An assessment of the potential of irradiation as a postharvest control treatment against the banded fruit weevil, *Phlyctinus callosus* (Coleoptera: Curculionidae): effects on adult weevils and host fruit ('Flavor Fall' pluots)

by

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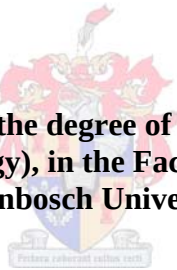

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Abstract

The export of South African fruit to some of its biggest international markets may be rejected if the phytosanitary pest, *Phlyctinus callosus* (Coleoptera: Curculionidae) is found in fruit consignments. An alternative to methyl bromide fumigation is needed and one of the most promising of the alternative treatments is phytosanitary irradiation as it is environmentally friendly, does not leave residues on food or in the environment and it is effective against a wide variety of insects.

Field-collected weevils were treated with five doses of gamma irradiation (5, 10, 20, 40 and 80 Gy) and the fecundity and fertility of mating crosses of treated males and females with treated and untreated individuals of the opposite sex, were determined to evaluate the effect on *P. callosus* reproductive ability post-treatment. Results indicated that irradiation treatment did not affect fecundity, but fertility was significantly affected, decreasing as the irradiation dose increased. Females were more susceptible to the irradiation treatment than males, and after treatment with 80 Gy, eggs laid by females and mated with either treated or untreated males, did not hatch. A generic dose of 400 Gy for all insect pests except tephritid fruit flies and pupae and adult Lepidoptera is currently approved by USDA-APHIS (United States Department of Agriculture – Animal and Plant Health Inspection Services) for use on certain commodities. Results from the present study support the development of a species-specific dose for *P. callosus*, as well as the development of a group generic dose for the Curculionidae that is lower than 400 Gy. Effective phytosanitary irradiation treatments are only feasible if the treatment does not adversely affect fruit quality and the marketability of export fruit.

Therefore, an investigation of the effects of irradiation disinfestations treatments on the quality of the new pluot cultivar, 'Flavor Fall' was made. Packed cartons were treated with three doses of gamma irradiation: 400 Gy, 900 Gy and 1400 Gy. After treatment fruit underwent a PD 7 dual temperature cold storage regime for 42 days and a shelf-life

simulation for 7 days. The impact of insect-proof bags, sometimes required by importing countries to keep insects off packaged fruit, was also investigated. Respiration rate of the fruit was measured throughout and fruit quality evaluations were done after cold storage and after shelf-life. The results indicated that quality parameters measured at the end of cold storage, which would be after the fruit arrives at the export markets, were above the minimum standards for overseas markets. Gel breakdown was unacceptably high after the higher temperature exposure of shelf-life for fruit treated with the 900 and 1400 Gy doses. The insect-proof bags reduced shrivel, but resulted in higher incidence of gel breakdown. The use of irradiation, together with the use of the insect-proof bag, has potential as an alternative postharvest mitigation treatment for plums.

Lastly, an investigation into potential rearing methods for *P. callosus*, including recommendation for the future, was made as the availability of a sustainable rearing method that ensures a consistent supply of high quality *P. callosus* adults would enable continuous research with greater numbers of this pest. The information generated in this study provides a greater understanding of the radiation biology of, not only this curculionid species, but the Curculionidae as a group, and is valuable in advancing the development of alternative postharvest control measures against this phytosanitary pest.

Opsomming

Suid Afrikaanse vrugte uitvoere na van die grootste internasionale markte mag weg gewys word as die fitosanitêre pes, *Phlyctinus callosus* (Coleoptera: Curculionidae) in die versending gevind word. ‘n Alternatief vir metiel bromied beroking word benodig en een van die mees belowende alternatiewe behandelings is fitosanitêre bestraling aangesien dit omgewings vriendelik is, nie residue op kos of in die omgewing los nie, en effektief is teen ‘n wye verskeidenheid van insekte.

Veldversamelde kalanders is behandel met vyf dosisse gamma bestraling (5, 10, 20, 40 en 80 Gy) waarna die vrugbaarheid van paringskruisings bepaal is deur kruisings tussen behandelde manlike en vroulike kalanders met behandelde en nie-behandelde individue van die teenoorgestelde geslag te maak, en so die na-behandelings effek op die voortplantings vermoeë van *P. callosus* te evalueer. Die resultate het getoon dat die bestralings behandeling geen invloed gehad het op die hoeveelheid eiers wat gelê is nie, maar dat die uitbroei van eiers aanduidend geaffekteer is deur die behandeling. Die hoeveelheid eiers wat uitgebroei het, het minder geraak soos die bestralings behandeling toegeneem het. Vroulike kalanders was meer sensitief vir die behandeling en na 80 Gy, of hul gekruis is met behandelde of nie-behandelde mannetjies, het geen eiers uitgebroei nie. ‘n Generiese dosis van 400 Gy vir alle insekte, uitsluitend tephritiese vrugte vlieë en papies en volwasse Lepidoptera is huidiglik goedgekeur deur die USDA-APHIS (United States Department of Agriculture – Animal and Plant Health Inspection Services) vir sekere kommoditeite. Die resultate van die huidige studie ondersteun die ontwikkeling van ‘n spesie-spesifieke dosis vir *P. callosus*, so ook die ontwikkeling van ‘n generiese groep dosis vir Curculionidae wat laer as 400 Gy is. Effektiewe fitosanitêre bestralings behandeling is slegs moontlik indien die behandelings dosis nie nadelig vir vrugkwaliteit en die bemarking van uitvoer vrugte is nie.

Dus is die effek wat bestralings bestryding behandeling op die kwaliteit van ‘n nuwe pluot kultivar, ‘Flavor Fall’ ondersoek. Vrugte verpak in kartonne is met drie dosisse gamma bestraling behandel: 400 Gy, 900 Gy en 1400 Gy. Na behandeling is die vrugte deur ‘n PD 7 dubbel temperatuur koelopbergings regime van 42 dae en rak-lewe simulasie vir 7 dae gesit. Die impak van insek-bestande sakke wat insekte van die verpakte vrugte weg hou en soms deur invoerende lande ‘n vereiste is, is ook ondersoek. Respirasie tempo van die vrugte is getoets en vrugkwaliteit evaluasies is gedoen na koelopberging en rak-lewe. Die resultate het getoon dat die kwaliteits maatstawwe wat getoets is na koelopberging (wat tipies is wanneer die vrugte by die uitvoer mark arriveer), almal bo die minimum standaarde van die uitvoer markte was. Gel-afbraak was onaanvaarbaar hoog na blootstelling aan die hoër temperature tydens rak-lewe vir vrugte wat behandel is met 900 en 1400 Gy. Die insek-bestande sakke het verrimpeling verminder, maar die voorkoms van gel-afbraak vermeerder. Die gebruik van bestraling, tesame met die insek-bestande sakke, het potensiaal as alternatiewe na-oes behandeling vir pruime.

Laastens is ‘n ondersoek ingestel vir moontlike teeltegnieke vir *P. callosus* en aanbevelings gemaak vir toekomstike studies. Die beskikbaarheid van ‘n volhoubare teeltegniek wat konstante, hoë kwaliteit *P. colossus* individue verskaf sal aaneenlopende navorsing met groter getalle van die pes moontlik maak. Die inligting wat deur hierdie studie gegenereer is help om die bestralings biologie, nie net van hierdie curculionid spesie nie, maar die Curculionidae as ‘n groep te verstaan, en is kosbaar in die bevordering van ontwikkeling van alternatiewe na-oes beheer meganismes teen hierdie fitosanitêre pes.

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Chapter 1

Introduction and literature review

Phlyctinus callosus

History and Distribution

The banded fruit weevil, *Phlyctinus callosus* (Coleoptera: Curculionidae), was first described by Schonherr in 1926 whilst describing the subgenus *Peritelus*. The authority for the description of *P. callosus* sometimes appears in the literature as Boheman 1834, but Schonherr remains the valid authority as he was the first to describe this species (Barnes 1989).

Phlyctinus callosus is indigenous to South Africa and has been a pest of fruit in the south-western Cape region since 1896 (Barnes 1989). This region has a temperate climate with hot, dry summers and wet winters. *Phlyctinus callosus* has only been recorded below a latitude of 33° south (Barnes 1987) and is a major pest of pome fruit, stone fruit, and vines (Annecke and Moran 1982). Many years ago vines where cultivated in the Elgin area of the south-western Cape. It is believed that *P. callosus* was initially a pest of vines and as the vines were phased out, and Elgin became a pome fruit production area, a host transfer also took place. Initially *P. callosus* was kept under control with lead arsenate sprays and later, with dichlorodiphenyltrichloroethane (DDT), that was also used to control another major pome fruit pest, the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae). These chemicals were later replaced by organo-phosphates and carbamate insecticides and with that, the control of *P. callosus* became less successful. The use of an automatic spray machine and the changeover from clean cultivation to sod culture may also have played a role. Automatic spray machines made the process of applying insecticides much faster and easier, but in

comparison to hand application techniques, the insecticides do not reach into all the crevices of the tree and less are placed on the trunk and scaffold branches. As weevils are known to hide under bark and in curled up leaves, the insecticides very often do not reach them. Sod culture provides soil-dwelling weevil larvae with roots of weeds and grasses for feeding (Barnes and Swart 1977).

Phlyctinus callosus has spread from South Africa to New Zealand and Australia, where it also has a limited distribution, only occupying the warmer parts of the North Island, and Nelson in the South Island of New Zealand, and all the Southern Australian states (Butcher 1984; Kuschel 1972). It is not present in the United States of America and Europe (CABI 2013) which are some of South Africa's biggest export markets, and therefore, *P. callosus* is a pest of quarantine concern, posing a phytosanitary risk to these regions.

Morphology

Adults reach 10 mm in size, are wingless because of fused elytra, greyish-brown in colour, with a lighter coloured V-shaped pattern dorsally across the rear end. The elytra have distinct lumps at the rear end past the V-shape and each of these lumps bear setae. The rostrum is cork shaped and the tip is black and shiny (Annecke and Moran 1982). The females lay creamy-white eggs that are 0.9 mm in length. In the three or four weeks of egg laying, females lay approximately 5 eggs per week, however, batches of up to 70 eggs per week have been counted for one female. As they mature and the larvae develop inside the eggs, the eggs turn black at the ends (Butcher 1984). Larvae are creamy-white with an orange head capsule and black mandibles. They have long hairs on their body, and larvae can reach up to 6 mm long in later instars (Walker 1978). Pupae are 7-8 mm long, have hooked bristles and form in an earthen cocoon.

Biology

The life cycle of *P. callosus* includes below and above ground stages. Females lay their eggs in the soil or organic matter. Barnes and Pringle (1989) found that eggs were oviposited in moist plant tissue, dead or alive, of various weed species on the orchard floor. These included *Pennisetum clandestinum* (Poaceae), *Trifolium repens* (Fabaceae), *Cyperus esculentus* (Cyperaceae) and *Plantago lanceolata* (Plantaginaceae) (Barnes and Pringle 1989). According to Barnes (1987) there are three distinct egg laying periods; spring (September to October) and summer (November to January) resulting in first generation adults (first- and second phase); and autumn (February to April) resulting in second generation adults. After an incubation period of 7 to 10 days larvae hatch from the eggs and burrow into the soil where they feed on roots of weeds (Van Den Berg 1971). Larvae are mostly found in the top 10 cm of soil where they develop through six to eight instars in approximately 83 to 107 days (Barnes and Pringle 1989). An earlier study of *P. callosus* development by Walker (1978) indicated that larvae can develop through as little as four, or as many as 11 instars. Mature larvae pupate in the soil in an earthen cell, and after approximately 14 days adults start to emerge (Barnes 1988).

The adults climb up the trunk of the tree where they start feeding on twigs, leaves and fruit (Barnes and Giliomee 1992). Adults are nocturnal, only feeding at night. During the day they hide under loose bark or in curled up leaves (Myburgh et al. 1973).

Seasonal cycle

Phlyctinus callosus can have one or two generations per year, depending on weather conditions and agricultural practices. Barnes (1989) studied the difference in *P. callosus* lifecycles on two apple farms in the south-western Cape of South Africa that were 5 km apart. Applethwaite farm had light sandy soils and irrigation by sprinkler, whereas Arieskraal

farm had red, coarse soils and drip irrigation. Results indicated that on Applethwaite there were two generations per year, whilst on Arieskraal there was only one.

When there are two generations per year the eggs that were laid by second generation females the previous autumn results in mature larvae that overwinter and give rise to adults that emerge in October. This emergence lasts about four to six weeks and gives rise to the first phase of the first generation adults. Overwintering second generation females from the previous season lay their eggs in September/October. These eggs hatch in December/January and give rise to the second phase first generation adults. The first phase first generation females (from overwintering larvae) oviposit in December/January and in February/March these eggs hatch and the second generation adults start emerging. These second generation females oviposit between March and April where after they overwinter in the covercrop. These eggs give rise to overwintering larvae that result in first phase first generation adults that again emerge in October/November (Barnes 1989). Overwintering females once again lay eggs in spring, that hatch in summer and give rise to second phase first generation adults.

When there is only one generation per year the life cycle is similar to that of the two generations per year up to about December/ January. In August/September overwintering females oviposit. In October the first phase first generation adults start to emerge. During December/January second phase first generation adults sometimes emerge and first phase first generation females oviposit. At this stage the life cycle differs from the two generations per year cycle. First phase first generation females have a small or absent oviposition phase in February. March to June/ July are when their ovipositioning reaches maximum levels. Because of lower soil moisture content in the interrow when drip irrigation is used as was on Arieskraal farm, eggs laid by first generation females only hatch with the onset of autumn rains and larval mortality is high, resulting in a small or absent second generation (Barnes 1989).

Hosts and feeding damage

Phlyctinus callosus is a phytophagous pest and feeds on a wide variety of hosts. Even between countries the preferred hosts of *P. callosus* differs. In South Africa *P. callosus* is a pest of apples, pears, vines, nectarines, plums and blueberries (Marais and Barnes 1989; Bredenhand et al. 2010). In New Zealand it prefers carrots and parsnips, and also bulbs or corms of some ornamental plants, even though grapevines and apples are grown in New Zealand (Butcher 1984). In Australia it is a pest of grapevines, apples and nectarines, as in South Africa, but in Tasmania it is only a pest of root vegetables (Fisher and Learmonth 2003).

Phlyctinus callosus adults damage the fruit, stems and leaves of fruit trees. They start chewing away at the leaves from the edges primarily, although they sometimes do chew holes in the rest of the leaf. Leaf damage caused by *P. callosus* is only of significance in nurseries and young plantings where adults can completely defoliate a tree and may cause death. Adults also chew the petioles of fruit and leaves, which cause them to wilt or drop prematurely (Barnes and Swart 1977). The damage to fruit varies from superficial damage to the skin of fruit, to holes chewed out of the flesh of the fruit. Damage to the skin leaves a grayish-brown lesion on the fruit that may sometimes bulge, as cell growth continues under the scar (Annecke and Moran 1982). Damage caused to the fruit in the earlier part of the season is repaired to a great extent, and only russeted lesions remain. Damage caused to the fruit when it has nearly reached full size leaves shallow, corky lesions (Barnes and Swart 1977). *Phlyctinus callosus* causes most damage to fruit and leaves near the base of the tree, close to the trunk. This is because adults normally move to the covercrop during the day and when they climb up the trunk when night falls, they start feeding on the fruit closest to the trunk (Barnes and Swart 1977).

Preharvest control practices

Since *P. callosus* adults are flightless and can only climb into trees, trunk barriers or exclusion barriers are the most successful way to limit adult damage. Barriers prevent the adults from reaching the tree canopy once they emerge from the soil. To ensure the success of this method, grasses and weeds need to be kept short and trellis wires also need to be surrounded by the exclusion barriers. If not, adults will use these as bridges to climb into trees. Trunk or exclusion barriers are fluffy batting strips tied around the bottom of the trunk of the tree with the fluffy side facing outwards. Weevils find it difficult to move across this fluffy batting and get stuck in it if they try to. Trunk barriers treated with pesticides like fenvalerate have proven to be even more successful (Barnes et al. 1995). Trunk barriers that incorporate sticky glue, instead of batting material, have also been used with some success (Barnes et al. 1995). In addition to trunk barriers, clean cultivation and herbicides applied underneath the trees will also deprive weevils of roots of weeds which are the main food source of the larvae (Barnes and Swart 1977).

Insecticidal control of *P. callosus* adults can be done with trunk or foliar sprays, but timing is essential. Early in the season trunk drenches can be used as a preventative method. Various insecticides are available for the different host crops of *P. callosus*. Not all are suitable for all host plants and in some cases the doses at which they are applied differ between these. It is advisable to refer to a specialist when considering insecticidal control. Insecticides such as acephate, alpha-cypermethrin, azadirachtin, beta-cypermethrin, esfenvalerate and fenvalerate can be used on pome fruit trees as preventative applications against this pest and lamda-cyhalothrin as trunk treatment as soon as adults are noticed (NDA 2007). For grapes, deltamethrin and tralomethrin is used as a preventative application in mid-October and esfenvalerate, fenvalerate, lambda-cyhalothrin, permethrin and zeta-cypermethrin is applied when the pest or damage is noticed (NDA 2007). For stone fruit acephate is used as a

preventative application and beta-cypermethrin, cypermethrin, deltamethrin, gamma-cyhalothrin, lamda-cyhalothrin and tralomethrin can be applied when the pest or damage is noticed (NDA 2007).

Postharvest control practices

Adult *P. callosus* sheltering in the calyx or stem ends of fruit or inside bunches of grapes enter the packing shed on fruit that has been harvested. They are often not washed off the fruit by packline sprayers, and are not noticed by pack shed staff. In this manner, they can end up in fruit cartons and can be exported to countries where they have the potential to become a pest of several crops.

Phlyctinus callosus has been detected in consignments of grapes exported from South Africa to the USA since the late 1960's. Findings of *P. callosus* in fruit consignments destined for the USA are one of the main reasons why export fruit has been rejected in the past (Myburgh and Kriegler 1967; CABI 2013). *P. callosus* is one of the main phytosanitary pest problems in the South African table grape export program to Israel. The risk of *P. callosus* manifesting as a pest of several crops grown in Israel is high, and consequently strict control measures are in place to manage the risk (Opatowski 2001).

There are currently not many options for postharvest control against *P. callosus*. Fumigation with methyl bromide substituted ethylene dibromide fumigation in 1984 when the latter was removed from the chemical register for use in the USA, because it was linked to cancer (Anon. 1993). Methyl bromide fumigation has since been the only effective postharvest mitigation treatment to ensure that live *P. callosus* do not reach export countries. However, in the 1990's, at the Montreal Protocol, after it was found that methyl bromide is an ozone depleter, it was decided that the production and use of methyl bromide treatments will only be allowed for quarantine and pre-shipment purposes until alternatives are found (Anon.

1993). The use of methyl bromide fumigation is also becoming more expensive, adding to the pressure to develop sustainable alternative postharvest treatments (Neven 2010).

Alternative treatments that are being investigated include controlled atmospheres, extreme temperatures, and irradiation. Johnson and Neven (2011) combined two of these and investigated the potential of using controlled atmospheres and extreme temperatures to control *P. callosus*, as well as the grain chinch bug, *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae), another key phytosanitary pest in South Africa. Two different heating rates were used, namely 12°C/h and 24°C/h, from a starting temperature of 23°C to a final temperature of 45°C with a gas mixture of 1% O_2 and 15% CO_2 in nitrogen. They found that this method is effective against both these pests, especially *P. callosus*. *Phlyctinus callosus* required 30 minutes less treatment time at the faster heating rate than *M. diplopterus* for 100% mortality to be reached. Although extreme temperatures and controlled atmosphere has shown great potential as postharvest mitigation treatments, and such treatments are applied on a commercial scale to certain export fruits (USDA-APHIS 2008), there are drawbacks. Heated treatments can negatively affect fruit quality, particularly if the insect pests require longer treatment times, such as *M. diplopterus*. Therefore, research into other methods that may be more suitable to certain fruit types and pests is required and ongoing. One of the most promising of the alternative mitigation treatments is the use of irradiation. Irradiation is environmentally friendly, it does not leave residues on food or the environment, and is effective against a wide variety of insects (Molins 2001). Irradiation works by breaking chemical bonds in DNA and causing either reproductive sterility at lower doses or mortality at higher doses (Ducoff 1972; Koval 1994). Irradiation as a postharvest mitigation treatment is relatively new and there are many cultivars of fruit and species of insects that have not yet been tested with this method.

The aims of this study are to evaluate the potential of irradiation as a postharvest mitigation treatment against *P. callosus*, and to determine the effects of this means of mitigation on stone fruits, one of the hosts of it. In the following sections of this chapter irradiation and how insects and food products react when treated with different irradiation doses will be discussed. Different aspects of plums, which are one of the host plants of *P. callosus*, and how this fruit is affected by irradiation will also be discussed by referring to previous plum irradiation studies.

Irradiation

History and Background

In 1895 W. C. Roentgen discovered X-rays, and the following year, A. H. Becquerel discovered radioactivity (Thorne 1991). In 1896 it was speculated that irradiation could be used to kill micro-organisms in food, but it was not until 1921 that irradiation became practically applied for that purpose when B. Schwartz obtained a US patent for the use of irradiation to control a worm infection in humans caused by the parasite *Trichinella spiralis* (Owen) (Trichocephalida: Trichinellidae) that was present in meat (Thorne 1991).

In 1948 research on food irradiation started at the Low Temperature Research Station in Cambridge, England (FDA 1986). Throughout the 1950's to 1970's research into food irradiation increased dramatically. In the 1950's the USA military sponsored food irradiation research as part of President Eisenhower's "Atoms for Peace" policy (Anon. 1986). Since 1970 various research groups were launched, such as the International Food Irradiation Project (IFIP) and the International Consultative Group for Food Irradiation (ICGFI) that completed studies on all aspects of food and commodity irradiation (Eale 1988).

In 1986 the United States Food and Drug Administration approved up to 1kGy of irradiation for disinfestation treatment of fresh fruit and vegetables (Thorne 1991). This meant that

irradiation became more accepted, and research into this manner of postharvest mitigation became more relevant. Specific pests could now be tested against irradiation and thus broaden the spectrum of pests for which irradiation can be used as postharvest mitigation treatment.

Importance of dose and source when using irradiation

When ionizing radiation passes through matter such as food or insects, it loses energy to the molecules in the matter of that object. The molecules in the matter absorb the energy lost by the radiation molecules and become ionized or excited. This leads to chemical changes taking place in the food or organism and may lead to secondary effects such as off flavours in the food or sterilization of the organism (Molins 2001). Depending on what the purpose of the treatment is, lower or higher doses of irradiation can be used. Lower doses are usually used to inhibit sprouting of vegetables (0.05 to 0.15 kGy), delay ripening of fruit (0.20 to 0.50 kGy) or to disinfest commodities of insect pests (0.20 to 1.00 kGy). Higher doses are used to control parasites (0.03 to 6.00 kGy), microbes (0.50 to 5.00 kGy), pathogens (3.00 to 10.0 kGy), and bacteria (up to 50.0 kGy) (Anon. 1986).

The longer a product is exposed to the radiation source, the higher the dose it receives. Thus, to get a product exposed to a lower dose takes less time than it does getting it exposed to a higher dose. There are a few factors that have to be taken into account when a certain dose is needed: 1) the type of source, its strength and the layout of the irradiation facility; 2) product configuration at time of exposure; and 3) conveyor speed (Molins 2001).

There are three sources of irradiation that are permitted to be used for treatment of food. These are gamma rays that are produced by radioisotopes such as cobalt-60 and cesium-137, machine generated electron beams and X-rays (Wilkinson 1986; Guise 1989; Molins 2001). Gamma rays are electromagnetic radiations that are produced when certain radioisotopes

decay, such as cobalt-60 and cesium-137. Cobalt-60 is obtained by irradiating cobalt metal in a nuclear reactor. Cesium-137, on the other hand, is present as a fission product in the fuel elements used in nuclear reactors (Guise 1989). Cesium-137 has a much longer half-life than cobalt-60. The half-life of cesium-137 is 30 years, whilst it is just 5.2 years for cobalt-60. Consequently, 12% of a cobalt-60 source must be replenished every year for it to maintain its original strength (WHO 1994). However, Cobalt-60 is the primary radioisotope used at the moment, as it is readily available, the technology for production and encapsulation is highly developed, and it has a better penetration power than cesium-137 (Molins 2001).

Electron accelerators are used to produce high-energy electron beams. These machines use electricity and linear accelerators to accelerate electron beams to very high speeds, close to the speed of light, thus producing high voltages. High-energy electron beams are in no way related to the nuclear industry and can be switched on and off very easily. One drawback is that the penetration power of high-energy electron beams is at most 5-10 cm (Cleland and Pageau 1987). Therefore, it is not very practical for use in the fruit industry where packaged boxes or pallets of fruit need to be treated. Electron beams can be converted to X-rays. X-rays have been shown to have a high degree of penetration, even higher than cobalt-60 and cesium-137. For this reason it would be practical to use for the treatment of packaged fruit, but the efficiency of electron beam to X-ray conversion is at most 4-6% (Cleland and Pageau 1987).

Product configurations and conveyor speed are strongly related when the exposure time of the product to the irradiation source needs to be calculated. The positioning of the product on the transport system is crucial as the radiation field around the source is constant. To ensure the entire product receives the same amount of irradiation throughout the product the positions at which the product is exposed to the source need to be set points (Molins 2001). It is vital that the whole pallet of cartons receives the minimum desired dose to ensure that

insects present in the cartons, no matter where, receive their sterilizing dose. The typical dose uniformity ratios at commercial irradiation facilities are 1.5 to 3.0. This means that, for example, to get a dose of 600 Gy on the inside of a stacked pallet a dose of 900 to 1800 Gy needs to be applied to the pallet (Follett et al. 2007).

Effects of irradiation – insects

Irradiation of insects as a postharvest mitigation treatment is one of the most promising uses of this technology. Doses of irradiation below 1 kGy (1000 Gy) are very effective as a disinfestation technique against various pests (Molins 2001).

Irradiation affects the life cycle of insects in various ways. It may lead to mortality, reduced longevity, delayed moulting, lower fecundity, aspermia, reduced fertility, delayed development, cessation of feeding and locomotion, and inhibition of respiration (Molins 2001). For example, irradiation can have a significant effect on digestion as it affects the midgut of the insect by killing the columnar cell lining in the midgut. This leads to infection by microorganisms that lead to the midgut being unable to absorb food, which causes reduced feeding by the insect and eventually death (Ashrafi et al. 1971). Ahmed et al. (1989) found that movement of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) was greatly affected after treatment with 300 Gy as a result of the reduced feeding. Low doses may sometimes lead to the opposite of the above mentioned effects. This may be due to a shock reaction to the sub-lethal dose that prompts the insect to feed and reproduce as quickly as possible (Molins 2001).

Tolerance to radiation normally increases as the developmental stage of the insect progresses. Thus, adults are usually the most tolerant, followed by pupae, larvae, and then eggs, being the least tolerant (Kader 1986). The reason for this is that actively differentiating insect cells are very sensitive to radiation. Adult cells are static with very little divisions and are therefore

more resistant (Molins 2001). Cell divisions in developing larvae and pupae are slightly higher than in adults, while division and tissue differentiation is at its highest in developing embryos in eggs. The effects of irradiation also vary with the age of the developmental stage. Usually, the earlier the radiation is applied in each developmental stage, the more profound the effect will be. This will also affect further development of that specific stage of development and its transition into the next stage. Eggs that receive irradiation will not hatch or result in malformed or sterile adults. When larvae receive irradiation it may prolong the larval stage. Diapausing larvae are more resistant to irradiation, but adults from these normally die after pupation. The more active an insect is, i.e. in a non-diapausing state, the more profound the effect (Molins 2001). Also, generally, female insects are more sensitive to irradiation than males (Tilton and Burditt 1983; Ahmed et al. 1976)

Sensitivity to irradiation also varies from one insect order to the next. Most insects become sterile when treated with dosages between 50 to 750 Gy, while some moth species need up to 1000 Gy (Follett 2008; Kader 1986). Coleoptera are generally the most sensitive order and Lepidoptera, especially adult moths, the most tolerant. Moths have diffused centromeres in their chromosomes, whereas beetles have monocentric chromosomes. Monocentric chromosomes obtain breakages at lower irradiation doses than diffused centromere chromosomes do, and therefore moths require higher doses for sterility to be achieved (Molins 2001). To achieve mortality of insects with irradiation may require very high doses that could be detrimental to the commodity that they are present on. However, it is unnecessary to aim for mortality when using irradiation for postharvest mitigation, as sterility also prevents establishment of the pest in the importing country. Sterility is more obtainable, and can be achieved at only 50 Gy in certain beetles and up to over 1000 Gy in some moths (Molins 2001).

In 2006 the United States Department of Agriculture – Animal and Plant Health Inspection Services (USDA-APHIS) approved generic doses of 150 Gy for tephritid fruit flies, and 400 Gy for all other pests, except pupa and adult Lepidoptera (USDA-APHIS 2006). These generic doses were based on reviews of previous studies on the effects of irradiation on insects. Here are some examples of these studies: Brower (1973) found that the bruchid beetle (*Callosobruchus maculatus*) needed 70 Gy to be sterilized. Bhuiya et al. (1991) reported that 350 Gy was enough to sterilize the angoumois grain moth (*Sitotroga cerealella*) (Olivier). Tilton and Burditt (1983) worked on the khapra beetle (*Trogoderma granarium*) (Everts) and found that the sterilizing dose for males was 160 Gy and for females it was only 50 Gy (Molins 2001). Wit and Vrie (1985) found that the beet armyworm (*Spodoptera exigua*) (Hubner), the green peach aphid (*Myzus persicae*) (Sulzer), and a certain species of thrips (*Frankliniella pallida*) (Uzel) needed dosages of 100 to 200 Gy to stop development and prevent reproduction. The comstock mealybug (*Pseudococcus comstocki*) (Kuwana) became sterile at 400 Gy (Dohino and Masaki 1995). Melon thrips (*Thrips palmi*) (Karny) became sterile after 400 Gy and 1500 Gy was needed to obtain mortality of this insect (Hara 2002). Yellow flower thrips (*Frankliniella schultzei*) (Trybom) treated with 250 Gy showed the following deviations: non-emergence of eggs and pupae, inhibition of larval development and sterility of adults (Yalemar et al. 2001).

Today the USDA-APHIS approved dosages are implemented in a number of approved postharvest treatments. However some fruit have borderline quality problems when the 400 Gy generic dose is applied and therefore lowering the radiation dose for specific pests and commodities may be beneficial (Follett 2009).

Effects of irradiation – food

The major food components namely, water, carbohydrates, proteins, lipids, vitamins and minerals, are affected in various ways when subjected to radiation treatment.

Water is a major component of almost all foods. When pure water is irradiated a number of highly reactive entities are formed. These include hydroxyl radicals, aqueous electrons and hydrogen atoms. Hydroxyl radicals are powerful oxidizing agents and aqueous electrons and hydrogen atoms are reducing agents. Food that contains water will undergo oxidation and reduction reactions because of these molecules (Stevenson 1992).

When carbohydrates are irradiated, numerous radiolytic products are produced. Browning of the sugars may be observed because of optical rotation in the chemical structure of the sugars. A mixture of gasses may also be formed such as hydrogen (H_2), carbon dioxide (CO_2), methane (CH_4), carbon monoxide (CO), and water (H_2O). Presence of other food constituents may dampen the effect of irradiation on carbohydrates. Diehl et al. (1978) observed that the presence of proteins in wheat flour changed the way radiolytic products were formed when carbohydrates were irradiated, and that much higher doses were needed to cause degradation of starch in flour.

Proteins that are irradiated are not affected severely from a nutritional point of view, as amino acids, the building blocks of proteins, rarely become damaged (Eggum 1979). Meat that is irradiated sometimes has changes in flavour and colour (Millar et al. 2000a; Millar et al. 2000b). Changes to nucleic acids, deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) can include base modification, denaturation of the DNA helix, and single- and double-strand breaks (Deeble et al. 1991).

When vitamins are irradiated in a solution, considerable reduction of these micronutrients may occur, but when foods are irradiated, the effects on the vitamins are not as extreme

(Diehl 1991; Thayer et al. 1991). Stevenson (1994) found that other food preservation techniques such as heat also destroy vitamins to mostly the same extent as irradiation does. Most vitamins, water-soluble and fat-soluble, only become reduced or damaged at doses of irradiation higher than 10kGy. These doses are much higher than those required to disinfest insect pests in fruits and vegetables. A joint study group from the Food and Agriculture Organisation, International Atomic Energy Agency and World Health Organization found that consumers need not be worried about radioactivity or radiolysis products present in irradiated foods as the amount of radioactivity present in irradiated food is similar to that found in non-irradiated food (FAO/ IAEA/ WHO 1999)

Research on the use of irradiation for different purposes has been done on a wide range of products. Kader (1986) summarised the effects of ionizing radiation on various fresh fruits and vegetables. Doses between 50 to 150 Gy have been used to prevent sprouting of tubers, bulbs, and root vegetables. Doses above 150 Gy may have some undesirable effects such as decreased wound-healing ability, tissue darkening, increased sugar content in potatoes and higher susceptibility to postharvest pathogens. Doses between 50 to 150 Gy also prevent elongation and curvature of asparagus, but may have negative effects on quality and storage life. Postharvest growth of mushrooms can be controlled and fresh appearance maintained by exposure to doses between 60 to 500 Gy. Above 500 Gy undesirable changes in colour and taste of mushrooms may arise. The ripening and senescence of tropical fruits such as banana, mango, papaya and guava, can be altered using doses between 250 to 350 Gy. Ethylene can be used at a later stage to ripen these fruit to a preferred level. Research on the irradiation of orchid flowers, day-lily flowers, and citrus has also been done. Orchid flowers tolerated between 150 to 750 Gy before negative effects appeared, depending on cultivar (Kikuchi 2000). Yang et al. (2002) suggested that fresh day-lily flowers should not be treated by irradiation to inhibit the flower blooming as the doses needed to achieve this will be

detrimental to the epidermis of the flower. Khalil et al. (2009) investigated the effect of irradiation on the Pakistani blood red oranges and concluded that treatment with 500 Gy is an effective postharvest technique to minimise the changes in physiochemical and sensory quality of these oranges during storage.

Witbooi and Taylor (2008) investigated the effects of irradiation on some horticultural products that included 'Royal Gala' and 'Granny Smith' apples, 'Packham's Truimph' and 'Forelle' pears, and 'Thompson Seedless' and 'Sunred Seedless' table grapes. Fruit was irradiated at dosages of 300, 600, 900, 1200 and 2500 Gy and then went through a cold storage regime after which quality assessments were made. The trial was carried out over 3 seasons and results varied. Some of the negative effects found with these fruit after exposure to the different doses included higher incidences of internal disorders and lower levels of greasiness on the apple cultivars and berry split, lower berry firmness and stem desiccation with the grapes. They concluded that although doses of 900 Gy is possible for table grapes and pears, apples would require doses lower than 600 Gy.

The reason for the treatment of horticultural product with irradiation is primarily as a method of postharvest control of insect pests and pathogens. If doses lower than that which would cause damage to the fruit could be used to control insect pests and pathogens that may be present in packaged export fruit it would have great potential as a non-toxic postharvest mitigation treatment.

Plums

History

Stone fruits, which include plums, peaches, apricots, cherries and almonds, belong to the genus *Prunus*, which is part of the Prunoideae subfamily of the Rosaceae (rose) family (Ertekin et al. 2006). Plums are one of the most taxonomically diverse fruits and are adapted

to a wide range of climatic and edaphic conditions. The two most commonly produced plums are *Prunus domestica* L. and *Prunus salicina* (Lindl), the European plum and the Japanese plum, respectively. Reports of plums in literature can be traced back 2000 years, yet it is thought that plums have been produced for 4000 years. The European plum originated in Southern Europe or Asia, close to the Caucasus Mountains and the Caspian Sea (Cullinan 1937). European plums are now produced in most regions with temperate climates. The Japanese plum became established in Japan approximately 200 to 400 years ago, but originated in China (Tromp et al. 2005).

The European plum is the primary plum produced for consumption throughout the world. The fruit size, colour and shape vary. This species of plum include green, yellow, red and blue fruit, and the shape may be round or oval. The fruit has good eating quality and can be free or clingstone (Tromp et al. 2005). The Japanese plum, on the other hand, is mostly produced in temperate or semi-arid regions. The fruit is bigger and more attractive than the European plum, but its flavour is inferior. The colour and shape of the fruit also varies considerably, but are mostly clingstone. Trees of the Japanese plum are more vigorous and have greater production than the European plum (Tromp et al. 2005). Countries such as Egypt, Japan, Pakistan, South Africa, Australia, New Zealand and China produce mainly Japanese plums, although European plums are grown in the cooler parts of these countries (Yoshida 1987; Ramming and Cociu 1991; Faust and Surányi 1999).

Plum production

The world produced 11.4 million tonnes of plums in 2011 (Hortgro 2012). China contributed 5.9 million tonnes to this. The Serbia, Romania and the USA are the other prominent producers. South Africa produced 66736 tonnes of plums in 2011 and 75% of this fruit was exported. The rest was used for the local market (22%) or was processed (3%). Europe and

Russia received 50% of the plums that South Africa exported, the United Kingdom 26% and the other 24% went to the Middle East, Far East, Asia, Indian Ocean Islands and other African countries. 'Laetitia', 'Songold', 'Sapphire', 'African Delight' and 'Angeleno' are the primary cultivars that are produced and the primary export cultivars are, by far, 'Laetitia' and 'Songold'. The Western Cape Province produces the most plums in South Africa, although plums are produced in all eight other provinces (Hortgro 2012).

Plums are the most tolerant of the stone fruits to poor drainage (Tromp et al. 2005). It is not easy to say exactly what soil requirements each specific fruit species has because it depends on the different rootstocks used, but it seems that in general stone fruit prefer lighter soils with sufficient moisture holding capacity and good drainage (Tromp et al. 2005). In South Africa the vigorous 'Marianna' and peach/almond hybrid rootstocks are used due to poor soil conditions and trees are planted at high densities of 1667-2222 trees per hectare, 1.5m apart and 4m between rows (Cook 2004). In South Africa plum trees are pruned in winter according to the 2:1 rule and new branches are bent to a 45° angle and tied to a trellis to prevent wind damage (Cook 2004).

Postharvest storage plum disorders

Plums, apples and pears are all climacteric temperate fruit, but certain cultivars of plums can be suppressed climacteric, such as 'Angeleno' (Candan et al. 2011). Plums do not have a very long postharvest life when compared to apples and pears (Kader 1992). Using low temperature is one of the most effective means to delay postharvest ripening of plums (Kader and Mitchell 1989) by reducing ethylene production, respiration rate, pigment changes, softening, increase in total soluble solids (TSS) and reduction in titratable acidity (TA) (Crisosto et al. 2007). Plums can be stored with dual or single temperature regimes depending on the cultivar. Temperatures below 0°C but above freezing point are used. For example,

'Songold' plums are stored using a dual temperature storage regime of -0.5°C for 10 days, followed by 18 days at 7.2°C (Taylor et al. 1995). A single temperature regime would typically consist of 35 to 42 days at -0.5°C (or between -2°C and 0°C). 'Larry Ann', 'Angeleno', 'Southern Bell', 'Lady Red' and 'Purple Majesty' are examples of plum cultivars that can be stored with either single temperature or dual temperature regimes (Hortgro 2008).

Chilling injury is a concern with many plum cultivars depending on the storage temperature and span (Taylor 1996). Chilling injury includes symptoms such as flesh translucency or "gel breakdown" and browning of the flesh. These symptoms usually appear when the fruit is on the shelf in the supermarket and are exposed to temperatures between 10 to 20°C. Flesh translucency or "gel breakdown" is a translucent and gelatinous area in the flesh around the stone characterized by a loss of juiciness (Taylor 1996). Chilling injury is related to modifications in membrane permeability associated with membrane-lipid transition from a flexible liquid-crystalline to a solid-gel structure (Lyons 1973) .

Candan et al. (2011) looked at how 1-methylcyclopropene (1-MCP) would affect the symptoms of chilling injury if applied before cooling to four cultivars of Japanese plums namely, 'Royal Zee', 'Linda Rosa', 'Friar' and 'Angeleno'. They found that 1-MCP could significantly reduce the symptoms of chilling injury by inhibiting ethylene production. Modified atmosphere packaging (MAP) is another means of delaying ripening. MAP has selective permeability to CO_2, O_2 and water vapour, thus leading to increased CO_2, decreased O_2 and higher water vapour inside the packaging as the fruit respiration continues. As the gas composition in the packaging changes, the respiration rate will drop, causing a delay in the ripening process, by delaying change in colour and minimising firmness and acidity losses (Díaz-Mula et al. 2011).

Plum irradiation studies

When South African fruit are exported to certain countries, a mandatory 22 days at -0.55°C are required to disinfest fruit of phytosanitary pests (USDA-APHIS 2013). However, certain plums need a dual-temperature regime to maintain postharvest quality, therefore this is not a viable method for all cultivars (Viljoen 2011). If irradiation can be shown to be effective as an insect disinfestation method, and is not detrimental to the fruit itself, the mandatory cold storage would not be necessary and the fruit could be shipped at whatever temperatures are most beneficial to it (Taylor and Brock 1998). Research into the irradiation of plums is minimal, but it has shown promise with the cultivars that have been tested thus far.

Taylor and Brock (1998) investigated the effects of irradiation on two plum cultivars namely, 'Laetitia' and 'Songold'. They treated the fruit with 300, 330 and 500 Gy to achieve absorbed doses of 150, 300 and 400 Gy, respectively. The fruit was then put into cold storage for 35 days at dual temperature and then 7 days at 10°C after which quality assessments were done and O_2 consumption and CO_2 and ethylene (C_2H_4) production were measured. 'Laetitia' plums exhibited a 30% increase in shrivel between 330 Gy and 500 Gy, suggesting that doses less than 330 Gy could be used to treat 'Laetitia' plums without undesirable levels of shrivel. With 'Songold' plums they found that gel breakdown was significantly higher with all absorbed doses in comparison with the untreated controls and that irradiation adversely affected internal quality of 'Songold' plums after being stored for six weeks. For both cultivars they found that irradiation did not have a significant effect on the production of CO_2 and C_2H_4 or the consumption of O_2. But with 'Songold' there was a tendency towards higher CO_2 production and O_2 consumption as the dose increased.

More recently Viljoen (2011) investigated the potential of using irradiation to treat 'Songold' plums similar to the way that Taylor and Brock (1998) did, but looked at how applying 1-

MCP, in the form of Smartfresh™, 5 and 12 days after cooling at -0.5°C would affect the negative effects that irradiation with 400, 600, 800 Gy had on these fruit. He found that by applying Smartfresh™ the amounts of shrivel and gel breakdown of the irradiated fruit was greatly reduced. Also flesh firmness was higher in Smartfresh™ treated fruit. He concluded that by applying Smartfresh™, the undesirable effects of irradiation on 'Songold' plums could be minimised.

Thesis outline and study objectives

The overall aim of my project is to expand on the potential postharvest treatments available for use against *P. callosus,* to help improve market access and opportunities for export of South African fruit. My specific objectives are 1) to evaluate the potential of irradiation of adult *P. callosus* as a postharvest mitigation treatment; 2) determine the effects of irradiation on the fruit quality of the new pluot cultivar 'Flavor Fall' (Pluots are interspecific hybrids of complex crosses of plum and apricot, with predominantly plum parentage, typically with a smooth skin (Crisosto et al. 2007)); and 3) investigate methods for rearing *P. callosus* in the laboratory, as a laboratory colony would enable faster progress of such research that is currently dependant on the seasonal availability of this insect pest. These objectives are dealt with in the following three chapters.

- Chapter 2 describes the trial in which field-collected *P. callosus* adults were subjected to a range of irradiation doses, and the fecundity and fertility of different mating crosses of treated and untreated adults were determined.
- Chapter 3 presents the trail in which the pluot cultivar, 'Flavor Fall', is exposed to different doses of irradiation to evaluate the effects of this treatment on the quality parameters and storage potential of the fruit.

- Chapter 4 is presented as a review of previous rearing studies on this pest, where I will also present my findings of a rearing trial and propose recommendations for establishing a laboratory colony of *P. callosus*.

Chapter 5 is the concluding chapter where I will summarise my findings from the previous chapters and evaluate the overall aim of this study.

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Chapter 2

The potential of irradiation as a postharvest disinfestation treatment against *Phlyctinus callosus* (Coleoptera: Curculionidae)

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Abstract

Phlyctinus callosus (Boheman) (Coleoptera: Curculionidae) is a pest of major phytosanitary concern for some of South Africa's biggest export markets such as the United States of America and Europe, since this pest does not occur there. At present, fumigation with methyl bromide is the only postharvest disinfestation treatment against this pest and therefore sustainable alternatives are needed. One such alternative is irradiation treatment of whole pallets of packed fruit to sterilize insects that may be present within the cartons. Wild adult *P. callosus* weevils were treated with 5, 10, 20, 40 and 80 Gy of gamma irradiation and then cross mated to breed with either treated or non-treated adults of the opposite sex. Fecundity and fertility were monitored and recorded. Trials were conducted during the 2009/10 and 2010/11 fruit harvesting seasons. The results from both seasons indicated that irradiation did not affect fecundity but fertility was significantly affected, decreasing as irradiation dose increased. Egg hatch was zero for mating crosses that involved females weevils treated with a dose of 80 Gy gamma irradiation. Probit analysis indicated that, in the first season the estimated LD95 for crosses involving treated males and treated females was 30 Gy, while in the second season it was 49.5 Gy. Respective estimated LD99s were 47.9 and 169.4 Gy. Ultimately, a dose lower than the current generic dose of 400 Gy, approved for irradiation disinfestation treatments, would control *P. callosus* should they occur in packed export fruit.

Key words: irradiation, *Phlyctinus callosus*, phytosanitary, fecundity, fertility

Introduction

Insect pests with restricted global distributions pose a phytosanitary risk in the international trade of fresh agricultural products. A key phytosanitary pest that countries importing fruit from South Africa have to take into consideration is the curculionid, *Phlyctinus callosus*, (Boheman), commonly known as the "banded fruit weevil". *P. callosus* is indigenous to the Western Cape of South Africa where it has been a pest of deciduous fruit since 1896 (Barnes 1989), and is a major pest of pome fruit, stone fruit and vines (Annecke and Moran 1982). Apart from South Africa, *P. callosus* is also found in parts of Australia and New Zealand (CABI 2013). The pest status of *P. callosus* in the regions in which it occurs, coupled with its limited global distribution makes this curculionid a pest of phytosanitary / quarantine concern to the many countries where it is not present.

In the past fumigation with methyl bromide was the only commercial postharvest disinfestation treatment available for use against *P. callosus*. However, with the need for environmentally-friendly techniques, an alternative postharvest disinfestation treatment, that is fast becoming an accepted, safe, non-toxic, and effective treatment, is phytosanitary irradiation. Irradiation for the purpose of insect sterility for the sterile insect technique is widely used in preharvest pest management, and in recent years insect sterility as a postharvest control measure against phytosanitary pests has also been used. In 2006 the United States Department of Agriculture - Animal and Plant Health Inspection Services (USDA-APHIS) approved generic doses of 150 Gy for the treatment of tephritid fruit flies and 400 Gy for all other pests, except pupa and adult Lepidoptera (USDA-APHIS 2006). As a result of these approved generic doses, worldwide use of irradiation as a disinfestation treatment now includes treatments for a variety of fresh fruits and vegetables imported from Hawaii, India, Mexico, Thailand and Vietnam, into the United States, as well as mango,

papaya and litchi from Australia into New Zealand. A detailed review of how these markets developed is given in Hallman (2011). Recently irradiation became an approved option for the export of fresh grape, litchi and persimmon from South Africa to the United States (USDA-APHIS 2013). *P. callosus* is a key phytosanitary pest of South African grapes, as well as other fresh fruit commodities, and has been intercepted in fruit consignments imported into the USA from South Africa in the past.

Subsequent to the approval of generic doses, continued research on the effect of irradiation on specific plant pests has led to the USDA-APHIS approving minimum doses for 23 specific phytosanitary insect pests (USDA-APHIS 2013). The list includes ten tephritid fruit fly species (Diptera), six species of Lepidoptera, four curculionid species (Coleoptera), two species of scale (Hemiptera) and one mite species (Acari). Approved species-specific doses for fruit flies range between 60-150 Gy, 100-250 Gy for Lepidoptera species, 92-300 Gy for Coleoptera, 150 Gy for the scale species and 300 Gy for the spider mite. The generic dose of 400 Gy for all insects, except adult and pupae Lepidoptera, which is accepted by USDA-APHIS, is not accepted by the International Plant Protection Convention (IPPC) as an international standard phytosanitary treatment for quarantine pests. However, the 150 Gy minimum absorbed dose for Tephritidae is, and is included in the International Standards of Phytosanitary Measures (ISPM) as ISPM #28, together with 13 species-specific treatment schedules (IPPC 2011). Hallman et al. (2010) discusses the submission and review process of USDA-APHIS approved treatments proposed to the IPPC for consideration before 2011. Adopted treatments included the generic dose for Tephritidae and species-specific doses for seven species of fruit fly, two Lepidoptera and one Coleoptera species. In 2011, three additional treatments were adopted by the IPPC, one for *Ceratitis capitata* (Weidemann) and two for two more species of Coleoptera (IPPC 2011).

All approved species-specifc treatments are at dosages lower than the generic dose previously approved by USDA-APHIS. Lowering the 400 Gy generic dose prescribed by USDA-APHIS may be beneficial when considering the cost and quality problems that can arise when certain fruits and vegetables are treated with this dose. Lowering the generic dose will also broaden the spectrum of fresh agricultural products that can be treated with irradiation. Follett (2009) advocated that future irradiation research should focus on 'lowering dose levels below 400 Gy for specific commodities with a limited number of quarantine pests', as well as 'developing generic doses below 400 Gy for other important groups of quarantine pests'. Candidate species for research that comprise the important pest groups for which generic treatments should be developed are discussed by Hallman et al. (2010). The authors present four main pest groups: weevils, Lepidoptera larvae, Sternorrhyncha or Coccoidae and Acari. Hallman (2011) also presents a number of cases indicating the usefulness of generic doses for important pest groups such as mealybugs, scales and weevils.

Previous irradiation studies on weevils suggest that a generic dose lower than 400 Gy is applicable to this group of pests. The sweet potato weevil, *Cylas formicarius elegantulus* (Summers) and the West Indian sweet potato weevil, *Euscepes postfacius* (Fairmaire), were unable to reproduce after exposure to 140 Gy and 145 Gy gamma irradiation, respectively (Follett 2006). The diaprepes root weevil, *Diaprepes abbreviates* L., was unable to reproduce after a treatment with 50 Gy (Gould & Hallman 2004). Hallman (2003) found that 92 Gy was sufficient to control the plum curculio, *Conotrachelus nenuphar* (Herbst). Sterility studies on the mango seed weevil, *Sternochetus mangiferae* F. have shown that females treated with 50 or 70 Gy laid sterile eggs (Seo et al. 1974), and also emerging adults from fruit treated with 100 and 300 Gy did not lay eggs (Follett 2001), but due to the cryptic feeding nature of this pest and the lack of an artificial diet, large-scale studies needed to confirm this have not yet been done.

In the present study the effect of irradiation on the reproductive success of *P. callosus* adults was investigated to establish whether this phytosanitary pest can also effectively be controlled at irradiation doses lower than the prescribed 400 Gy generic dose.

Materials and Methods

Insects

Field-collected, actively reproducing *P. callosus* adults were obtained from two farms in the Elgin/Grabouw area of the Western Cape, South Africa. Weevils were collected using corrugated cardboard bands placed around the trunks of apple trees. Adult weevils seek shelter during the day, and cardboard bands provide good sheltering sites. Bands were checked weekly and all sheltering weevils were taken to the laboratory where the sexes were separated and kept in 40 x 30 x 30 cm Perspex cages (± 25°C, ± 70% RH, photoperiod 14:10 [L:D] h). *Coprosma repens* (Rich) twigs were placed in the cages to serve as food, cotton eye-pads as oviposition sites and rolled up moist paper towels for shelter. Females were allowed to lay eggs for three days before experimental trials commenced.

Treatments

Irradiation treatments were carried out during two consecutive seasons: 2009/10 and 2010/11. Each trial consisted of a control (0 Gy), five irradiation doses (5, 10, 20, 40 and 80 Gy) and three mating crosses. The mating crosses were: 1) treated females and non-treated males (Tf/Nm); 2) non-treated females and treated males (Nf/Tm) and 3) treated females and treated males (Tf/Tm). There were three replicates of 20 adults of each sex per irradiation dose and mating cross in the first season (n = 120 individuals per treatment), and two replicates of 20 adults of each sex per dose and mating cross in the second season (n = 80 individuals per treatment). Irradiation treatments were carried out at the SIT Africa irradiation facility in Stellenbosch, South Africa. The radiation source was a 500 Curie ^{60}Cobalt gamma irradiator

[Owners ARC Infruitec-Nietvootbij; Source origin Russia; Installed by NECSA (South African Nuclear Energy Corporation) in 2000]. Reference standard dosimetry was done in 2001 and 2007 by iThemba labs, South Africa. Routine dosimetry was done with Fricke dosimeters and analysed with a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) at 500 nm absorbance to verify dose accuracy and measure variation. The unit delivered a gamma ray dose rate of ±1.98 Gy/min at the time of this research. Just prior to irradiation, weevils were removed from cages and placed in 100 mm petri dishes with a few *C. repens* leaves. After treatment weevils were transferred from the petri dishes into 1 litre plastic containers, with either treated or non-treated adults of the opposite sex (according to the mating cross). Weevils were kept in the same conditions as described above for field-fresh weevils brought into the laboratory. The oviposition pads were removed from the cages every two to three days, and replaced with fresh, moistened pads. Removed pads were inspected for eggs which were collected, counted and transferred to moist filter paper inside petri dishes. The petri dishes were sealed with parafilm and kept at 25°C in complete darkness. Eggs were checked seven days after collection, and the number of hatched larvae was recorded. Egg collection was done until adults weevils died.

Statistics

Regression analyses were done to determine the relationship between the radiation dose and the number of eggs laid (fecundity), and the number of larvae that hatched (fertility), for both seasons. Dummy variable linear regression (Gujarati 1970a, 1970b) was used to determine whether or not there were differences in the regressions for fertility between the three mating crosses and between the two seasons. The full model of this regression assumed separate slopes and intercepts for all combinations of mating crosses and seasons. This was compared to a reduced model, selected by inspecting the regression coefficients of the full model, where common slopes and/or intercepts were assumed for some of the combinations (namely,

Tf/Nm and Tf/Tm in 2009/10 and 2010/11 seasons). The F-test was used to determine whether or not the reduced model differed from the full model, as described by Gujarati (1970a, 1970). Thereafter a Probit analysis (Finney 1971) was done to determine the relationship between dose and mortality of the eggs. Tests were conducted to determine whether or not the probit regression lines were coincidental. If not, they were tested for parallelism. The LD95 and LD99 values were determined, as well as the relative sensitivity to the irradiation doses (Finney 1971).

Results

Control groups of insects that were not irradiated laid 26.33 ± 4.6 eggs per female in the first season and 20.08 ± 1.1 in the second season. Within expectations, for each mating pair in the control groups more than 90% of the eggs laid hatched. The fecundity and fertility data of each mating cross treated with the different irradiation doses in both seasons, as well as the regression analysis results between irradiation dose and fecundity and fertility, are shown in Table 1. The low r^2 values associated with fecundity indicate a poor correlation between dose and the mean number of eggs laid per female, which is also not significant in any of the crosses or either of the seasons. Thus, treatment with ionizing irradiation up to 80 Gy did not affect oviposition. The high r^2 values associated with fertility of crosses involving treated females indicate a strong correlation between dose and the mean number of eggs hatched per female. As dose increased, fertility decreased. This correlation was stronger and significant ($p < 0.05$) in the second season as opposed to the first. In both seasons however, treatment with 80 Gy ionizing irradiation resulted in zero egg hatch in crosses with treated females.

Table 1. Mean number of eggs laid per female (fecundity) (FEC) and mean number of eggs hatched per female (fertility) (FER) for the 2009/10 and 2010/11 seasons for each mating cross of treated females and non-treated males (Tf/Nm), non-treated females and treated males (Nf/Tm) and treated females and treated males (Tf/Tm) after each treatment of irradiation. For each mating cross the r^2 values, degrees of freedom (df) and *P*-values determined by regression analysis are also shown. The standard error of mean is given in brackets below each fecundity and fertility data point.

Cross	Tf/Nm				Nf/Tm				Tf/Tm			
Season	2009/10		2010/11		2009/10		2010/11		2009/10		2010/11	
Dose (Gy)	FEC	FER	FEC	FER	FEC	FER	FEC	FER	FEC	FER	FEC	FER
5	23.63 (9.78)	20.2 (8.45)	2.83 (0.98)	1.03 (0.73)	14.48 (4.73)	13.33 (4.53)	3.75 (0.05)	2.58 (0.43)	17.45 (9.00)	14.28 (7.13)	3.45 (0.45)	0.93 (0.13)
10	12.1 (2.48)	5.73 (1.10)	4.15 (1.55)	1.25 (0.15)	30.95 (8.02)	26.63 (7.08)	4.18 (0.43)	2.53 (0.08)	20.73 (5.60)	7.58 (2.24)	3.83 (2.53)	0.83 (0.48)
20	16.05 (3.25)	4.37 (0.82)	1.98 (0.13)	0.35 (0.05)	27.38 (8.93)	22.68 (7.09)	3.68 (0.28)	2.1 (0.25)	15.47 (0.45)	3.77 (0.49)	4.28 (0.58)	0.7 (0.20)
40	39.72 (2.74)	0.88 (0.44)	3.35 (0.45)	0.48 (0.48)	23.47 (7.67)	22.53 (5.08)	4.1 (0.15)	2.23 (0.03)	31.63 (6.27)	0.17 (0.02)	2.23 (1.13)	0.1 (0.05)
80	7.22 (2.22)	0 (0.00)	2.93 (0.68)	0 (0.00)	16.8 (4.28)	13.12 (3.52)	2.78 (1.38)	1.65 (0.80)	9.02 (3.04)	0 (0.00)	3.03 (0.68)	0 (0.00)
r^2	0.05	0.48	0.01	0.9	0.12	0.17	0.6	0.18	0.11	0.61	0.26	0.87
df	4	4	4	4	4	4	4	4	4	4	4	4
P-value	0.73	0.2	0.88	0.01	0.57	0.49	0.12	0.47	0.59	0.12	0.38	0.02

There was no difference between the reduced and full models, therefore dummy variable regression analysis results for fertility of the different mating crosses at each irradiation dose in each season are presented in Figure 1 as the reduced model. The reduced model produced a good fit to the data ($F = 38.02$; $df = 7.54$; $p < 0.001$). In both seasons, for the crosses that involved treated females (Tf), the slope values [Tf/Nm; Tf/Tm, 2009/10 (-1.89) and Tf/Nm; Tf/Tm, 2010/11 (-0.57)] indicates a strong negative relationship between irradiation dose and fertility. This means that there is a good correlation between the decrease in number of eggs that hatched and the increasing irradiation. The dummy variable data depicted in Figure 1 predicts that in the 2009/10 season approximately 38 Gy was enough to prevent egg hatch of crosses that involved treated females. In the 2010/11 season, according to Figure 1, 56 Gy was sufficient to stop eggs produced from crosses that involved treated females from

hatching. For the crosses that involved only males that were treated (Tm) and non-treated females (Nf), the maximum treatment dose of 80 Gy that was applied was not enough to stop reproduction. At 80 Gy percentage egg hatch per female was still above 50% in both seasons. To further analyse the relationship between irradiation and reproductive success, probit regression lines for egg mortality were compared. In the 2009/10 season the value for the slope of the probit regression (0.9308), in the case of the cross involving Nf, was much less than that for the two probit regression lines involving Tf (3.3023 & 3.2175). In the 2010/11 season the value for the slope of the probit regression for the cross involving Nf (0.5356)

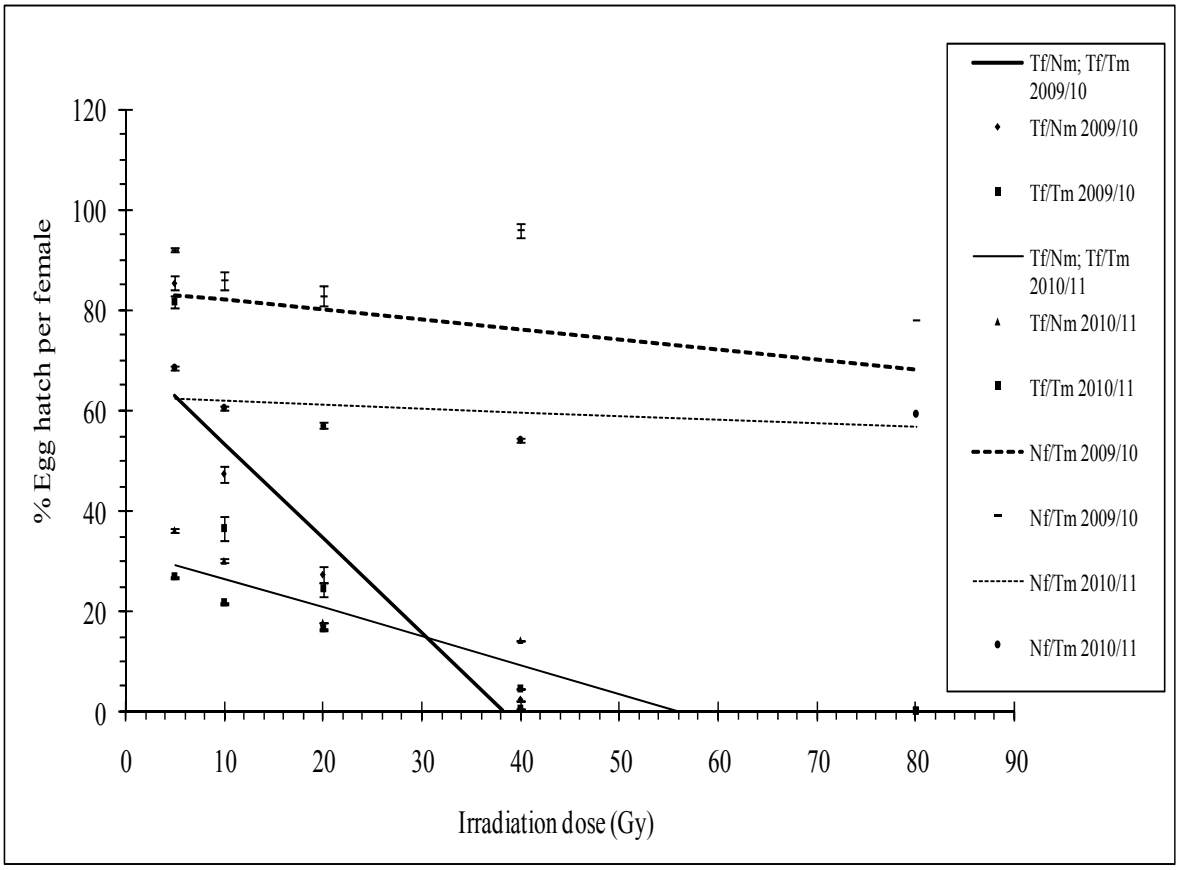

Figure 1. Reduced model of percentage egg hatch per female (fertility) for the 2009/10 and 2010/11 seasons for each mating cross of treated females and non-treated males (Tf/Nm), non-treated females and treated males (Nf/Tm) and treated females and treated males (Tf/Tm) after each treatment of irradiation.

*Tf/Nm; Tf/Tm 2009/10, Slope: -1.89; Intercept: 72.35. Tf/Nm; Tf/Tm 2010/11, Slope: -0.57; Intercept: 32.07. Nf/Tm 2009/10, Slope: -0.20; Intercept: 84.10. Nf/Tm 2010/11, Slope: -0.08; Intercept: 62.77.

Table 2. Probit analysis results (slope ± SE, intercept ± SE, x^2 , degrees of freedom (df), *P*-value, LD95 and LD99 (fiducial limits)) on fertility data for treated female and non-treated male (Tf/Nm) and treated female and treated male (Tf/Tm) mating crosses in 2009/10, and treated females and treated males (Tf/Tm) in the 2010/11 season.

Season	Cross	Slope ± SE	Intercept ± SE	x^2	df	*P*-value	LD95 (Fiducial limits)	LD99 (Fiducial limits)
2009/10	Tf/Nm	3.27 ± 0.06	1.59 ± 0.07	51.42	2	0.001	35.40 (30.62 to 41.97)	57.24 (43.19 to 84.49
	Tf/Tm	3.27 ± 0.06	1.84 ± 0.07	0.51	1	0.476	30.03 (25.64 to 35.18)	47.94 (36.13 to 70.96)
2010/11	Tf/Tm	1.27 ± 0.19	4.50 ± 0.22	14.30	8	0.074	49.51 (31.59 to 118.77)	169.37 (52.61 to 15724.0)

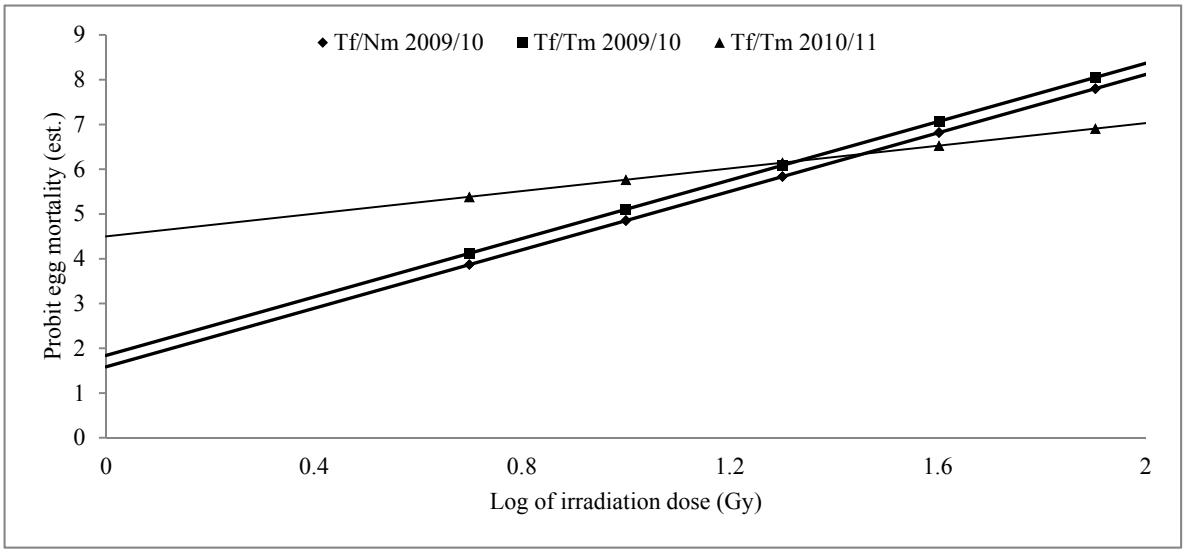

Figure 2. Probit egg mortality estimate for treated females and treated males (Tf/Tm) and treated females and non-treated males (Tf/Nm) in 2009/10, and treated females and treated males (Tf/Tm) in the season 2010/11, plotted against the log of the dose (Gy).

was less than half the value of the slope for the other two crosses that involved Tf (1.2744 & 1.2664). Therefore, in both seasons, the treatment using Nf was not included in the comparison of the probit regression lines. The probit regression lines for Tf/Nm and Tf/Tm crosses made in 2009/10 were not coincidental, but they were parallel (Table 2; Figure 2). The Tf/Nm cross in 2010/11 season had a very high chi-square goodness of fit value (χ^2 = 67.51; df = 8; $p < 0.0001$) indicating a very poor fit and thus the estimation of the probit regression lines could not be made. For the Tf/Tm cross in the second season, the chi-square goodness of fit value indicated a good fit (Table 2; Figure 2).

The relative potency of the radiation treatment indicated that, in the 2009/10 season, 1.19 times the dose required to produce a given level of egg mortality for the Tf/Tm cross would be required to produce the same level of mortality in the Tf/Nm cross (Table 2). For example to obtain 99% egg mortality with the Tf/Nm cross 57.24 Gy would be required, in comparison with 47.94 Gy needed with the Tf/Tm cross. This comparison could not be made with the 2010/11 results. Although the estimated LD99 for the second season was 169.37 Gy, the observed mortality was 100% at 80 Gy.

Discussion

Mating crosses involving treated females were unable to reproduce after treatment with a relatively low dose of 80 Gy gamma irradiation. Postharvest treatments are targeted at the most tolerant life stage found in the shipped commodity, but unlike other disinfestations techniques, irradiation does not need to kill the pest to ensure quarantine security. It is focussed on eliminating reproductive capacity, and therefore can instead be targeted at the most sensitive sex of the most developed stage that may be shipped with a commodity. Age and developmental stage are important parameters influencing radiotolerance, as the further developed the life stage the higher its radiotolerance (Bakri et al. 2005; Hallman et al. 2010).

Adults are therefore the most radiotolerant life stage, as was shown for the plum curculio, *Conotrachelus nenuphar* (Hallman 2003). *C. nenuphar* eggs were prevented from developing beyond early instars by treatment with 20 Gy irradiation. Larvae treated with 40 Gy did not develop into pupae and adults required 80 Gy to prevent reproduction. *C. nenuphar* however can occur on fruit as eggs, larvae or adults, and therefore all life stages needed to be tested to determine radiotolerance and potential for irradiation as a postharvest mitigation treatment. No distinction was made between male and female *C. nenuphar* adults. *P. callosus* adults however are the only life stage that affects the fruit, as the egg and larval stages are confined to the soil of the orchard floor. *P. callosus* adults are also only found on the exterior of the fruit, thus irradiation under hypoxic atmospheres is not a factor for this species, as it is for other phytosanitary weevil species (Heather and Hallman 2008).

In this study, a distinction was made between the sexes, highlighting females as being more sensitive to the detrimental effects of irradiation. Generally female arthropods are more sensitive than males (Hallman 1998, Bakri et al. 2005, Robinson 2005), but exceptions have been reported. Sharp (1995) found that for the sweetpotato weevil, *Cylas formicarius elegantulus* (Summers) 270 Gy did not prevent female reproduction while 150 Gy prevented male reproduction. However, previously, Dawes et al. (1987) observed the opposite finding for this species in which it still conformed to the observation of lower radiotolerance in females. The difference in radiotolerance between males and females may be due to female reproductive processes (egg maturation and development) being more complex than male reproduction (spermiogenesis) (Hallman 1998, Bakri et al. 2005). In addition, females carry significantly less reproductive cells (gametes) than males, therefore the same level of treatment would result in a greater effect in females than in males. At the genetic level, radiation-induced mutations in DNA lead to chromosomal breaks that perpetuate and accumulate during cell division ultimately causing cell death (Robinson 2005). The stage of

development of reproductive cells at the time of irradiation is also an important consideration (Carpenter et al. 2005) in assessing radiotolerance.

With regard to the development of irradiation disinfestation treatments, the fact that female *P. callosus* adults are more sensitive than males means that research need only be concentrated on sterilizing the female regardless of whether both sexes are present in packaged fruit. Females irradiated with 80 Gy and mated with either treated or non-treated males did not produce viable eggs. It is unknown whether treated females were carrying already-fertilised eggs when treated, since wild-caught females were allowed to oviposit for three days before irradiation treatment was started. Research has shown that after treatment with a sterilizing dose, gravid females either lay eggs that do not hatch or hatching neonates do not develop (Follett et al. 2007). Gravid female *P. callosus* adults will need to be treated to confirm that irradiation with 80 Gy results in non-viable eggs or offspring. Large-scale testing with >10 000 individual *P. callosus* adults is required to confirm a species-specific dose, and for this, a laboratory colony is needed. However, effective rearing methods for *P. callosus* are not yet available. A laboratory colony will also allow further research on the radiation biology of *P. callosus* and determine the most effective measure of prevention of reproduction. Determining a measure of prevention of reproduction is important in reviewing literature towards the proposal of a generic dose for a group (Hallman et al. 2013). Survival of neonate *P. callosus* larvae that do hatch after treatment with sub-sterilizing dosages should be determined in order to monitor the development of the F_1 generation.

Dosages that are less than the 400 Gy minimum dose, as is currently required by USDA-APHIS would be advantageous in maintaining fruit quality. Applying the minimum dose of 400 Gy to all parts of a standard pallet-load of packed fruit may result in some fruit near the exterior of the load receiving at least twice that dose. Exceeding 1000 Gy is prohibited by USDA-APHIS for fresh fruit irradiation. South African export pome, stone and grape

cultivars have been tested for the effect of phytosanitary irradiation on fruit quality. The storage quality of, 'Forelle' and 'Packham's Triumph' pear cultivars was acceptable after irradiation at 300, 600 and 900 Gy, however apple cultivars 'Royal Gala' and 'Granny Smith' showed signs of adverse effects on fruit quality at 600 and 900 Gy (Witbooi and Taylor 2008). In the same study table grape cultivars, 'Thompson Seedless' and 'Sunred Seedless' showed adverse effects at 900 Gy. Stone fruit cultivars, "Songold' plums and "Flavor Fall' pluots, both showed adverse effects on storage quality after irradiation at dosages ranging from 400 – 900 Gy (Viljoen 2011, Duvenhage et al. 2012).

A generic dose less than 400 Gy for Curculionidae was first proposed in 1998 at 200 Gy (Hallman 1998). In 2011 the proposed dose was 150 Gy (Hallman 2011), but more recently a review of generic phytosanitary irradiation treatments proposed that a generic dose for Curculionidae should be no less than 165 Gy (Hallman 2012). Accepted pest-specific doses for weevils include: *C. nenuphar* at 92 Gy, *E. postfasiatus* and *C. formicarius elegantulus* at 150 Gy and *S. mangiferae* at 300 Gy (USDA-APHIS, 2013), while the IPPC accepts the same for *C. nenuphar* and *E. postfasiatus*, but requires 165 Gy for *C. formicarius elegantulus* (IPPC 2011). More research is required to establish an acceptable dose for sterility in *S. mangiferae*. The current dose of 300 Gy is based on no adult emergence from treated fruit, but the dose for sterility would be less than that, nearer to 100 Gy, as suggested by Seo et al. (1974) and Follett (2001). An effective dose for the mango pulp weevil, *Sternochetus frigidus* is close to 165 Gy (Hallman 2012). Stored-product pest weevil species belonging to the genus *Sitophilus* require up to 100 Gy for sterility (Franco et al. 1997; Aldryhim and Adam 1999). Results from the present study indicate that radiation-induced sterilization of *P. callosus* adults at 80 Gy supports the development of irradiation as a disinfestation treatment for this species, as well as the Curculionidae as a group, at a dosage as low as 165 Gy.

It is also important to consider the development of a specific versus a generic dose. In cases where specific weevil pests of concern are associated with specific regulated commodities, focussing research on obtaining specific doses for those pests may be more valuable than developing generic doses. Data obtained from radiation studies can be used in supporting proposals for group generic doses, but species-specific doses are useful in countries where specific species are of concern. In such cases, if a species-specific dose that is less than the 400 Gy generic dose is effective, this will already improve treatment applicability and support the use of a dosage less than 400 Gy for the group as well.

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Chapter 3

Irradiation as a postharvest quarantine treatment for a new pluot cultivar

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Abstract

The effects of irradiation treatment on the quality of 'Flavor Fall' pluots (*Prunus salicina* x *armeniaca* Lindl.) were tested to determine if this is a feasible alternative postharvest mitigation treatment to disinfest fruit of quarantine pests. Packed cartons of fruit were treated with three doses of irradiation (400, 900 and 1400 Gy) where after the fruit was cold stored for 42 d with a dual temperature regime and then 7 d to simulate shelf-life conditions. The effect of two different bag types used inside the carton was also tested; a high density polyethylene perforated bag used commercially to package grapes, and a low-density polyethylene insect-proof bag with much smaller perforations. Respiration rates and ethylene production were determined at various time points during the trial, and quality parameters such as hue angle, shrivel, flesh firmness, decay and gel breakdown were measured throughout the process. The results indicated that the respiration rate of the fruit generally increased as the irradiation dose increased, except after shelf-life where it decreased after reaching a maximum at 400 Gy. Even at the highest dose most of the quality parameters measured at the end of cold storage were above the minimum arrival standards for overseas markets, except for shrivel, a condition that is common in the 'Flavor Fall' cultivar. Shrivel was higher than the maximum allowed, and although fruit contained in the insect-proof bags had half the amount of shrivel than fruit in the commercial bag, it was still above the maximum 10% allowed for export fruit. After further optimization of the approach, a system using insect-proof bags and irradiation would be an effective non-toxic alternative to currently used mitigation treatments for plums.

Keywords: irradiation, postharvest treatment, quarantine, pluot, plum

Introduction

International trade in fresh agricultural products is invariably jeopardized by quarantine risks posed by phytosanitary pests that may be present in consignments. The export of stone fruit is affected by a variety of insect pests (Tephritids, Diaspidids, Tortricids and Curculionids) that may occur in the exporting country. Another limiting factor of international trade is the lack of appropriate postharvest mitigation treatments to deal with these pests. In the past methyl bromide was heavily relied upon as a fumigant that would kill any pest of quarantine concern, but environment-friendly alternative postharvest disinfestation treatments are now required.

Alternative mitigation treatments for postharvest purposes include biofumigants, hot and cold temperature treatments, controlled atmospheres and irradiation (Neven 2010). Tephritid fruit flies, one of the major pests of stone fruit, are highly susceptible to cold treatment, and as a result, standard cold sterilization treatments are used to treat plums exported to the USA. Mitigation treatments approved to control fruit flies range from 1.11 - 2.22°C for 14 -18 d (USDA-APHIS 2010). However, in commercial trade these cold sterilization temperatures are not beneficial for the correct ripening of stone fruit, and may also lead to chilling injury of some plum cultivars. Most plum cultivars exported from South Africa, such as 'Pioneer', 'Sapphire', 'Songold' and 'Laetitia' plums, need a dual temperature storage regime to ensure optimum quality and ripening (De Kock and Taylor 2011). Some plum cultivars, e.g. 'Angeleno' and 'Larry Ann', can be stored at a single temperature of -0.5°C for between 4 and 7 weeks (Anon. 2009). For these two latter cultivars cold sterilization may be a feasible option, but for dual temperature plum cultivars it is not.

Irradiation for postharvest disinfestation has been investigated for various fruits and vegetables and shows great promise in that it sterilizes insects at doses that are low enough not to be detrimental to most fruits and vegetables (Kader 1986; Follett 2007). The United

States Department of Agriculture – Animal and Plant Health Inspection Services (USDA-APHIS), together with other international regulatory bodies, such as the International Atomic Energy Agency (IAEA) and the International Plant Protection Convention (IPPC), have issued guidelines for irradiation treatments to meet export and quarantine restrictions (Molins 2001).

Moy et al. (1983) investigated how the sensory qualities of irradiated plums would differ from non-irradiated plums, and found that there were no significant differences in the taste of the fruit when treated with 30 krad (300 Gy). In their investigation on irradiation effects on the quality of 'Songold' and 'Laetitia' plums, requiring dual temperature regimes, Taylor and Brock (1998) observed low flesh firmness and increased gel breakdown in 'Songold' plums at all irradiation doses tested. In 'Laetitia' plums, they measured high levels of shrivel only at the highest dose of 400 Gy. The authors also found that, for both cultivars, the dose of irradiation did not significantly affect O_2 consumption or CO_2 and C_2H_4 production. Another study conducted by Viljoen in 2011 evaluated the impact of Smartfresh™ on irradiated stone fruit. The active ingredient in Smartfresh™, 1-methylcyclopropene, delays ripening by blocking the effects of exogenous and endogenous ethylene (Martínez-Romero et al. 2003). Viljoen (2011) found that Smartfresh™ lowered the incidence of reduced flesh firmness and decreased gel breakdown after irradiation, but shrivel was higher in Smartfresh™-treated plums after shelf-life. The author suggested that this may be due to non-Smartfresh™-treated fruit ripening more rapidly, and thereby having reduced occurrence of shrivel symptoms. Results from the above two trials indicate that, although certain quality characteristics may be negatively affected by irradiation, this treatment does have potential as a postharvest treatment for plums. Further investigation is required, and more cultivars have to be tested, as well as supplementary treatments, such as Smartfresh™, which may counteract the negative effects of irradiation.

A new pluot (*Prunus salicina* x *armeniaca*) cultivar, 'Flavor Fall', was harvested for the first time in South Africa in 2011. Pluots are interspecific hybrids of complex crosses of plum and apricot, with predominantly plum parentage (therefore, and hereafter, also referred to as plums), typically with a smooth skin (Crisosto et al. 2007). 'Flavor Fall' is a dark red/purple plum, with a slightly oblong shape. A number of factors make this cultivar a good candidate for export. 'Flavor Fall' can be stored at either single or dual temperature regimes (unpublished results: M. Jooste 2011), and has an exceptionally long storage capacity of 42 d (Oosthuizen 2011). It is also harvested quite late in the stone fruit season, thereby extending the export season. As a new cultivar, its characteristics and responses to postharvest treatment and handling are yet to be documented.

In addition to postharvest disinfestation treatments, other physical control measures to keep pests off packed fruit are sometimes required by importing countries. A physical barrier, such as insect-proof (IP) bags, in which fruit is wrapped in inside each carton, is one such control measure. A high-density polyethylene bag with 2 mm perforations is currently used commercially, but certain markets require bags made to particular specifications and propose an IP bag, made of low-density polyethylene with 0.8 mm perforations. The effect of these IP bags on fruit quality is not known, particularly in the application of various postharvest treatments such as irradiation. The objectives of the current study were thus, to determine the effects of an irradiation postharvest treatment on 'Flavor Fall' plums, as well as the impact of IP bags on post-treatment fruit quality.

Materials and Methods

Fruit material and trial layout

Plums were purchased from a commercial orchard in Robertson, Western Cape Province, South Africa. On the day of commercial harvest and packing, plums were transported in an uncooled, covered vehicle to the laboratory in Stellenbosch within 2.5 h. To confirm that

plums were at the correct maturity recommended for export, a 50-fruit sample was used to determine maturity indices of the fruit at harvest. A total of 64 cartons of plums were used for the irradiation treatment trial. Each carton contained about 60 fruit, packed in two layers. The 64 cartons comprised 32 cartons in which plums were packed in the commercially-used (CU) bags, and another 32 in which plums were contained in the IP bags. The entire trial consisted of; application of irradiation treatment the day after harvest, followed by a cold storage regime of -0.5 °C for 10 d, 7.5 °C for 7 d and -0.5 °C for 25 d, and finally a shelf-life period at 10 °C for 7 d. The bags were removed from the cartons before the shelf-life period commenced. The respiration rate of the fruit was determined at harvest, after irradiation treatment, after cold storage and after shelf-life. Fruit quality evaluations were done after cold storage and after shelf-life.

Maturity indexing

Maturity indices were determined using the above mentioned 50-fruit sample obtained from the orchard. On both cheeks of each fruit, surface colour was measured as hue angle using a calibrated Minolta CR-400 Chroma meter (Konica Minolta, Japan) and flesh firmness (N) was determined with an electronic fruit texture analyser (GÜSS GS-14, Strand, South Africa) fitted with an 11.0 mm tip. To measure the total soluble solids (TSS) (% Brix), all of the 50 fruit was destoned, juiced and a drop of juice from the pooled sample was placed onto a temperature-controlled digital refractometer (Palette PR-32 ATAGO, Bellevue, USA). Titratable acidity (TA), given as malic acid %, was measured using a weighed 10 g aliquot of the pooled juice sample which was titrated with 0.1M NaOH to a pH end-point of 8.2 using an 719 S Titrino automated titrator, fitted with a Metrohm AG 760 sample changer (Herisau, Switzerland).

Gamma irradiation treatment

The day after the fruit was harvested it was transported, unrefrigerated, from the laboratory to the Hepro irradiation facility in Montague Gardens. At the facility four cartons were stacked one on top of the other and placed onto mini pallets. Stack dimensions were 39 x 29 x 42 cm. Dosimeters with a dose range of 150 – 3030 Gy were placed in four positions (1, 20, 37 and 41 cm from the top of the pallet) on the outside of the stack of cartons. There were two pallets per dose/bag combination. For each bag type, 8 cartons received no irradiation as the control group, and 400, 900 and 1400 Gy as the treated groups. These dosages of irradiation were chosen for various reasons; 400 Gy is the dosage prescribed by USDA-APHIS for treatment of all insects except the pupa and adult stages of Lepidoptera, 900 Gy was used to ensure that the minimum absorbed dose of 400 Gy was obtained on the inside of the stacks, and 1400 Gy, as an extreme dose, if it is ever needed in future and to determine what effect that might have on fruit. Pallets were treated for 8, 20 and 31 min for each of the respective treatment dosages. The control cartons were kept aside and not exposed to the irradiation source. Treatments were carried out at room temperature (23°C). After treatment, dosimeters were removed and read with a spectrophotometer at 552 nm (Thermo Fisher Scientific Inc., Waltham, USA). The dose uniformity ratio (ratio of maximum/minimum dose) was <1.6 for 400 Gy treatments, <1.5 for 900 Gy and <1.4 for 1400 Gy. After treatment the fruit was transported back to the laboratory for respiration rate and ethylene production determinations, and then put into cold storage to start the dual temperature storage regime.

Respiration rate and ethylene production determinations

For determination of the respiration rate (done at harvest, after irradiation, after cold storage and after shelf-life), 10 fruit from three of the eight cartons making up the control and each treated group were used; five fruit from the top layer and five fruit from the bottom layer. The fruit were acclimated to room temperature of 23°C. Thereafter each group of 10 fruit was placed into airtight 5 L glass jars for 1 h (total of 24 jars) after which a sample of the air in the

headspace of the jars was collected using an airtight syringe. Ethylene production (µL C_2H_4 $kg^{-1}h^{-1}$) and respiration (mg CO_2 $kg^{-1}h^{-1}$ and mL O_2 kg^{-1} h^{-1}) were measured by injecting the gas into a gas chromatograph (Model N6980, Agilent Inc., Wilmington, U.S.A.) fitted with a PorapakQ and a Molsieve packed column, and flame ionization and thermal conductivity detectors. The oven temperature was held constant at 80°C. The volume of free space in the jar, as well as the mass of the fruit, was used to calculate ethylene and CO_2 production rates and O_2 consumption rate. After each determination of respiration rates the 10 fruit were placed back into their respective cartons.

Fruit quality evaluations

After the cold storage period, fruit quality evaluations were carried out on half of the fruit in each carton, taken from both layers. The remaining fruit was stored for the shelf-life period and subsequent fruit quality evaluations. Hue angle and flesh firmness were determined, as described above, for five fruit per carton, both after cold storage and after shelf-life. Shrivel and decay was measured on all the designated fruit by physical examination. Shrivel was recorded when it reached over the shoulder of the fruit, and decay when fungal growth and slipskin were observed on the fruit. Internal disorders such as overripeness, gel breakdown and internal browning were determined by cutting open all the designated fruit, except the 5 fruit used for the determination of hue angle and flesh firmness, and examining the inside. Internal disorders were determined by one person throughout the trial to ensure consistency. Overripeness was recorded when fruit were abnormally soft with excessive amounts of free juice and the sub-epidermal mesocarp tissue was translucent. Gel breakdown was recorded when the inner mesocarp tissue, around the stone, was translucent, and internal browning when a brown discolouration of the mesocarp tissue was noted.

Statistical analysis

Data were analysed by one-way analysis of variance (SAS/STAT software v.x.y; SAS Institute Inc., Cary, USA). Where applicable, single degree of freedom, orthogonal polynomial contrasts were fitted to the data. Mean separation was conducted using the LSD test (alpha = 5%). For shrivel, gel breakdown and decay, the analysis was done using logit transformed data.

Results

At-harvest maturity, respiration rates and ethylene production

According to the maturity indices (c.f. South African export standards; DAFF 2012) as well as respiration and ethylene production rates (Table 1), plums were harvested at the correct stage of ripeness. In addition, initial respiration and ethylene production rates could be taken as base measurements to evaluate the effects irradiation treatment, cold storage and shelf-life on these parameters.

Table 1. At-harvest maturity indices, respiration rates and ethylene production of 'Flavor Fall' plums sampled from Robertson in the 2011 season.

Maturity index	Mean ± SD (n = 50)
Hue angle	22.70 ± 14.00
Flesh firmness (N)	67.18 ± 1.10
TSS (%)	18.18 (Pooled sample)
TA (%)	1.30 (Pooled sample)
Respiration rate	**Mean ± SD (n = 24)****
CO_2 production (mg CO_2 kg^{-1} h^{-1})	31.71 ± 4.27
O_2 consumption (mL O_2 kg^{-1} h^{-1})	69.29 ± 13.69
C_2H_4 production (µL C_2H_4 kg^{-1} h^{-1})	0

* TSS – total soluble solids; TA – titratable malic acid

**n = number of gas samples taken for each measurement

Respiration rates and ethylene production after irradiation treatment

Respiration rates generally increased with irradiation dose. In addition, plums packed in IP bags showed higher respiration activity than those in the CU bags (Table 2). However, compared to O_2 consumption, changes in CO_2 production were more pronounced and statistically significant. This applies for both bag types. Nevertheless, plums in the IP bags showed significantly higher rates of CO_2 production than fruit in the CU bags. Irradiation and bag type showed no interactions. At this stage, no ethylene production was evident. After cold storage (Table 3) there was still no ethylene production, and neither the irradiation dose, nor the bag type, had any significant effect on the lowered respiration rate of fruit in cold storage.

After the fruit was exposed to higher temperatures during the shelf-life period, production of ethylene was observed (Table 4). Significantly less ethylene was measured at the higher doses (900 and 1400 Gy). Once again the irradiation dose and bag type did not significantly affect respiration activity. A noted increase in CO_2 production, although not significant, at 400 Gy was seen, where after it dropped as the dose increased.

Table 2. Respiration rates and ethylene production measured on 'Flavour Fall' plums after irradiation treatment of 400, 900 and 1400 Gy, in the different bag types.

Treatment	CO_2 production (mg CO_2 kg^{-1} h^{-1})	O_2 consumption (mL O_2 kg^{-1} h^{-1})	Ethylene production (µL C_2H_4 kg^{-1} h^{-1})
Irradiation (Gy)			
0	22.24a	67.31	0.00
400	27.42a	65.92	0.00
900	34.14b	74.74	0.00
1400	37.25b	87.12	0.00
Bag type			
Commercial bag	27.44a	70.60	0.00
Insect proof bag	33.09b	76.94	0.00
Source of variation (Pr>F)			
Irradiation	0.0002	0.2442	-
Contrast: Irradiation linear	<0.0001	0.0647	-
Contrast: Irradiation quadratic	0.3878	0.4505	-
Bag type	0.0085	0.4288	-
Irradiation x Bag type	0.4652	0.8475	-
Irradiation $LSD_{5\%}$	5.6071	23.604	-
Bag type $LSD_{5\%}$	3.9648	16.69	-

* Significant differences between values are indicated by lowercase letters.

Table 3. Respiration rates and ethylene production measured on 'Flavour Fall' plums after cold-storage regime of -0.5°C for 10 days, followed by 7.5°C for 7 days and -0.5°C for 25 days.

Treatment	CO_2 production (mg CO_2 kg^{-1} h^{-1})	O_2 consumption (mL O_2 kg^{-1} h^{-1})	Ethylene production (µL C_2H_4 kg^{-1} h^{-1})
Irradiation (Gy)			
0	10.26	53.85	0.00
400	13.06	50.52	0.00
900	13.84	47.74	0.00
1400	13.60	66.23	0.00
Bag type			
Commercial bag	14.32	50.94	0.00
Insect proof bag	11.06	58.22	0.00
Source of variation (Pr>F)			
Irradiation	0.5002	0.2084	-
Contrast: Irradiation linear	0.2193	0.2141	-
Contrast: Irradiation quadratic	0.3913	0.1019	-
Bag type	0.0955	0.2616	-
Irradiation x Bag type	0.1532	0.9901	-
Irradiation $LSD_{5\%}$	5.5133	18.857	-
Bag type $LSD_{5\%}$	3.8985	13.334	-

Table 4. Respiration rates and ethylene production measured on 'Flavour Fall' plums after cold storage of -0.5 °C for 10 days, followed by 7.5°C for 7 days and -0.5°C for 25 days plus shelf-life of 7 days at 10°C.

Treatment	CO_2 production (mg CO_2 kg^{-1} h^{-1})	O_2 consumption (mL O_2 kg^{-1} h^{-1})	Ethylene production (µL C_2H_4 kg^{-1} h^{-1})
Irradiation (Gy)			
0	35.78	77.12	1.79a
400	40.51	75.86	1.39a
900	34.02	78.66	0.92b
1400	31.37	86.00	0.63b
Bag type			
Commercial bag	37.35	77.70	1.16
Insect proof bag	33.48	81.13	1.21
Source of variation (Pr>F)			
Irradiation	0.0817	0.3348	0.0003
Contrast: Irradiation linear	0.0642	0.1197	<0.0001
Contrast: Irradiation quadratic	0.1874	0.3458	0.5208
Bag type	0.1182	0.4152	0.7061
Irradiation x Bag type	0.9955	0.0707	0.0023
Irradiation $LSD_{5\%}$	7.0452	12.403	0.4402
Bag type $LSD_{5\%}$	4.9817	8.7704	0.3113

*Significant differences between values are indicated by lowercase letter

Compared to freshly harvested fruit, CO2 production declined by approximately 30% after 1 d of storage in untreated controls; it was only 30 % of the initial rate after cold storage.

Fruit quality evaluations

No significant results for overripeness and internal browning were seen, both after cold storage and after shelf-life, and therefore these fruit quality parameters are not presented in the results. Across the dosages there was no significant difference in colour development after

cold storage (Fig 1a). Hue angle ranged from 21.54 to 22.52. After shelf-life however, although colour development in fruit treated with up to 900 Gy irradiation did not differ significantly from the control (14.68), fruit treated with 1400 Gy did have a significantly greater hue angle (16.02). Significant differences in shrivel and flesh firmness were observed both after cold storage and after shelf-life (Fig 1b and c). Shrivel increased as the irradiation dose increased to 900 Gy. At 1400 Gy shrivel decreased, but was still higher than in the control fruit after cold storage. While after shelf-life, 1400 Gy treated fruit had shrivel levels similar to that of control fruit. Flesh firmness decreased as irradiation dose increased. Irradiation dose had no significant effect on percentage decay (Fig 1d) after either cold storage or the shelf-life period.

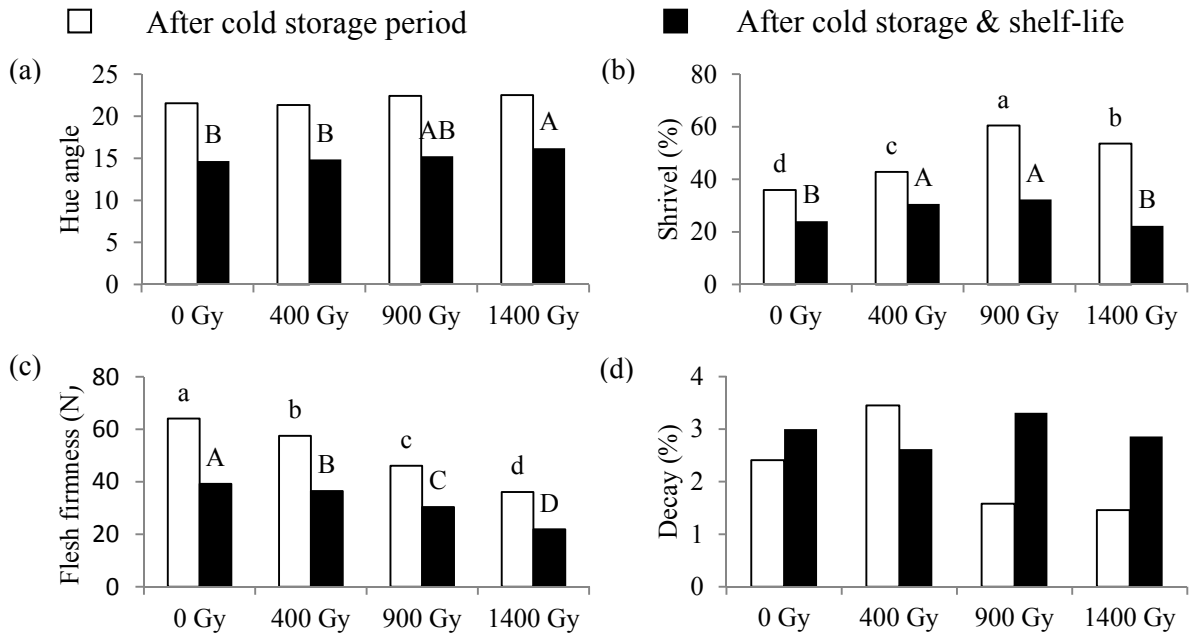

Figure 1. The effect of irradiation dose (Gy) on (a) hue angle, (b) shrivel, (c) flesh firmness and (d) decay of 'Flavour Fall' plums measured after cold storage of -0.5°C for 10 days, followed by 7.5 °C for 7 days and -0.5 °C for 25 days and again after shelf-life of 7 days at 10°C.

* Significant differences, (P< 0.05) according to the LSD test, between values measured after cold storage (*acs*) are indicated with lowercase letters and values measured after cold storage plus shelf-life (*asl*) are indicated by uppercase letters. Statistical analysis was done separately for after cold storage parameters and after cold storage plus shelf-life parameters.

The effect of the two different bag types on fruit quality parameters are presented in Figure 2. Percentage shrivel was the only parameter in which significant differences between the two bag types were observed, both after cold storage and after shelf-life (Fig 2b). At both time points, fruit packaged in the CU bag had almost double the amount of shrivel than the fruit packaged in the IP bag. Marginally higher percentages of decay were observed in the IP bags, but percentage decay did not at any point exceed 4% (Fig 1d).

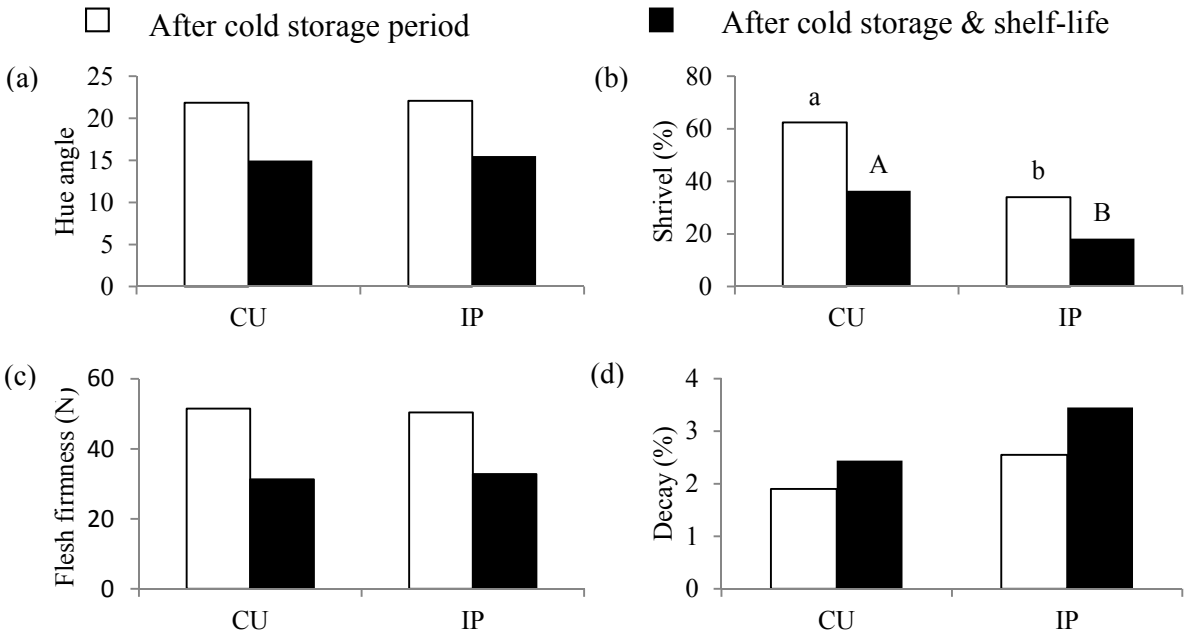

Figure 2. The effect of two different bag types - commercially-used (CU) bags and insect-proof (IP) bags, on (a) hue angle, (b) shrivel, (c) flesh firmness and (d) decay of 'Flavour Fall' plums measured after cold storage of -0.5 °C for 10 days, followed by 7.5°C for 7 days and -0.5 °C for 25 days and again after shelf-life of 7 days at 10°C.

*Significant differences, (P< 0.05) according to the LSD test, between values measured after cold storage (*acs*) are indicated with lowercase letters and values measured after cold storage & shelf-life (*asl*) are indicated by uppercase letters. Statistical analysis was done separately for after cold storage parameters and after cold storage plus shelf-life parameters.

For all fruit quality parameters measured there was no interaction between irradiation treatment and bag type at either time point. However, while in general gel breakdown increased as the irradiation dose increased an interaction between irradiation dose and bag type was noted after the shelf-life period (Figure 3). The control and 400 Gy treatment had similar gel breakdown percentages, irrespective of the type of bag used. With the 900 Gy treatment the IP bag produced a significantly larger percentage of gel breakdown than the CU

bag (32%, as opposed to 10%). At 1400 Gy unacceptably high percentages of gel breakdown were observed (50% in the IP bags and 57% in the CU bags). Here the difference between the effect of the two bag types were smaller and not significant.

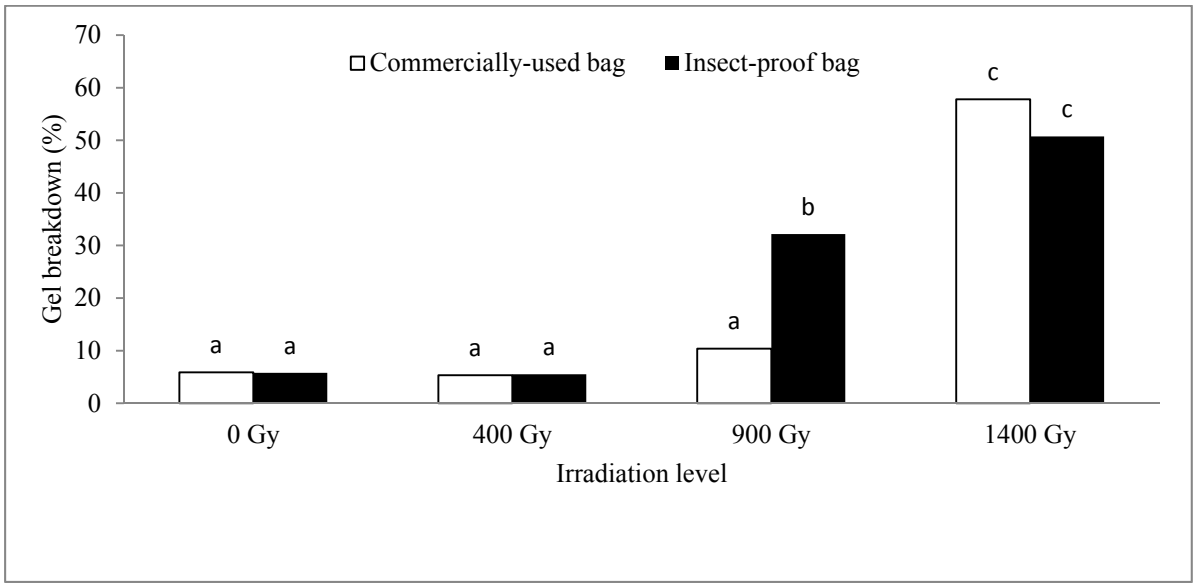

Figure 3. Interaction between the two bag types - commercially-used (CU) bags and insect-proof (IP) bags, and the irradiation dose on the percentage gel breakdown observed on 'Flavour Fall' plums after cold storage of -0.5 °C for 10 days, followed by 7.5°C for 7 days and -0.5°C for 25 days plus shelf-life of 7 days at 10°C.

*Significant differences between values are indicated by lowercase letters. P*irr* = <0.0001, P*bag* = 0.0822, P*irrXbag* = 0.0158, LSD5% = 10.6902

Discussion

Both treatments tested in this study, irradiation and bag type, had effects on the respiration rate, as well as the fruit quality parameters of 'Flavour Fall' plums. The linear increase in respiration rate of the fruit, as the irradiation dose increased, has also been observed in other studies of the effects of irradiation on fruit (Gunes et al. 2000). The mechanism of increase in respiration after irradiation is not well known, but Massey and Bourke (1967) found that irradiation stimulated the catabolism of acetate to CO_2 in carrot tissues, and concluded that this might explain the increase in CO_2 production after irradiation. Taylor and Brock (1998) speculated that changes in the amounts of O_2, CO_2 and C_2H_4 that are noticed with irradiated fruit are rather associated with fruit ripening. Fruit treated with higher doses of irradiation

produced less ethylene. Production of less ethylene associated with higher dosages of irradiation was also noted in the irradiation of sliced apple and grated carrot (Chervin et al. 1992; Gunes et al. 2000). Ethylene synthesis starts with methionine as the main substrate and requires ATP and enzymes such as ACC synthase and ACC oxidase. Gunes et al. (2000) postulated that the inhibitory effect of irradiation on ethylene production might be due to a change in substrate concentration, enzyme activity or energy level.

Shrivel results from cumulative water loss through the skin of the fruit from the moment the fruit is harvested (LaRue and Johnson 1989; Crouch 1998). The significant differences in shrivel, due to irradiation dose, as well as bag type, noticed at the end of cold storage and end of shelf-life is the result of a number of contributing factors. The higher respiration rate related to the higher irradiation dosages (900 and 1400 Gy), would result in respiration heat making the fruit slightly warmer than the surrounding cold room air. This causes a small, positive temperature gradient around the fruit, favouring water loss from the fruit (Saltveit 2002; Kays and Paull 2004), which only manifested as shrivel after the shelf-life period, during which the bags were opened. The bags reduce air movement over the fruit, and also moisture loss compared to there being no barrier (Moelich and Taylor 2012). The same trend of an increase in shrivel at 900 Gy, and then a decrease at 1400 Gy, both after cold storage and shelf-life, was also seen in the respiration rate (CO_2 production). The reduced respiration heat would have resulted in a smaller temperature gradient, less moisture loss and ultimately the reduced amount of shrivel noted. The difference in percentage shrivel between the two bag types was possibly caused by the IP bags being less permeable than the CU bags, and thus restricting water vapour movement from the inside of the bag. This probably also caused the higher, albeit insignificant, percentages of decay in the IP bags.

Another interesting phenomenon is the decrease in shrivel measured from after cold storage to after shelf-life. This might be due to the fruit ripening faster after exposure to higher

temperature (10°C) during shelf-life. Saladié et al. (2007) and Ghiani et al. (2011) observed this during the ripening of melting peaches and 'Ailsa Craig' tomatoes, and proposed that the lower incidence of shrivel was due to cell wall disassembly, in conjunction with a loss in the turgidity of the mesocarp cells during fruit ripening at the higher storage temperature.

Furthermore, the loosening of cells due to the degradation of cell wall material as fruit matures and ripens results in reduced flesh firmness (LaRue and Johnson 1989; Tromp et al. 2005). In addition, lower flesh firmness was seen in 'Flavour Fall' plums as the irradiation dose increased. Similar results were seen in apples, where Kovacs et al. (1997), explained that irradiation increases the activity of polygalacturonase enzyme and releases neutral carbohydrates from insoluble cell walls, thus causing fruit to become softer.

Gel breakdown manifests as a gelatinous breakdown between the stone and the mesocarp and is associated with a loss of juiciness (Taylor et al. 1995). The irradiation dose had a significant effect on gel breakdown after cold storage and even more so after the shelf-life period. According to Taylor et al. (1995), pectic substances might play a role in the development of gel breakdown in plums. Pectic substances are implicated in the development of wooliness in peaches because of their ability to bind water into gel complexes. Irradiation is known to affect carbolytic enzymes (Kovacs et al. 1997) and therefore could cause pectic substances in the cell wall area to bind cell fluids into gels, which subsequently manifests as gel breakdown in the mesocarp of the fruit. Gel breakdown was far more evident after shelf-life where the temperatures were higher, causing fruit to ripen faster and cell membranes to become more permeable to water and other cell fluids with which the cell wall pectins could bind. Voisine et al. (1991) found that free radicals generated during irradiation of cauliflower heads (*Brassica olera*) caused senescence-like deterioration of cell membranes. The IP bag may also have an impact on gel breakdown, since at 900 Gy, gel breakdown was three times

that produced by CU bags. This must be taken into consideration if the IP bag is to be used in future.

Conclusion

Shrivel was the most problematic of all the quality parameters that were measured. Even the control fruit had levels of shrivel that were above the maximum 10% allowed for export fruit, both after the cold storage period and after shelf-life. Since control fruit exhibited these high percentages of shrivel, it appears that 'Flavour Fall' plums are prone to shrivel. Although the IP bags reduced the amount of shrivel, in comparison with the CU bags, it was still above the maximum 10% allowed. Gel breakdown was also problematic. Although the percentage gel breakdown after cold storage was below 3%, the percentages after shelf-life were unacceptably high for fruit treated with 900 Gy and 1400 Gy. The combination of 900 Gy and the IP bag was particularly problematic. This is important, as the 900 Gy dose may have to be applied commercially to palletized fruit to achieve a 400 Gy dose on the inside of the fruit stack. At the highest irradiation dose however, all of the other fruit quality parameters evaluated on 'Flavour Fall' plums, at the end of cold storage, which is after the fruit arrives at the export markets, were within the minimum arrival standards for export. However, further research in the prevention of shrivel associated with irradiation is needed, especially for cultivars that are prone to shrivel. Since IP bags reduced shrivel, but increased gel breakdown, investigating different forms of packaging might help relieve the shrivel and gel breakdown problems.

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Chapter 4

A review on insect rearing for research purposes with focus on the banded fruit weevil, *Phlyctinus callosus* (Coleoptera: Curculionidae) and results from preliminary rearing trials

Introduction

One of the major aspects hampering the development of insect pest management strategies is the seasonality of these organisms. An insect's seasonal cycle dictates which life stage is available at certain times of the year. Therefore, research on these insect pests is confined to a limited period when enough individuals of the required life stages are available in the field, unless a reliable rearing method has been developed. The adult stage of the banded fruit weevil, *Phlyctinus callosus* (Boheman) (Coleoptera: Curculionidae), for example, is only found in orchards from October to early March (Barnes 1989). It is in this limited time period that adults can be field-collected to perform laboratory trials. Reliable insect rearing techniques that produce a year round supply of a particular pest is the foundation for the discovery and development of most pest management strategies. The lack of postharvest mitigation treatments for *P. callosus* can be attributed to the fact that there is no sustainable rearing technique available to produce a constant supply of *P. callosus* adults.

This chapter is a mini review on insect rearing for pest management and will include descriptions of different rearing techniques used for specific pests, followed by a summary of previous attempts made at rearing *P. callosus*, description of a trial conducted to test a combination of the previous methods to rear *P. callosus*, and finally, recommendations for future development of *P. callosus* rearing techniques.

Basic principles of insect rearing

There are three different types of rearing systems: small scale-single species; medium scale-multiple species; and mass-rearing-single species (Leppla 2004). In small scale-single species systems all the work is usually performed by one person. The insect species is fed on either host plants or an artificial diet and natural environments and oviposition substrates are duplicated or slightly modified from nature. Medium scale-multiple species rearing systems are usually found at research stations. A small staff is needed for this type of rearing system and isolation is vital as diet preparation, egg treatment, larval rearing, harvesting of pupae, and adult colony maintenance can be combined for similar species. Single species-mass-rearing systems are designed to fit factory-like production. Here controlled environments, artificial diets, oviposition substrates and mechanised equipment are used and different processes are performed by separate work units (Leppla 2004).

Whether using any one of the above three rearing systems, the basic principles of insect rearing involve obtaining wild adults and developing a production system that produces a constant supply of high quality individuals that can be used for educational, personal, agricultural and medical research purposes (Leppla 2004). The key ingredients to establish a successful rearing procedure, whether it is for small colonies or large scale production, include finding high quality wild individuals with broad genetic variability, identifying a synthetic diet on which to rear these individuals, and creating a rearing procedure that mimics the optimal developmental conditions of the specific insect species.

The genetic variability of a founding population determines its potential for adapting to the rearing environment and producing subsequent generations of high quality insects (Hoy 1979, Bartlett 1984, 1985). A general rule to achieve acceptable genetic variability is to obtain a total of 200 field-collected individuals from five to ten different locations, keep them separate to eliminate diseases and natural enemies, isolate pairs of adults to verify that they reproduce,

and use surface-sterilized eggs to infest larval rearing containers (Hoy 1979, Bartlett 1984, 1985). Genetic variability is of utmost importance in single species-mass-rearing systems.

When developing an insect diet it is recommended to review literature on diets for related species, or species with similar feeding characteristics. The next step would be to present the target insect with the established diet of a similar species and thereafter modifying it with natural host material. If there are no established diets for similar insect species then the early stages of diet development are more complicated (Cohen 2004). Looking at the natural food of the target insect, the feeding mechanism and the rearing conditions might be a starting point when no diet for species with similar feeding habits are available. Thorough knowledge of the feeding mechanisms of the target insect in its natural habitat is crucial to develop a diet, whether experimenting with an artificial diet of a similar species or creating a new one (Cohen 2004).

A rearing facility is a great advantage when creating an environment in which to optimally produce high numbers of the target species. Abiotic environmental conditions that are optimal for the target insect need to be created and maintained. Temperature, light and humidity are the basic components which must be adjusted to an optimal range at which the target insect will reproduce (Fisher 2009).

Three different types of rearing systems – Success stories

As early as 1935, scientists rearing *Heliothis zea* (Ochsenheimer) (Lepidoptera: Noctuidae) on corn plants in a greenhouse (small scale-single species), recognised that laboratory colonies of these insects were an important instrument to ensure a constant supply of the pest for research purposes (Raulston and King 1984). When artificial diets were developed it became possible to rear *Heliothis* moths under continuous laboratory culture, and since then

they have been reared by many scientists, and each modified the technique to make it more efficient than the method that was used before. In the 1960's work was focussed on ingredients and composition for optimal production, whereas in the 1970's focus shifted towards cost efficiency due to rearing on a larger scale (Raulston and King 1984). The correct method of dispensing diet and transferring the larvae onto it required some research and Burton et al. (1966) found that disposable plastic cups worked better than glass vials. Further research was done and the process was later automated (Harrell et al. 1969). One of the effective formats of oviposition cages for the *Heliothis* moths was a 3.8 litre ice-cream carton. About 10 pairs of moths could be contained in these cartons without affecting fecundity and fertility (Burton 1969). A cloth was placed over the top of the carton with streamers hanging from it and serving as oviposition substrates. Moths were fed diluted honey or a sucrose solution. Callahan (1962) showed how light, temperature and humidity are critical to obtain maximum mating and oviposition in *H. zea*. All the dietary and technological advances allowed continuous colonization of *Heliothis* spp. in the laboratory, but Sparks and Harrell (1976) emphasized that to continue production and prevent colony collapse, the correct environment and disease control are of utmost importance.

The Chevron Chemical Company in Richmond, California, U.S.A. was a good example of a medium scale-multiple species rearing system. At this plant, 17 different insect species from 7 orders were reared for insecticide screening purposes (Wheeler 1984). Insect species vary in their susceptibility to any one insecticide class and therefore screening against many insecticide types is desirable to avoid missing a potentially useful insecticide. The insects used to start colonies were collected from their preferred host crop or natural habitat. The number of insects collected for each species had to be large enough for preliminary standards testing after three generations. At the facility the insectary consisted of a series of six rooms isolated from a central hallway by anterooms. Here, both artificial diets and natural diets were

used. Artificial diets were maintained by following prescribed preparation methods and by using materials of consistent quality. Natural diets consisted mainly of host plants that were grown in greenhouses or outside beds. Contamination control is very important in a multi-species rearing facility such as this one. Facility design and material flow was particularly important in keeping the insects free from contamination. The last factor that had to be considered was the quality control of the reared insects. The most important aspect in an insect rearing facility for insecticide screening is that insects have the required susceptibility to insecticides (Wheeler 1984). At this facility, the dosage-mortality of test insects was measured against standard insecticides to determine insect quality.

One of the most important implementations, and examples of single species-mass-rearing is the rearing of tsetse fly, *Glossina* spp. (Diptera: Glossinidae) adults for the Sterile Insect Technique. The tsetse fly is the sole vector of animal and plant trypanosomiasis (sleeping sickness). This disease has had devastating effects on the development of human and agricultural resources in Africa for many years. Research has been conducted at the Entomology Section of the Agricultural Biotechnology Laboratory at Seibersdorf, Austria, and in Nigeria, where a tsetse fly eradication programme was initiated (Lindquist 1984). The mass-rearing procedure conducted on two species of tsetse flies (*G. morsitans* (Weidemann) and *G. palpalis* (Weideman)) consisted of a few phases. First, the equipment was designed, methods were developed, and the reproductive ability of the insects was studied under standard environmental conditions using live animals – *in vivo* rearing (Lindquist 1984). Thereafter work started on creating an *in vitro* feeding system. Silicone membranes covering defibrinated pig and cow blood were used to simulate feeding on a live animal (Lindquist 1984). Further research has made it possible to freeze-dry blood for at least one year, and ship it anywhere in the world where tsetse flies need to be reared (Lindquist 1984). Research at Seibersdorf has also resulted in the development of a semi-defined synthetic diet suitable for

the rearing of a number of species of tsetse flies (Lindquist 1984). In 1977 the Federal Nigerian Government and the International Atomic Energy Agency (IAEA) agreed to a project for the Biological Control of tsetse fly by the Sterile Insect Technique, known as BICOT. Both *in vivo* and *in vitro* rearing systems were used to maintain a colony of about 70 000 females, which produced approximately 5 000 males every week for irradiation and release in the field. (Lindquist 1984). In 2010 the tsetse fly eradication programme had already been implemented on about 10 000km² of land, and the project intended to expand the operations to around 25 000km² in the following 1-2 years (IPC 2011).

Rearing the boll weevil, *Anthonomus grandis grandis* (Boheman) (Coleaptera: Curculionidae), at the United States Animal and Plant Health Inspection Service's Robert T. Gast Boll Weevil Rearing Facility (Roberson and Wright 1984) is an example of a successful single species-mass rearing production system for Curculionidae. The boll weevil develops on cultivated wild cotton and therefore is a pest. They are mass-reared at the above mentioned facility for research and proposed field testing of sterilized weevils (Roberson and Wright 1984). At this facility the weevils are fed on an artificial diet. Environmental conditions for larvae are maintained at 31°C, 55+/-5% RH and 24h dark. Eggs hatch within three days, and after 13 days of larval feeding adults emerge. The first adults to be collected are used for research purposes and the rest are kept at 28°C, 50+/-5% RH and a 20:4h L:D cycle to produce eggs.

Rearing *Phlyctinus callosus*

In the following section, I present three studies in which methods for rearing *Phlyctinus callosus* were tested. All three are examples of small scale-single species rearing systems. The motivation to rear this pest was different for all three authors and will not be discussed in detail here, instead I will focus on the method of rearing and the rates of success achieved.

The first study into rearing methods for *P. callosus* was done in 1980, when Walker (1980) determined the number of larval instars produced, and investigated the effect of temperature on *P. callosus* growth and survival. One first-instar larvae was placed in a small hole bored into discs of carrot root. The discs were placed on moistened filter paper inside a petri dish which was sealed with tape to minimize evaporation. The petri dishes were placed inside darkened boxes, incubated at 20°C and were inspected on a weekly basis for exuviae, on which basis the instars were determined. Head capsule widths of each instar were also measured throughout. It was reported that larvae were successfully reared with this method, however, the success rate was not clear. Walker (1980) also reported that head capsule width is a suitable criterion for separating larval instars. The second part of the experiments involved rearing adult weevils from eggs obtained from adults collected in the wild. The eggs were placed on moist filter paper which was then placed on the soil surface of potted carrot seedlings. The filter paper was covered with a lid to prevent desiccation and new carrot seeds were sown weekly to sustain the feeding source. These pots were kept under 5 different temperatures (10.5, 15, 20, 25 and 30°C) with a 12:12h L:D cycle. Two cabinets with different light intensities (5166 and 1184 lx) were used and the pots rotated between the cabinets to receive equal amounts of both light intensity. The pots were inspected at regular intervals to examine how many larvae survived and just before pupation the pots were covered with wire or plastic gauze cylinders to confine emerging adults. The results showed that at 10.5, 25 and 30°C no adults were collected. The 20°C treatment resulted in approximately 6% survival rate and the 15°C treatment approximately 1.5% survival rate to the adult stage.

Barnes (1987) modified the methods described by Walker (1980) to further understand the development of the embryonic and larval stages of P. *callosus*. Adults were collected from the wild, kept in the laboratory at 25°C and fed a mixed diet of leaves from weeds,

ornamentals and apple trees. Females oviposited on moistened filter paper discs and eggs were transferred to a moistened filter paper strip placed inside a "snap-cap" plastic vial and kept at 25°C in an unilluminated incubator. When eggs hatched the larvae were transferred, individually, to a carrot root disc with a 3mm hole in. The carrot disc was then placed hole-down on damp filter paper in a petri dish and the lid closed, but not sealed as Walker (1980) did. Larval development was inspected two to three times a week and fresh carrot disks provided each time. Larvae were reared in this manner until adults emerged. For the head capsule measurements some of the larvae from each instar were decapitated and the width of their head capsules measured under a stereo-microscope. Adult yield in this study was 9%. The greatest mortality of larvae, as with the study by Walker, were during the first three instars – 70% of larvae had died by the third instar. The author found that the use of larval head capsule widths to determine the age of larvae were complicated due to the overlapping of head capsule widths between instars.

Another study into the rearing of *P. callosus* was conducted by Ferreira (2010). The objective of this study was to successfully rear mature *P. callosus* larvae that could be used in further research on biological control methods for *P. callosus*. The author used field collected adults, kept in a laboratory at 25°C and fed on *Coprosma repens* (Rich) (Gentianales: Rubiaceae) leaves. Layered moistened cotton wool was used as oviposition sites for females. Eggs were collected daily and transferred to petri dishes with moist filter paper where after the petri dishes were kept in the dark at room temperature. When larvae hatched, after approximately 7 days, they were used to evaluate the different diets and rearing methods. Three different artificial diets: a basic diet, based on that described by Fisher and Bruck (2004) for the black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae); the basic diet with sawdust added; and a codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) diet described by Guennelon et al. (1981) were tested. Also, four different forms of carrot (carrot

disc with hole, carrot disc with hole and another disc on top, grated carrot, and long flat pieces of carrot); fully-grown carrots potted in two different soil types; pots with carrot seedlings, chrysanthemums, and fully-grown carrots were all tested using neonate larvae to determine whether these larvae could grow until they were mature. The results indicated that the basic diet was most effective with 30% of neonate larvae surviving. The author found that fungal and bacterial growth on the diets were evident and may have haltered the development of the larvae. Therefore formalin was also added to the basic diet and the basic diet with sawdust, but this contributed to larval mortality. Weevils are extremely susceptible to fungal and bacterial inhibitors that are added to artificial diets (Bass and Barnes 1969, House et al. 1971). Of the different forms of carrot, the discs were the most effective. The two carrot disc approach gave 60% survival of the larvae, but this was not significantly higher than the one disc with hole in (54%). The other two forms of carrot resulted in significantly lower survival rates. When fully-grown carrots were planted in two different soil types the coarse sand was by far better than the sandy loam soil resulting in an average survival rate of 87.5% for the coarse sand and only 10% for the sandy loam soil. In the experiment where carrot seedlings, chrysanthemums and fully-grown carrots planted in pots were compared, the fully-grown carrots were by far the most successful with a survival rate of 79.3%.

Although Walker (1980) and Barnes (1987) were able to rear *P. callosus* adults, the success rate was far too low (1.5% and 9% respectively). Ferreira (2010) reared mature *P. callosus* larvae with a relatively high success rate of 87.5%. A sustainable method with a higher adult weevil survival rate is necessary to supply a continuous population for pest control studies. Below, I describe my attempt at modifying the methods described above in order to investigate more sustainable methods of rearing *P. callosus*.

Materials and Methods

Phlyctinus callosus adults were collected from pome fruit orchards in the Elgin/Grabouw area of the Western Cape, South Africa. Corrugated cardboard bands were placed around the trunks of apple trees and adult weevils collected from it as the weevils used it for shelter during the day. Weevils were placed in Perspex cages (40 x 30 x 30 cm) in the laboratory and kept at 25°C, +/-70% RH with a photoperiod of 14:10 [L:D] h. *Coprosma repens* twigs were supplied as food and rolled paper towels for shelter. Moistened cotton eye-pads were placed in the cages as oviposition sites for females. The eye-pads were collected and replaced every three to four days and eggs collected from it. Eggs were transferred to moist filter paper inside petri dishes and sealed with parafilm to prevent desiccation of the eggs. The eggs were kept at 25°C in the dark, and after 7 to 10 days larvae emerged and were used for the rearing experiment.

The experiment existed of eight treatments and three replicates. The treatments comprised of three variables: potting soil or riversand (both freeze sterilized) as medium, drainage chips (freeze sterilized) or no drainage chips at the bottom of the containers, and 150ml of water two times a week or 250ml of water once a week. The treatments were as follows:

- riversand, drainage chips, and 150ml water twice weekly
- riversand, drainage chips, and 250ml water once a week
- riversand, no drainage chips, and 150ml water twice weekly
- riversand, no drainage chips, and 250ml water once a week
- potting soil, drainage chips, and 150ml water twice weekly
- potting soil, drainage chips, and 250ml water once a week
- potting soil, no drainage chips, and 150ml water twice weekly
- potting soil, no drainage chips, and 250ml water once a week

Three litre plastic buckets were used as containers and drainage holes were drilled into the bottom of each bucket. Mature carrots purchased from a local retailer were planted in the substrate in the buckets to serve as food for the burrowing larvae. Twenty neonate larvae were washed into each bucket close to the planted carrots. The buckets were placed in an insectary which was maintained at 25°C and 24h darkness. When the first emerging adults were noticed *C. repens* twigs were placed inside the buckets as food source for adults until they were collected twice a week. At the same time buckets were covered with netting to prevent emerging adults climbing out of the buckets. When emerged adults were collected they were transferred to perspex cages and maintained in the same manner as described above for the field-collected adults.

Results and Discussion

Survival rate from larval inoculation until adult emergence was very low, only 1.2%. There are a number of reasons that could have contributed to this poor success rate. Although the soil was freeze-sterilised before use, major fungal and bacterial growth occurred, as the carrots started to decompose in the soil. The carrots purchased from the retailer were not all of equal quality and sterilising the carrots before they were planted might have delayed the onset of the fungal growth, but might have negatively affected the larvae. It is known that weevils are extremely susceptible to fungal and bacterial inhibitors that are added to artificial diets (Bass and Barnes 1969, House et al. 1971) and therefore neither the carrots nor larvae were sterilised in any way before they were added to the soil. Sowing carrot seeds over a period and adding the larvae when the first of the sown carrots reaches maturity (Ferreira 2010, Walker 1980) might be worth investigating further as the carrots would not decompose as rapidly as harvested carrots that are replanted and the larvae would have fresh material when the first carrots start withering. In Ferreira (2010) the fully-grown planted carrots were the most successful of the techniques experimented with, however, in that study only mature

larvae were needed, and these were collected after 5 weeks. When waiting for adults to emerge the collection period might be between 6 to 8 weeks, by which time fully-grown and replanted carrots were badly decomposed in the current trial. Walker (1980) also tried this approach to collect adult weevils, but placed eggs on filter paper on top of the soil surface. It is recommended that washing the larvae into the soil close to the actively growing carrots be experimented. Another contributing factor to the poor survival rate in this trial was the method of applying the watering regime which was done by measuring the correct amount of water into a cup and pouring the water onto the soil surface. This caused compaction of the soil and might have resulted in an anaerobic environment for larvae, as well as difficulty for emerging adults. It is recommended that a more subtle way of applying water to the soil, by way of a misting spray for example, be used.

In conclusion, a rearing method to ensure a constant supply of *P. callosus* adults is still to be developed. The rearing trials for this pest that are discussed in this review cover a broad spectrum of methods, and although none of them are greatly successful it does amount to a good basis to work from for further research on rearing methods for this species. It does seems as though the survival problem lies between the final larval instars, pupation and adult emergence, as Ferreira (2010) was able to rear mature larvae with a relatively high success rate. A topic that might be worth investigating and incorporating more thoroughly in the rearing process is the seasonal cycle of this insect. Barnes (1989) found that for *P. callosus* the transition from one phase to another and the number of generations per year is closely correlated with environmental changes. The optimal temperature and light conditions for rearing this pest is well investigated, but in the wild nothing is perfectly optimal and “change is the only constant” (quote by Greek philosopher, Heraclitus (Laertius and Hicks 1925)). Therefore a temperature regime instead of a constant optimal temperature would be useful investigating as this may provide the stimulus to change from one phase to the next. The

larvae of *P. callosus* feed on the roots of weeds such as *Listroderes costirostris*, *Pennisetum clandestinum*, *Trifolium repens*, *Cyperus esculentus* and *Plantago lanceolata* that are present on the orchard ridges of pome fruit, stone fruit and vines (Barnes and Pringle 1989). It is worth experimenting with these weeds as a source of feeding during rearing trials, as it is the natural food source of *P. callosus* larvae.

Seeing as *P. callosus* is a pest with great phytosanitary significance and the limited postharvest disinfestation techniques that are available for this pest, a laboratory colony would aid research on this topic immensely, as currently research is limited to the summer months when adult *P. callosus* can be collected in the field. A sustainable, cost effective and less labour intensive rearing procedure that will ensure a constant supply of high quality adults should be the goal. To achieve this, trial and error is the best starting block. Therefore, although the above summarised attempts at rearing this pest did not deliver the answer, it does provide valuable information for the next ambitious rearing expert with new ideas.

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Chapter 5

General conclusions

The presence of *Phlyctinus callosus* in consignments of packed fruit has phytosanitary implications for the export of pome fruit, stone fruit and grapes from South Africa to some of its biggest export markets such as the United States of America (USA), since it does not occur there and has been intercepted in fruit consignments imported to the USA from South Africa. Irradiation is fast becoming an accepted, safe, non-toxic and effective option as a postharvest disinfestation treatment due to the United States Department of Agriculture – Animal and Plant Health Inspection Services approving generic irradiation disinfestations doses of 150 Gy for tephritid fruit flies and 400 Gy for all other pests, except pupa and adult Lepidoptera (USDA-APHIS 2006). To ensure that insects that may be residing within the centre of palletized fruit receive the minimum 400 Gy dose, a dosage of 1000 Gy or more may need to be applied to the pallet. It has been reported that such high doses have adverse effects on fruit quality and it is prohibited by USDA-APHIS for fresh fruit irradiation. Proving that *P. callosus* is rendered sterile and unable to reproduce after treatment with doses lower that the prescribed 400 Gy would support the proposition by Hallman (1998) to create a lower generic dose for Curculionidae.

In chapter 2, field-collected *P. callosus* adults were treated with varying doses of irradiation, up to a maximum of 80 Gy, and their reproductive ability evaluated afterwards in crosses involving treated and untreated individuals. Irradiation treatment up to 80 Gy did not affect the fecundity of *P. callosus* in any of the crosses, but there was a strong negative correlation between decrease in fertility and the increasing irradiation dose, especially in the irradiated females. Unlike other disinfestations techniques, irradiation does not need to kill the pest to

ensure quarantine security. It is only focussed on eliminating reproductive capacity. Mating crosses involving treated females were unable to reproduce after treatment with a relatively low dose of 80 Gy, whether crossed with treated or non-treated males. Where treated males were crossed with non-treated females, even the highest dose of 80 Gy was not sufficient to stop reproduction. This made it clear that female *P. callosus* are more sensitive to the detrimental effects of irradiation and thus research need only be aimed at sterilizing females regardless of whether both sexes are present in packaged fruit. Hallman (2012) proposed a dose increase from the previously proposed 150 Gy for Curculionidae, to a dose greater than 165 Gy. Based on the findings in this study, a generic dose, equal to or greater than 165 Gy will include effective phytosanitary sterilization of *P. callosus*. Thus, my findings support a dose, lower than the current minimum 400 Gy dose recommended by the USDA-APHIS, for this insect as well as the group.

In chapter 3 the effects of the minimum USDA approved radiation sterilization dose of 400 Gy for all pests except pupa and adult Lepidoptera were investigated on a new pluot cultivar 'Flavor Fall', which is a host of *P. callosus*. Fruit was treated with 400, 900 and 1400 Gy irradiation and exposed to a temperature regime that simulates cold storage during shipment and shelf-life at the retailer. Fruit quality evaluations done after cold storage and after shelf-life and respiration rate of the fruit (measured at harvest, after irradiation, after cold storage, and after shelf-life) were used to determine the effects of these irradiation doses on packaged fruit when used for insect disinfestation. Shrivel was the most problematic of all the parameters that were measured, but because even the control fruit had higher than acceptable amounts of shrivel, this could not be attributed to the irradiation treatment alone, but also the proneness of the 'Flavor Fall' cultivar to shrivel. The presence of gel breakdown after cold storage (-0.5°C for 10 days, 7.5°C for 7 days and -0.5°C for 25 days) was low (3%), but after shelf-life (10°C for 7 days) the amount of gel breakdown was unacceptably high for fruit

treated with 900 and 1400 Gy. This is particularly problematic as these higher doses may have to be applied to achieve the minimum 400 Gy USDA-APHIS sterilizing dose on the inside of commercially palletized fruit. This problem can be partially solved by developing a radiation-induced disinfestation treatment for *P. callosus*, as well as the Curculionidae as a group, at a dose as low as 165 Gy. Large scale testing needs to be done with more than 10 000 *P. callosus* adult individuals to confirm a dose for this species. A laboratory colony would make this possible, as using field collected adults is unreliable, labour intensive and expensive.

In chapter 4 the rearing of *P. callosus* was investigated, however an effective rearing method to continuously supply adults of this species has not yet been developed. A number of rearing methods have been tested and mature larvae were reared with a relatively high success rate, but very few adults were collected. It is worth investigating and incorporating the seasonal cycle of *P. callosus* more intensively into the rearing method as the transition from one phase to another, for this pest, is closely correlated with environmental changes. Another aspect worth looking at is using weeds that naturally occur on orchard ridges as a source of food for larvae.

Considering the phytosanitary status of *P. callosus* and the possibility of creating a generic group dose for Curculionidae, developing a reliable rearing method for this pest should enjoy high priority. Lower approved doses for *P. callosus* and the curculionid group would mean that lower doses of phytosanitary irradiation need to be applied to fruit and other fresh commodities when exporting to markets where these pests causes phytosanitary trade barriers. Establishing irradiation as an environmentally-friendly, safe, non-toxic and effective postharvest disinfestation treatment shows great potential, but applied doses need to be low enough not to be detrimental to the fresh commodity. For this to happen lower doses need to

be approved for specific species which in turn can be used to develop generic doses for groups of insects.

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