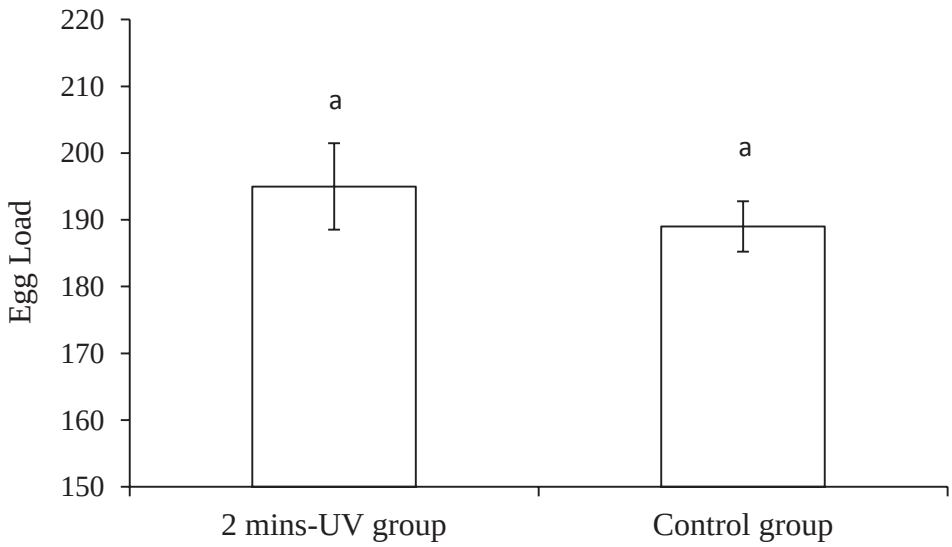

Figure 4.1: The average egg number of ALCM in UV-C treated group and control group. Columns with the same letters are not significantly different (P > 0.05).

4.3.3 Egg load of female ALCM in relation to body size

The fecundity of female ALCM in relation to body size dimensions (body length and body width) are shown in Figures 4.2 and 4.3 below. There was no significant difference in egg numbers in relation to body length (ANOVA: F = 3.74, df = 1, P = 0.0567), and egg numbers in relation to the body width (ANOVA: F = 0.39, df = 1, P = 0.5323) between treatments.

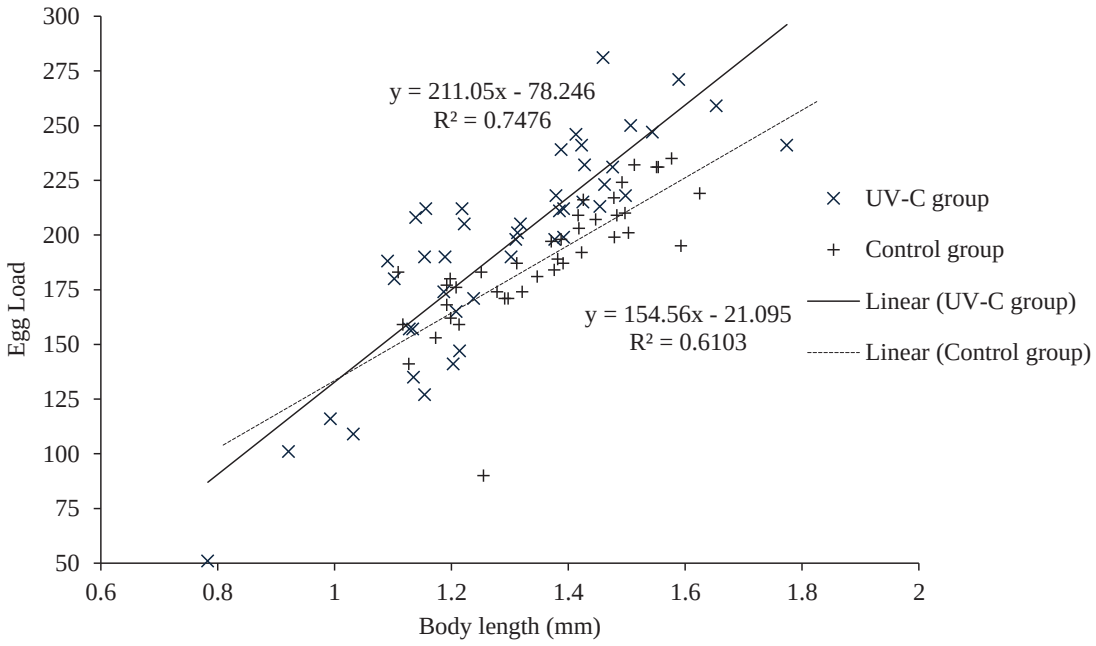

Figure 4.2: The egg load of ALCM in relation to body length (mm).

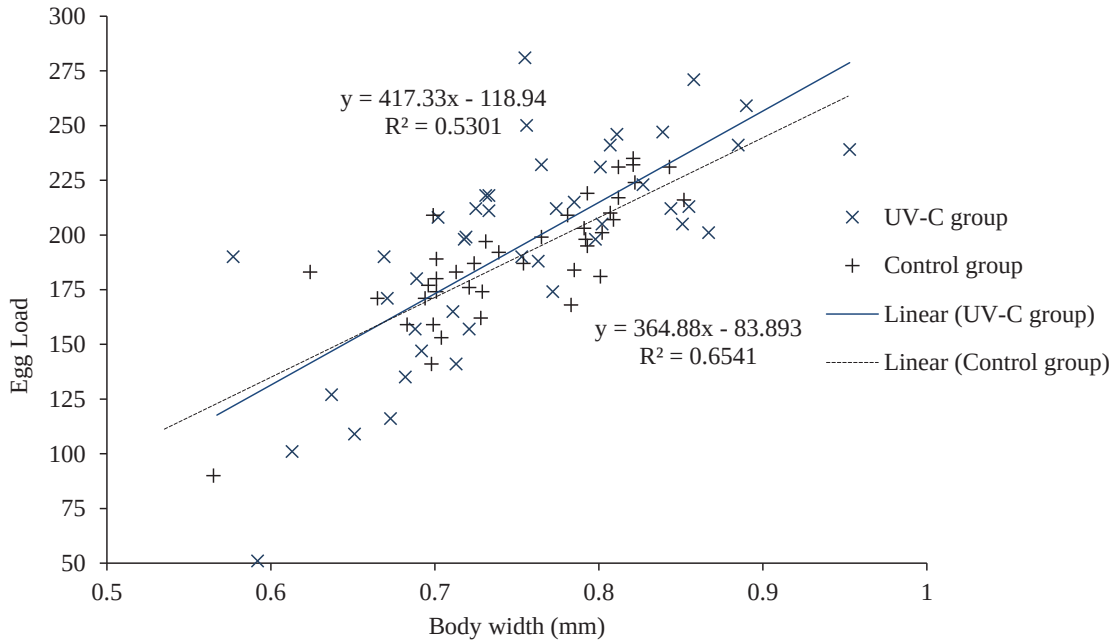

Figure 4.3: The egg load of ALCM in relation to body width (mm).

Based on the results, there were no significant differences in egg numbers in relation to body length and width between treatments. Based on Figure 4.2 and 4.3, it was found that the slopes of the regression lines were marginally significantly different in terms of the egg numbers in relation to the body length than that in relation to the body width. Hence, In terms of the egg numbers in relation to the body size, the ALCM body length had greater influence on the fecundity of ALCM comparing to the body width.

4.4 Discussion

The mature ALCM larvae collected were belonging to the first generation of ALCM (He and Wang, 2007). As discussed in the first experiment in Chapter 2, a thin layer of river sand was again observed and attached on the outer layer of ALCM cocoons. Previous studies had found that the mechanical properties of silk cocoons in many aspects (Zhao et al., 2005; Zhao et al., 2007; Teshome et al., 2012), and calcium oxalate crystals of cocoons have tremendous influence on the heat absorption and light transmission (Peigler, 1993). Thus, it is uncertain if the results of the effects of UV-C radiation are compromised due to the thin layer of river sand and silk cocoons. Further investigations regarding to these factors would be needed. Due to insufficient knowledge of the fecundity of female ALCM adult, the results obtained from this

experiment are inadequate to evaluate the roles of UV-C radiation on the fecundity of female ALCM. But this data shows the subtle differences are found between treatments.

Based on the results, in terms of the body size of ALCM, although the body size of female ALCM adults varied considerably, no significant difference was found between the 2 mins UV-C treated group and the control group (Table 4.2). It is likely that the short duration might not grossly affect the development of ALCM individuals. In addition to UV-C radiation, it was found that the body size of many insect species was influenced by environmental conditions, such as the quantity and quality of food eaten and temperature (Freeman and Geoghagen, 1987). In this experiment, all cocoons were maintained in the same conditions, and the female adults were dissected soon after emergence, the influence of those factors possibly affecting ALCM size was negligible. Further investigations would be needed to assess the body size of female ALCM maintained in the same conditions when they are transporting to overseas markets.

The results of egg load indicate that the female adults treated with 2 mins UV-C radiation had higher egg load comparing to the control group. However, no significant difference was found between those two groups (Figure 4.1). Based on the study of Whitcomb (1941), the average of egg capacity of ALCM is about 155.5. But this data is questionable due to the small sample size (n=12). For the fecundity of ALCM in relation to the body size between treatments, it is found that the UV-C group has larger R-squares and slopes of the regression lines, and it reveals that the UV-C group has a potential higher egg capacity with increased body size than the control group. It is possible that the short duration of UV-C radiation treatment might encourage the egg capacity of female ALCM.

Previous studies show that female size is positively correlated with the fecundity in many insect species (Taylor, 1975; Gilbert, 1984). Female midges are found to employ two different reproductive strategies to increase their fitness. They could either produce a few large eggs, or produce many small eggs. The difference between the larger and smaller eggs is the content of proteinaceous nourishment, where larger eggs have more proteinaceous nourishment with higher survival rate comparing to small eggs (Wheeler, 1996).

In addition, the survival rate and the fecundity of F_1 generation of UV-C treated female ALCM were not further measured or calculated in this experiment. The present study demonstrates the insufficient effect of UV-C radiation on the fecundity and the development of female ALCM adults. Nevertheless, it cannot be summarised that UV-C radiation has no effects on the F_1 generation of ALCM following treatment. In comparison to the evidence observed in this experiment, other herbivore systems indicate the negative effects on the longevity and reproduction of adult, and on its F_1 generation exposed to UV light, which would merit the further investigations regarding the ALCM species. In a previous study, it is reported that one of the effects of UV radiation increased oxidative stress on *Helicoverpa armigera* adults (Meng et al., 2009). Zhang et al. (2011) proved that UV radiation has negatively effect on the larval development of F_1 generation of *Helicoverpa armigera*.

In conclusion, depending on the previous studies and present observation, although the results of this experiment are not promising, further investigations which investigate the effects of UV-C radiation on the reproduction of the F_1 generation of ALCM would be needed.

CHAPTER 5: CONCLUSION

The key aim of this study was to evaluate the efficiency of UV-C radiation on the control of ALCM to support the development of postharvest insect pest management programmes for this pest in the New Zealand pipfruit industry. Fresh fruit contaminant by ALCM can lead to market barriers for the New Zealand's billion-dollar horticultural industry. To obtain the knowledge of the disinfestation effect of UV-C radiation on the mortality of ALCM, a series of UV-C radiation doses were applied in this study. No such study has been previously investigated, and thus this project could lead to a new tool in the control of unwanted organisms into the produce value chain. The following discussion summarises the knowledge and findings obtained from this study and assesses the feasibility if the UV-C radiation treatment can be applied by the industry. The suggestions for the present experimental improvement and future researches on the control of ALCM are discussed as well.

Summary of key findings

The objective of the first experiment was to evaluate the disinfestation effect of UV-C radiation on non-fruit attached ALCM cocoons in terms of the mortality rate, parasitism rate and the developmental duration of ALCM. It is found that the groups treated with UV-C radiation had higher mortality rates than that of the control groups. Admittedly, due to high parasitism rate by *P. demades*, the result of the total mortality of ALCM is not significant between treatments. Based on overall results, although the disinfestation effect of UV-C radiation is not remarkable, it is found that the emergence of ALCM from cocoons in the UV-C radiation treated groups was significantly delayed, and the number of emerged ALCM adults was quite low when comparing to the control groups. With the delayed emergence of ALCM, some external and/or internal factors which could negatively affect the survival rate of ALCM may be triggered.

The aim of the second experiment was carried out to evaluate the disinfestation effect of UV-C radiation on apple fruit-attached ALCM cocoons. Due to the small sample size, results obtained are not persuasive regarding to the effects of UV-C radiation on either the total mortality of ALCM, or the mortality of unparasitized ALCM. However, the group treated with 20 mins had the highest mortality rate. The insignificant mortality rate of cocooned larvae suggests that cocoons hidden in the apple calyx may be a

significant factor that limits the effect of UV-C on ALCM. In addition, results of postharvest quality test approve the role of UV-C radiation in improving the postharvest quality of apple fruits (Artes et al., 2009; Stevens et al., 2005).

The effect of UV-C radiation on the body size and egg load of ALCM females was assessed in the third experiment. Results of body size of ALCM indicate that low dose of UV-C radiation might not grossly affect the body size of ALCM individuals. Furthermore, the egg capacity of the ALCM females treated with UV-C radiation was not significantly different to that for the control group. However, after analysing the relationship between the fecundity and the body size of ALCM, it is found that the UV-C treated group has larger R-squares and slopes of the regression lines comparing to the control group, which reveal that the female adults from UV-C treated group have a potentially high egg capacity with the increased body size. It is possible that the short duration of UV-C radiation treatment might lead to increased egg capacity of female ALCM.

Future research

The high dose of UV-C radiation treatment was not applied in present study. Results of the second experiment indicated that the group treated with 20 mins had the highest mortality rate. However, 20 mins of UV-C radiation treatment is a significant treatment time. Therefore, further investigations are needed to confirm the effects of greatly increased UV-C doses on the mortality of ALCM cocoons and postharvest quality of apple fruits. Moreover, the optimal dose of UV-C radiation in the control of ALCM can be calculated to benefit future adoption of UV-C radiation during apple processing stage.

Being a fresh fruit contaminant, besides the decontamination of apple fruits by ALCM during apple processing stage, the infertility of its F_1 generation should also be taken into consideration in case ALCM is introduced to overseas markets accidentally. Although the third experiment fail to demonstrate the positive influence of UV-C radiation on the egg load of ALCM females, previous studies reported the negative effects of UV light on the longevity and reproduction of adult, and on its F_1 generation of other herbivore systems (Meng et al., 2009; Zhang et al. 2011), which benefit the further investigation regarding the effects of UV-C radiation on F_1 generation of ALCM.

In addition to UV-C radiation, the effects of many other postharvest treatment approaches such as heat treatment on ALCM control should be assessed as well. The disinfestation effect of these approaches have been approved by many studies (Smith and Lay-Yee, 2000; Shellie and Mangan, 2000; Hansen et al., 2006). Besides, the introduction of natural enemies of ALCM can reduce the need of chemical insecticides intervention, more important, it can reduce the possibilities of apple fruits being contaminated by ALCM at pre-harvest stage. All in all, the benefits of combining natural predators with UV-C radiation and other quarantine treatment approaches deserve further investigations for a better ALCM management.

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APPENDICES

APPENDIX I

The Optronics OL-756 Spectroradiometer (Optronics Laboratories, Florida, USA) was used to confirm the UV-C radiation dosage. The dose of each treatment applied in this experiment was equivalent to the dosage multiply the exposure time. The dosage 100 mm under UV-C lamps was measured in three different positions which were 45 cm from end, 15 cm from near end, and 15 cm from far end of UV box, respectively. The average dosage across tube was equivalent to 28.4579 W $m^{-2}s^{-1}$, or 0.0284579 KJ $m^{-2}s^{-1}$.

Table I: The measurement of the irradiance of UV-C lamps applied in the project.

Wavelength(nm)	Irradiance in watts cm^{-2} s^{-1}					Irradiance in watts m^{-2} s^{-1}	
	45 cm from end 'middle'	15 cm from near end	15 cm from far end	average tube	UV-C filtered	average tube	UV-C filtered
250	9.6384E-08	8.90947E-08	9.12027E-08	9.22271E-08	5.9929E-11	0.000922271	5.9929E-07
251.00	1.26669E-07	1.17143E-07	1.2339E-07	1.22401E-07	0	0.001224007	0
252.00	2.03114E-07	1.95363E-07	1.90347E-07	1.96275E-07	1.10423E-10	0.001962748	1.10423E-06
253.00	9.01164E-07	8.40157E-07	8.17422E-07	8.52914E-07	0	0.008529145	0
254.00	6.85152E-07	6.17741E-07	6.44908E-07	6.49267E-07	1.80452E-10	0.006492672	1.80452E-06
255.00	6.97093E-07	6.80069E-07	6.50318E-07	6.75827E-07	8.28971E-11	0.006758267	8.28971E-07
256.00	3.59636E-06	3.58088E-06	3.33146E-06	3.5029E-06	8.73192E-11	0.035028988	8.73192E-07
257.00	2.14364E-05	2.12084E-05	2.01202E-05	2.09217E-05	8.11709E-10	0.209216804	8.11709E-06
258.00	0.000791582	0.000791235	0.000733352	0.000772056	1.5415E-08	7.720562895	0.00015415
259.00	0.001937641	0.001830848	0.001874037	0.001880842	4.09108E-08	18.80842097	0.000409108
260.00	0.000154765	0.000140822	0.000154213	0.000149933	3.83955E-09	1.499334193	3.83955E-05
261.00	8.65377E-06	8.13956E-06	8.46035E-06	8.41789E-06	3.24097E-10	0.084178919	3.24097E-06
262.00	1.00531	9.86093	9.82021	9.91142	1.80199	0.00991	1.80199

	E-06	E-07	E-07	E-07	E-10	1417	E-06
263.00	4.67984 E-07	4.59722 E-07	4.55497 E-07	4.61068 E-07	1.50825 E-11	0.00461 0677	1.50825 E-07
264.00	1.83051 E-07	1.91784 E-07	1.81128 E-07	1.85321 E-07	7.39369 E-11	0.00185 3209	7.39369 E-07
265.00	1.16697 E-07	1.27156 E-07	1.17625 E-07	1.20493 E-07	4.95249 E-11	0.00120 4925	4.95249 E-07
266.00	8.52715 E-08	8.95136 E-08	8.27978 E-08	8.5861E -08	2.00708 E-10	0.00085 861	2.00708 E-06
267.00	6.72046 E-08	7.17069 E-08	6.69161 E-08	6.86092 E-08	2.76067 E-10	0.00068 6092	2.76067 E-06
268.00	7.63884 E-08	7.48181 E-08	7.38673 E-08	7.50246 E-08	0	0.00075 0246	0
269.00	1.40634 E-07	1.35961 E-07	1.28959 E-07	1.35185 E-07	1.54111 E-10	0.00135 1848	1.54111 E-06
270.00	2.24841 E-06	2.06159 E-06	2.03595 E-06	2.11532 E-06	2.3168E -10	0.02115 3162	2.3168E -06
271.00	2.41893 E-06	2.15813 E-06	2.29739 E-06	2.29148 E-06	1.23455 E-10	0.02291 4828	1.23455 E-06
272.00	1.5541E -07	1.35285 E-07	1.51546 E-07	1.47414 E-07	6.58726 E-11	0.00147 4137	6.58726 E-07
273.00	3.27079 E-08	3.03109 E-08	2.99081 E-08	3.09756 E-08	2.02252 E-10	0.00030 9756	2.02252 E-06
274.00	3.06487 E-08	2.96763 E-08	2.88352 E-08	2.97201 E-08	1.11692 E-10	0.00029 7201	1.11692 E-06
275.00	1.71583 E-07	1.51952 E-07	1.58601 E-07	1.60712 E-07	1.03374 E-10	0.00160 7119	1.03374 E-06
276.00	5.31805 E-08	4.73613 E-08	5.21258 E-08	5.08892 E-08	4.26435 E-11	0.00050 8892	4.26435 E-07
277.00	2.22882 E-08	2.24529 E-08	2.19754 E-08	2.22388 E-08	1.27855 E-10	0.00022 2388	1.27855 E-06
278.00	2.75814 E-08	2.40306 E-08	2.52911 E-08	2.56344 E-08	1.3524E -11	0.00025 6344	1.3524E -07
279.00	4.14848 E-08	3.99332 E-08	3.93217 E-08	4.02466 E-08	7.58294 E-11	0.00040 2466	7.58294 E-07
280.00	5.14134 E-07	4.77051 E-07	4.67943 E-07	4.86376 E-07	1.02919 E-10	0.00486 3761	1.02919 E-06
Total irradiance	0.00292 8243	0.00280 5688	0.00280 3429	0.00284 5787	6.39729 E-08		
Irradiance in W m^{-2} s^{-1}	29.2824 3455	28.0568 7936	28.0342 9296	28.4578 6896	0.00063 9729		

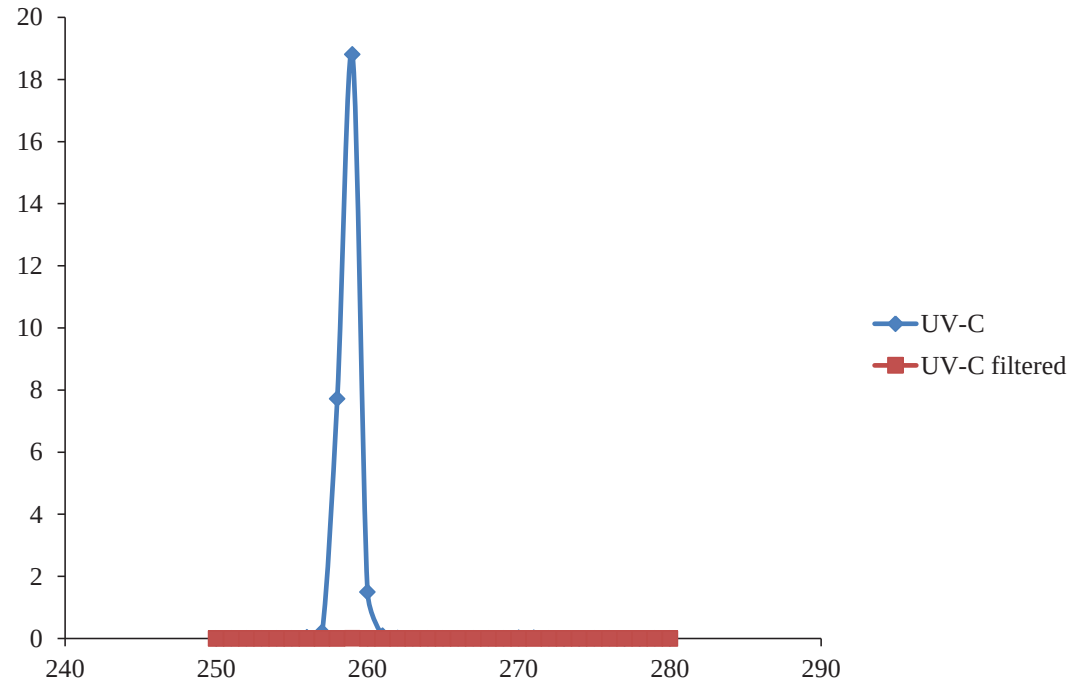

Figure I: The measurement of the irradiance of UV-C lamps in watts m^{-2} s^{-1}.

APPENDIX II

ANOVA analysis programme was used to analyse the mortality of fruit and non-fruit attached ALCM larvae, parasitized ALCM larvae, and unparasitized ALCM larvae in different dissections. Moreover, data of the developmental duration and the fecundity of ALCM was also analysed by ANOVA.

The following was the ANOVA code to analyse the total mortality of ALCM as an example (Percentage).

```
data a;
input Treat $ PLarvae unPLarvae total;
PL=arsin(sqrt (PLarvae/100));
unPL=arsin(sqrt(unPLarvae/100));
tot=arsin(sqrt(total/100));
cards;
2min  20    12.22 32.22
2min  16.67 15.56 32.23
2min  14.44 26.67 41.11
2min  21.11 17.78 38.89
2min  17.78 24.44 42.22
5min  11.11 35.56 46.67
5min  23.33 16.67 40
5min  26.67 14.44 41.11
5min  23.75 23.75 47.5
5min  22.22 15.56 37.78
10min 20    26.67 46.67
10min 26.67 18.89 45.56
10min 30    14.44 44.44
10min 25.56 18.89 44.45
10min 20    16.67 36.67
Con1  40    8.89  48.89
Con1  28.89 7.78  36.67
Con1  35.56 5.56  41.12
Con1  28.89 5.56  34.45
Con2  24.44 7.78  32.22
Con2  31.11 7.78  38.89
Con2  27.89 13.33 41.22
Con2  26.67 6.67  33.34
;

proc sort data=a;
by treat;
proc means mean stderr stddev data=a;
var  PLarvae unPLarvae total;
by treat;
run;

proc glm data=a;
    class Treat ;
    model PL=Treat /solution;
      means  Treat/LSD Duncan Tukey;
    output out=new p=yhat student=student residual=residual;
run;
proc freq data=new;
```

```
tables PL;
proc chart data=new;
vbar residual/levels=0.05;
run;
proc univariate plots data=new;
var residual;
histogram residual/normal(color=red fill);
run;

proc glm data=a;
    class Treat ;
    model unPL=Treat /solution;
      means  Treat/LSD Duncan Tukey;
    output out=new1 p=yhat student=student residual=residual;
run;
proc freq data=new1;
tables unPL;
proc chart data=new1;
vbar residual/levels=0.05;
run;
proc univariate plots data=new1;
var residual;
histogram residual/normal(color=red fill);
run;

proc glm data=a;
    class Treat ;
    model tot=Treat /solution;
      means  Treat/LSD Duncan Tukey;
    output out=new1 p=yhat student=student residual=residual;
run;
proc freq data=new1;
tables tot;
proc chart data=new1;
vbar residual/levels=0.05;
run;
proc univariate plots data=new1;
var residual;
histogram residual/normal(color=red fill);

run;
```

APPENDIX III

Table II.1: Each apple fruit in the group which was treated with 20 mins UV-C radiation was tested by Fruit Pressure Tester in average (kg).

Apple Number	Blush side (kg)	Green side (kg)
#2	6.5	5.4
#3	6.4	5.5
#7	9	6.8
#10	nil (rotted)	nil
#11	5.4	4.1
#27	8.6	6.2
#28	6.2	4.1
#33	7.3	5.4
#37	6.5	5.6
#38	8.2	6.7
#45	7.3	4.1
#48	6.5	4.3
#51	nil (rotted)	nil
#57	8.1	7.2
#75	7	6.6
Average	**7.15**	**5.53**

Table II.2: Each apple fruit in the control was tested by Fruit Pressure Tester in average (kg).

Apple Number	Blush side (kg)	Green side (kg)
#4	7.2	4.2
#8	6.7	4.8
#15	8.5	6.3
#19	nil (rotted)	nil
#24	nil (rotted)	nil
#26	6	5.1
#31	6.1	5
#35	6.2	5.3
#36	nil (rotted)	nil
#40	nil (rotted)	nil
#43	7.2	6.5
#49	6.9	6.3
#63	nil (rotted)	nil
#71	7	5.4
#89	5.3	3
Average	**6.71**	**5.19**