SURVIVAL OF THE NAVEL ORANGEWORM, *AMYELOIS TRANSITELLA* (LEPIDOPTERA: PYRALIDAE), ON PISTACHIO IN SOUTH AFRICA

by

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ABSTRACT

The navel orangeworm, *Amyelois transitella* (Pyralidae) (Walker), is the most damaging Lepidoptera larva found on pistachio nuts (*Pistacia vera* L., Anacardiaceae). Pistachios have only relatively recently been planted in South Africa at Green Valley Nuts, a division of the Industrial Development Corporation, near Prieska in the Northern Cape Province. The navel orangeworm causes direct damage to pistachio nut clusters by feeding on individual nut kernels and contaminating nuts with their faecal excretions. In the process the quality of the nuts is reduced and the nuts are rendered more susceptible to fungal infection. After harvest, navel orangeworm larvae overwinter inside fallen nuts on the orchard floor, as well as inside nuts left behind on trees. The prevalence of navel orangeworm in mummy nuts was studied from May to September in 2008 and 2009 at Green Valley Nuts. The potential survival of the larvae in these nuts was estimated from nuts sampled under trees of three different pistachio cultivars (Ariyeh, Sirora and Shufra). Orchard row management practices were investigated to determine the effect of cover crops, mulch and hydro-cooling on navel orangeworm survival. This was done by monitoring emergence cages and light traps for the presence of navel orangeworm adults emerging from mummy nuts. In both years, navel orangeworm was noted overwintering in mummy nuts. The highest occurrence of navel orangeworm over the two year study period was recorded in nuts from Sirora, a cultivar planted in an orchard lacking inter-tree row cover crops, mulch and hydro cooling. The results support the assumption that these orchard row management practices have a suppressing effect on navel orangeworm development, causing high mortality rates due to mummy nut decomposition. Research was also conducted to observe the life cycle and behaviour of the pest under laboratory conditions. The complete life cycle duration of the navel orangeworm ranged from 50 to 84 days. A single life cycle which gave rise to a next generation was successfully tracked.

Key words: Pyralidae, *Amyelios transitella*, pistachios, overwintering, survival, orchard management practices, life cycle

UITTREKSEL

Die navel orangeworm, *Amyelois transitella* (Pyralidae) (Walker), is die mees skadelike Lepidoptera larwe wat op pistashio neute (*Pistacia vera* L., Anacardiaceae) voorkom. Pistachios is relatief onlangs vir die eerste keer in Suid-Afrika by Green Valley Nuts, 'n afdeling van die Nywerheids Ontwikkelings Korporasie, naby Prieska in die Noord-Kaap Provinsie geplant. Die navel orangeworm veroorsaak direkte skade deur op individuele pistachio neutkerne te voed en neute te kontamineer met hul faekale uitskeidings. Hierdeur word die kwaliteit van die neute verlaag, terwyl die neute ook meer vatbaar raak vir swaminfeksies. Navel orangeworm larwes oorwinter na oestyd in neute wat van die bome afval en op die boordvloer beland, asook in neute wat op die bome agterbly. Die voorkoms van navel orangeworm in hierdie neute was vanaf Mei tot September in 2008 en 2009 ondersoek. Die prosedure om die potensiële oorlewing van die larwes vas te stel, was bepaal deur neute van drie verskillende pistashio kultivars (Ariyeh, Sirora en Shufra) te versamel. Boord bestuurspraktyke is ondersoek om die effek van dekgewasse, deklae en hidro-verkoeling op navel orangeworm oorlewing te bepaal. Dit is uitgevoer deur die monitering van hokke en ligvalle vir die teenwoordigheid van volwasse navel orangeworm. In albei jare is daar opgemerk dat die navel orangeworm in neute oorwinter. Die hoogste voorkoms van die navel orangeworm oor die twee jaar studie tydperk is in neute van die Sirora kultivar wat in ‘n boord waar dekgewasse, deklae en hidro-verkoeling ontbreek, geplant is, aangeteken. Die resultate ondersteun die aanname dat hierdie boord bestuurspraktyke 'n onderdrukkende effek op navel orangeworm oorlewing het, want dit lei tot hoë mortaliteit weens die ontbinding van die neute. Navorsing is ook uitgevoer om die lewensiklus en gedrag van die navel orangeworm onderhewig aan laboratoriumtoestande te bepaal. Die volledige lewensiklus van die navel orangeworm het ongeveer 50-84 dae geduur. 'n Enkele lewensiklus wat aanleiding gegee het tot 'n volgende generasie van die mot was suksesvol gevolg.

Sleutelwoorde: Pyralidae, *Amyelios transitella*, pistachios, oorwintering, oorlewing, boord bestuurspraktyke, lewensiklus

CHAPTER 1

Pistachio as a global nut crop

PISTACHIO AS A GLOBAL NUT CROP

1. Background

1.1. History

The pistachio (*Pistacia vera* L., Anacardiaceae) is native to the Middle East, where the trees were originally found growing wild in the high desert regions of Iran, Turkey and Afghanistan (Michailides & Morgan, 2004; Braga, 2006). Pistachios were first cultivated in western Asia from where it spread to the Mediterranean countries. Pistachio was introduced into the United States in 1930 where it was planted experimentally in California (Anonymous, 2007).

Pistachios are grown in several parts of the world that have similar climatic conditions to Iran, such as California and parts of Australia (W.A. Pistachios, 2005). Commercial cultivation of pistachios expanded in the 1970's and became a major industry in central California, where the pistachios are well adapted to the hot, drier climate. Other major pistachio producing areas include Iran, Turkey, Syria, India, Greece and Pakistan (Anonymous, 1997).

Large-scale cultivation of pistachios was only relatively recently established in South Africa and since 1993 approximately 1000 ha were planted at Green Valley Nuts (GVN), a division of the Industrial Development Corporation (IDC), located in the arid Northern Cape Province (Fig. 1). GVN is the first commercial pistachio farm in South Africa (Anonymous, 2009). Initially, experimental plantations were established at different locations around South Africa. The IDC selected Prieska because of its hot, dry summers and cold winters, plentiful water from the Orange River and good quality soils, since these are very suitable conditions for growing healthy pistachio trees (DWA, 1997).

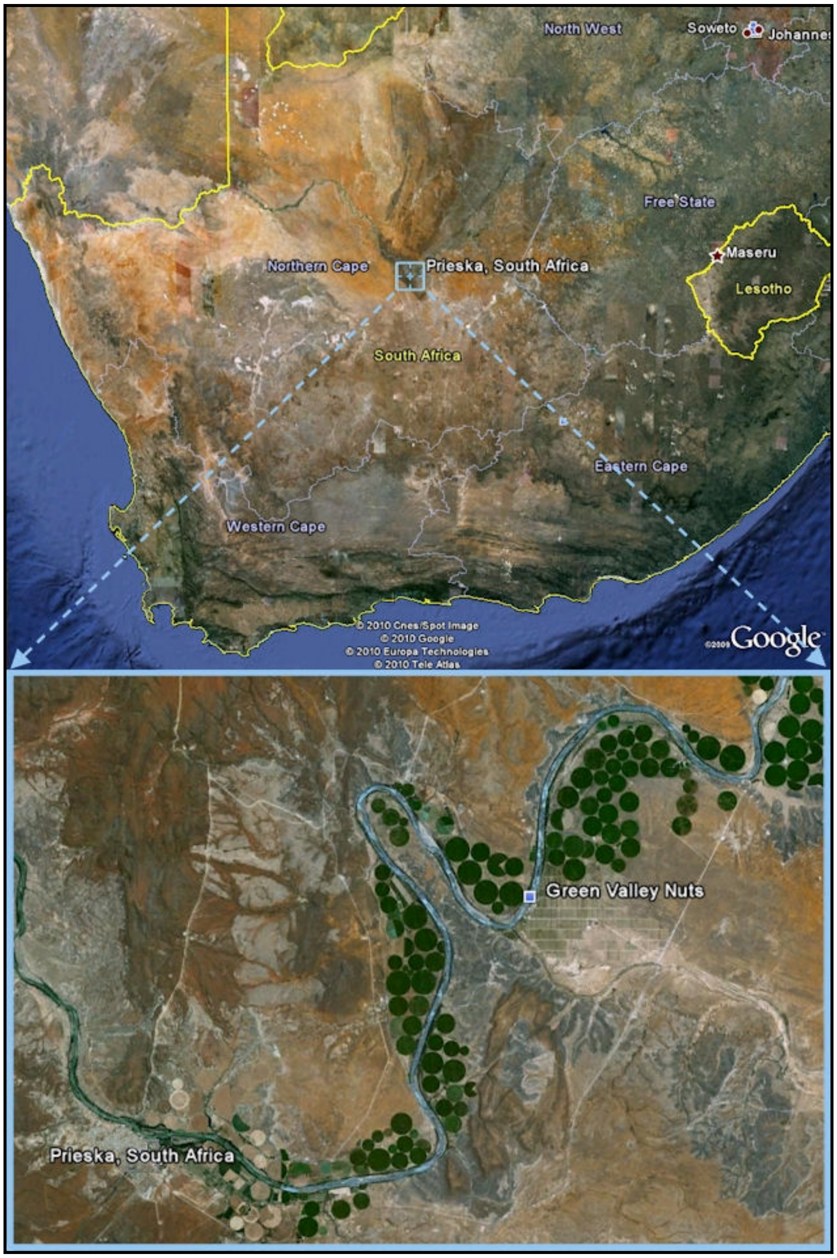

Figure 1. Location of Green Valley Nuts, near Prieska in the Northern Cape (Map from Google Inc., 2010).

1.2. Cultivation

Pistachios grow in grape-like clusters on the trees. The shell of each nut is surrounded by a fleshy hull (Anonymous, 1997). Male and female trees are present, with, as is normally the case, the male tree acting as pollinator and the female tree growing the fruit. Well-managed trees of about 10 years in age can provide about 10kg of nuts for harvest each year (W.A. Pistachios, 2005).

The trees flourish in locations with cold winters and long, hot summers and are therefore dependant on both heat and cold units. They are resistant to droughts and high summer temperatures, but intolerant of excessive dampness and high humidity (Anonymous, 1997). The nuts ripen in late summer or early autumn when the inner shell splits open. When the pistachios are ready for harvest, they are mechanically shaken from the trees and transported to the processing plant, to be hulled and dried immediately in order to prevent the shells from staining (Anonymous, 2005; Anonymous, 2007; Siegel, Kuenen, Higbee, Noble, Gill, Yokota, Krugner & Daane, 2008).

Pistachios are susceptible to aflatoxin contamination during the growth, harvesting and processing periods. Aflatoxin results from fungus growth on the nut that produces toxic and carcinogenic compounds. This contamination is reduced with proper drying and storage methods (Anonymous, 2005).

1.3. Production

The United States pistachio industry has grown rapidly in California over the last 30 years. Today, the United States is the second major producer of pistachios following Iran. In 2008, pistachios were the third largest U.S. tree crop after almonds and walnuts (Anonymous, 2005). The total world production of pistachios in 2007 was estimated at 521 921 metric tons, of which 44% was produced in Iran (230 000 ton), followed by USA (108 598 ton), Turkey (73 416 ton), Syria (52 066 ton) and China (38 000 ton) (FAO, 2007). In South Africa the pistachio industry has shown to have great potential and the country will probably be able to contribute approximately 3 000 ton to the world production of pistachios (Jooste, 2005).

1.4. Insect pests of pistachio

1.4.1. Hymenoptera

In regions of the Mediterranean and western Asia, a pistachio seed chalcid (Chalcidoidea: *Megastigmus pistaciae* Walker) has been recorded to be a primary pest of *Pistacia* species (Rice, Bentley & Beede, 1988). This insect is also found in other areas of the world where pistachios are cultivated. The pistachio seed chalcid causes damage by feeding directly on the pistachio nut. It overwinters as larvae in infested nuts and has the potential to reduce yields (UC IPM, 2007).

1.4.2. Hemiptera

Leaffooted plant bugs (Coreidae: *Leptoglossus clypealis* Heidemann and *L. occidentalis* Heidemann), stink bugs (Pentatomidae: *Thyanta pallidovirens* (Stål), *Chlorochroa uhleri* (Stål), *Chlorochroa ligata* (Say) and *Acrosternum hilare* (Say)), plant bugs (Miridae: *Phytocoris* sp., *Calocoris norvegicus* (Gmelin), *Neurocolpus longirostris* Knight, *Lygus hesperus* (Knight) and *Psallus vaccinicola* Knight) and the false chinch bug (Lygaeidae: *Nysius raphanus* (Howard)) are Hemipteran pests that have been found to damage pistachios (Rice *et al.*, 1988; UC IPM, 2007).

In 2003, pentatomid bugs were found to be abundant in the newly planted orchards at GVN. These orchards provide a kind of 'green oasis' in the harsh environment of the northern Cape, which can lead to the establishment of a vast array of primary and secondary pests, beneficials and neutral tourists in the orchards. Insect bio-monitoring surveys were conducted to identify potential pests and beneficial insects, and Hemiptera was recognized to be a potential pest species on pistachios (Louw, 2003 in Fourie, 2007). The powdery stink-bug, *Atelocera raptoria* Germar (Pentatomidae), was found to be a dominant species that can pose a major threat to pistachio production (Haddad & Louw, 2006).

Leaffooted bugs typically damage entire nut clusters and cause epicarp lesions and kernel necrosis, which can often lead to fungal infections under high humidity conditions. Stink bugs and plant bugs create similar signs of damage, also causing epicarp lesions. This leads to the blackening of nuts (necrosis of nut epicarp tissue) and often nut drop. Stink bugs can transmit pistachio stigmatomycosis and panicle and shoot blight. Newly planted pistachio trees and seedlings can wilt and die on account of false chinch bug feeding damage (UC IPM, 2007).

There are several species of Sternorrhyncha scale that have been recorded on pistachios from California. The most common of these include the European fruit lecanium (*Parthenolecanium corni* (Bouché)), the frosted scale (*Parthenolecanium pruinosum* Coquillett) and the black scale (*Saissetia oleae* (Olivier)) (Rice *et al.*, 1988). These soft scales produce large quantities of honeydew on the foliage and fruit of the trees during spring. The honeydew provides a substrate for the growth of sooty mold that reduces photosynthesis and subsequent plant growth. High populations of scales also hinder shoot growth and shell splitting (UC IPM, 2007).

1.4.3. Lepidoptera

The most common Lepidoptera pests found to cause damage on pistachios are the oblique-banded leafroller (Tortricidae: *Choristoneura rosaceana* (Harris)) and the tussock moth (Lymantriidae: *Orgyia vetusta* Boisduval) (Rice *et al.*, 1988). In both cases the larvae of these pests inflict feeding damage to the leaves and stems of the trees. The larvae of the oblique-banded leafroller destroy the leaves, reducing the photosynthetic ability of the trees, and feed on the stems, reducing crop yield. The tussock moth feeds on the foliage and heavy infestations can lead to the complete defoliation of trees (UC IPM, 2007).

Another highly destructive Lepidopteran pest on pistachio nuts is the navel orangeworm (Pyralidae: *Amyelois transitella* (Walker)). This pest was first recorded at Green Valley Nuts in 2006, where it was sampled in the nut drying baskets at the processing plant. Based on the implications this species could have on the budding South African pistachio industry, it was decided to further decipher the bio-ecology of the navel orangeworm, which subsequently gave rise to the focus of this dissertation. Pyralidae pests in general, with more focus on the navel orangeworm, will be discussed in further detail in the following paragraphs.

2. Pyralidae snout moths

2.1. General information

Pyralidae constitute the third largest family within the order Lepidoptera. The moths are small and delicate, with elongate or triangular front wings and broad hind wings. They are sometimes called snout moths due to the projecting labial palps. Pyralidae is divided into several subfamilies (Borror, Triplehorn & Johnson, 1989).

The navel orangeworm belongs to Phycitinae, a large subfamily with species that occur throughout the world. The best known species in this subfamily are seed feeders that attack stored grain, but some cause destruction to plants or are even predatory, feeding on various scale insects (Borror *et al.*, 1989).

2.2. Harmful Pyralidae

The Indian meal moth (*Plodia interpunctella* (Hübner)) and the Mediterranean flour moth (*Anagasta kuehniella* (Zeller)) often cause a large degree of damage when they attack stored food supplies (Borror *et al.*, 1989). The larvae of the Indian meal moth, also known as waxworms, cause direct damage by feeding and producing frass and webbing that reduce the quality of the product (Johnson, Valero & Hannel, 1997).

The cactus moth, *Cactoblastis cactorum* (Berg), which is native to South America, was introduced to Australia in the 1920s and to South Africa in the 1930s as a biological control agent against invasive *Opuntia* cacti (Cactaceae) (Zimmerman, Moran & Hoffmann, 2000). However, *C. cactorum* also have a significant impact on commercial plantations of *Opuntia ficus-indica* (L.) (cactus pear) (Habeck & Bennett, 1990). The insect feeds internally on the cladodes, causing structural damage to the plant that leads to reduced production (Lobos & De Cornelli, 1997). A rigorous control program has to be followed by cactus pear growers in South Africa to control infestations of the cactus moth, since entire plantations of cactus pear can be destroyed by the moth (Simonson, Stohlgren, Tyler, Gregg, Muir & Garrett, 2005). *C. cactorum* also caused a decline in the growth of spineless Opuntias that are a food source for cattle in South Africa (Habeck *et al.*, 1990).

Eldana saccharina Walker is an indigenous species to South Africa and a pest of sugarcane. It was first recorded in 1939 attacking sugarcane in the Umfolozi area. Presently *E. saccharina* has spread to large parts of the South African sugarcane region. It is absent only in the high altitude areas (Nuss, Bond & Atkinson, 1986). The insect tunnels into the lower parts of the sugarcane stalks where the sugar content is the greatest, leading to severe loss of crop yield (Leslie, 1994).

The sunflower moth (*Homoeosoma electellum* (Hulst)) is a serious pest of sunflowers mainly in the central and southern United States. The larvae feed on the corollas and floral ovaries and can be very damaging (Wilson, 1990). The olive leaf worm (*Palpita unionalis* (Hübner)) causes important damage on young olive trees in nurseries and shoots of old trees in orchards. The larvae feed mostly on the leaves, but can also feed on the fruits and seeds (Khaghaninia & Pourabad, 2009).

Cereal stemborers are a major pest that infests maize and other cereal crops in eastern and southern Africa (Mohamed, Khan, Overholt & Elizabeth, 2004). In South Africa, the African stemborer, *Busseola fusca* (Fuller), and the spotted stem borer, *Chilo partellus* (Swinhoe), are the most important pests of maize and grain sorghum. These pests are difficult to control, as it is expensive and requires expertise. The distribution of *C. partellus* has continuously increased since the pest has emerged on the African continent in 1932. Current studies have found that *C. partellus* is now expanding its distribution into the high elevations of the eastern Highveld region of South Africa and it seems to be displacing the indigenous *B. fusca*, the only stem borer already occurring there (ARC, 2006). According to the ARC (2006), nine indigenous parasitoids and hyperparasitoids have been recorded to attack both borer species, but they have not been able to reduce pest populations below economic damaging levels.

2.3. Beneficial Pyralidae

Pyralids are the most common Lepidoptera used for the biological control of weeds. The melaleuca moth (*Poliopaschia lithochlora* (Lower)) is very destructive on Australian broad-leaved paperbark (*Melaleuca quinquenervia* (Cav.)), a serious invasive weed in southern Florida, and can be a potential biological control agent for the eradication of this weed (Galway & Purcell, 2005). The alligator weed moth (*Arcola malloi* (Pastrana)) is a biological control agent that was introduced into the United States during the 1970's to control alligator weed. The larvae feed inside the stems of the plants, causing them to collapse (Hayes, 1996). The larvae of *Laetilia coccidivora* (Comstock) are predaceous on cochineal bugs (Dactylopiidae: *Dactylopius coccus* Costa). They feed on cochineal to obtain carminic acid for defensive purposes and disgorge the carminic acid-laden crop fluid when attacked by ants (Eisner, Ziegler, McCormick, Eisner, Hoebeke & Meinwald, 1994).

Emex australis Steinbell (Polygonaceae) is an annual, herbaceous plant, native to southern Africa, which has been declared a serious weed in parts of Australia. It is also a problem weed in vineyards, pastures, recreational areas and along roadsides. Larvae of the pyralid moth *Microthrix inconspicuella* Ragonot was collected in 1977 in South Africa to be evaluated as a potential biological control agent. Field studies in South Africa showed that *M. inconspicuella* may feed and develop on apple leaves in unsprayed orchards (Shepherd, 1990a). However, according to Shepherd (1990b), it appeared that there is no risk of larval damage to apple orchards if *M. inconspicuella* is introduced into Australia as a biological control agent against *E. australis*.

3. The navel orangeworm, *Amyelois transitella* (Pyralidae)

3.1. Distribution and host range

The navel orangeworm, *Amyelois transitella* (Walker), is native to southwestern United States and Mexico and was first described from specimens collected in Mexico in 1899 (Wade, 1961). It was recorded infesting injured and rotting navel oranges in Arizona in 1921, from which its common name originated (Rice, Barnett & Van Steenwyk, 1996).

The navel orangeworm is a scavenger insect that can infest various crops, but tree nuts are the hosts attacked most severely. Mature almonds, walnuts and pistachios experience great damage from this insect. Other hosts are also occasionally infested, but at very low levels compared to nut crops (Rice *et al.*, 1988; Rice *et al.*, 1996). It has been recorded to breed in the mummified fruits and nuts of at least 25 plant species (Shelton & Davis, 1994).

3.2. Description

Adult moths have silver grey forewings with transverse banding and irregular black markings (Fig. 2A), while the hindwings are lighter in colour. Typical of the Pyralidae, they have a snout-like projection at the front of the head. The size of the moths varies, ranging from 16 - 27 mm in length when measured with folded wings. The females are larger than the males (Wade, 1961; Rice *et al*., 1996; UC IPM, 2007).

The oval, flattened eggs (Fig. 2B) are creamy white when first laid and normally changes to a reddish colour within 24 to 48 hours. The surface of the egg is reticulated when viewed under high magnification, with several ridgelike markings (Wade, 1961; Rice *et al.*, 1988).

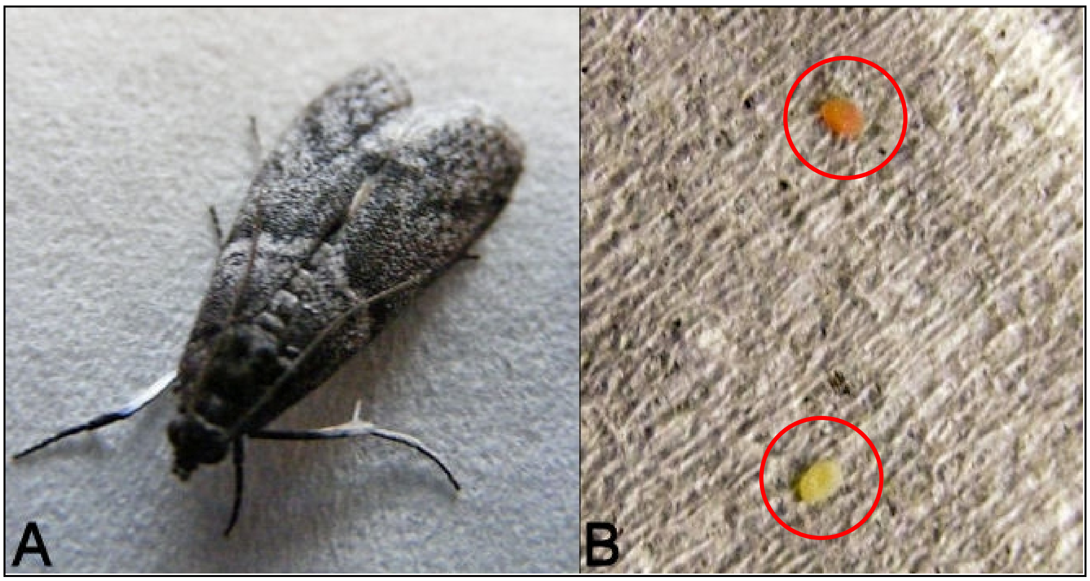

Figure 2. A – Adult navel orangeworm. **B** – Navel orangeworm eggs, showing the difference in colouring after time (*i.e.* creamy white when freshly laid (bottom) and orange to reddish when older (top)).

Young larvae are reddish orange at first, but the colour becomes lighter in later instars (Fig. 3A). It was found that this colouration can be influenced by the larval diet (UC IPM, 2007). Larvae range from 15 - 22 mm in length. The head capsule is reddish brown and they bear a characteristic crescent-shaped sclerite on each side of the thorax just above the second pair of legs. This sclerite is useful in distinguishing between the larvae of the navel orangeworm and the Mediterranean flour moth (*A. kuehniella*) (Wade, 1961; Rice *et al*., 1988).

Exerate pupae are dark reddish-brown in colour (Fig. 3B). They are typically caudally rounded and apically blunt in shape. Pupal length ranges from 7 - 12 mm, with the female pupae typically larger than the male pupae (Wade, 1961).

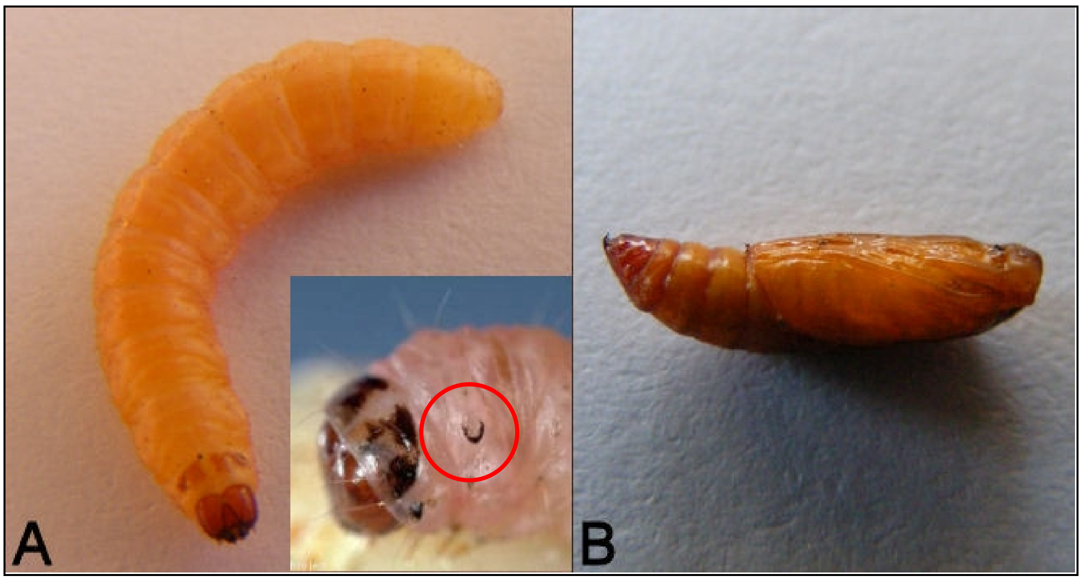

Figure 3. Navel orangeworm. **A** – Larva. Insert photo shows the crescent-shaped sclerite on the side of the thorax (Photo by Jack Kelly Clark, 2000). **B** – Pupa.

3.3. General life cycle

The navel orangeworm has no diapause stage in its life cycle and under favourable conditions is active all year round. Otherwise they exhibit the typical Lepidopteran life-cycle with five larval instars. They overwinter in various larval stages or as pupae in mummy nuts remaining behind in the orchard in the case of nut hosts. Mummy nuts or fruit are the only known source of shelter and sustenance for immature overwintering navel orangeworm. When the temperatures start to rise, the overwintering stages complete development and emerge as adults (Rice *et al*., 1988).

The moths survive for only a few days in warm, dry weather, but they can survive for several weeks in cool, wet weather. Split nuts and nuts with stretch marks are highly susceptible to navel orangeworm infestation, and egg laying on pistachios increases dramatically at the time the first nuts begin to split in January and February in the southern hemisphere (Rice *et al*., 1988).

Oviposition usually commences on the second day after adult eclosion and females will oviposit about 85 to 90 eggs. The eggs, which are laid within split nuts or on the surface of host nuts and stems, hatch in 3 to 23 days depending on the temperature (Rice *et al*., 1988). Eggs are unlikely to develop successfully when laid in temperatures that are too high or low, since this renders conditions unfavorable for egg development (Siegel *et al.*, 2008).

It was found that larval development in midsummer can be completed within 21 days. This development may extend over several months in autumn and winter. In hot weather, duration of the pupal stage may range from 4 to 5 days, but can last several weeks in late winter or early spring (Rice *et al*., 1988). There are between three and five overlapping generations per year and the population increases during summer and early autumn (Shelton *et al*., 1994).

3.4. Damage and influence on pistachio quality

The navel orangeworm is the most damaging Lepidoptera larva found on pistachios (UC IPM, 2007). The larvae infest nuts during and after hull split occurs. Postharvest loss is caused through direct feeding and contamination of the nuts with frass and webbing, which renders the nuts unmarketable (Zalom, Weakley, Hendricks, Bentley, Barnett & Connell, 1984; Wang, Johnson, Tang & Yin, 2005).

Small, pinhole size entrances into the nutmeat are the first signs of infestation. As the larvae grow, they feed upon the entire nut, leaving behind extensive amounts of frass and webbing. This reduces the quality of the nuts and leads to increased processing costs (UC IPM, 2007; Siegel *et al.*, 2008). Uncontrolled navel orangeworm populations may often infest 1 – 2 % of the yield. Some infestations can range as high as 10 % or more when nuts are harvested late. Pistachios sold in-shell may contain high levels of concealed damage, since infested pistachios that are only slightly damaged and partially consumed by larvae might not be removed during processing operations (Rice *et al*., 1988).

There are many different fungi that cause pistachio nuts to rot and these include numerous *Aspergillus* spp. and *Penicillium* spp. It is specifically *Aspergillus* spp. that produce aflatoxins and mycotoxins which are detrimental to human health (Doster & Michailides, 1999). Nuts damaged by navel orangeworm are more susceptible to contamination with toxin-producing fungi (Kuenen, Bentley, Rowe & Ribeiro, 2008; Gianessi, 2009). More specifically, navel orangeworm infestations are associated with increased levels of decay by *Aspergillus* spp., which subsequently leads to aflatoxin contamination (Doster *et al*., 1999). The site of damage on the nut caused by the feeding larvae remove the protective layers around the kernel, providing a point of entry for spores to contaminate the nut. Nuts contaminated by aflatoxins are a significant food safety risk due to its carcinogenic and teratogenic attributes (Beck, Higbee, Merrill & Roitman, 2008).

4. Management of the navel orangeworm

4.1. Cultural control

Two cultural procedures for managing navel orangeworm in pistachio orchards are early harvest and sanitation. Early, thorough harvesting of new nuts on the crop is done to prevent egg-laying by moths. The navel orangeworm is a scavenger insect that survives through the winter on mummy nuts and sanitation is the most effective control strategy for this pest in pistachios. This is done by removing the mummy nuts that remain on trees after harvest. These nuts must be destroyed to prevent the survival and development of navel orangeworm larvae and pupae inside the nuts, and to reduce egg-laying sites for moths emerging from the overwintering population (Zalom *et al.*, 1984; Rice *et al.*, 1988; UC IPM, 2007). The navel orangeworm has also been recorded surviving on the nuts that remain on the orchard floor. Tilling these nuts into the ground can drastically reduce the emergence of navel orangeworm and prevent females from laying eggs in them during the spring or early summer (Siegel *et al.*, 2008).

4.2. Biological control

In California, the navel orangeworm is parasitized by several parasites, such as *Goniozus legneri* Gordh (Bethylidae), *Pentalitomastix plethorica* Caltagirone (Encyrtidae) and *Copidosomopsis plethorica* (Caltagirone) (Encyrtidae) (Rice *et al.*, 1988; UC IPM, 2007). *G. legneri* is a gregarious larval ectoparasitoid, while *P. plethorica* and *C. plethorica* are egg-larval parasitoids (Meals & Caltagirone, 1995). Some control has been achieved in experimental plots with repeated applications of *Bacillus thuringiensis*, but the use of Bt is presently not recommended against the navel orangeworm as it is not effective enough as a control tactic (Rice *et al.*, 1996).

Entomopathogenic nematodes, such as *Steinernema carpocapsae*, can also be used to kill the overwintering larvae in the nuts. These nematodes can enter shallowly buried nuts and nuts on the soil surface, where they infect overwintering larvae (Siegel, Lacey, Higbee, Noble & Fritts, 2006).

4.3. Chemical control

There are several registered insecticides that can be used for navel orangeworm control in pistachios. It is best to apply insecticides just after first hull split or at the onset of stretch marks on the hulls (Rice *et al.*, 1988). The overwintering navel orangeworm population cannot be controlled with insecticides, because once the larvae are inside the nuts they escape the effect of the insecticides. The only effective method for controlling the navel orangeworm is with a combination of cultural control practices and insecticides (UC IPM, 2007).

4.4. Mating disruption

Several studies have been conducted to evaluate the potential of pheromone mating disruption as a pest management strategy for the control of the navel orangeworm. Some studies found that crop damage was reduced by 12 – 34 % in treated plots (Curtis, Landolt & Clark, 1985), whilst other research did not achieve reductions in damage compared to untreated plots (Gianessi, 2009).

5. Aim of study

1. The main purpose of the study was to investigate the influence of orchard management practices at Green Valley Nuts on the longevity of the navel orangeworm, to thereby gauge the survival of navel orangeworm under southern hemisphere conditions in general and South African conditions in particular for the first time.
2. Laboratory breeding trials to determine the life-cycle breakdown of Southern African navel orangeworm populations were also conducted,
3. General observations on the behavior of the navel orangeworm under local conditions were done, since this would contribute to orchard management decisions in the long term.

6. References

ANONYMOUS. 1997. California Rare Fruit Growers: Pistachio. Available online: http://www.crfg.org/pubs/ff/pistachio.html

ANONYMOUS. 2005. Pistachio profile. Available online: http://www.agmrc.org/commodities__products/nuts/pistachio_profile.cfm

ANONYMOUS. 2007. A brief history of pistachios. Available online: http://www.paramountfarms.com/nutrition/pistachio_history.aspx

ANONYMOUS. 2009. Green Valley Nuts: Pistachios. Leaflet.

ARC. 2006. Biological control: Cereal stemborers. Available online: http://www.arc.agric.za/home.asp?pid=952&sec=2067

BECK, J.J., HIGBEE, B.S., MERRILL, G.B. & ROITMAN, J.N. 2008. Comparison of volatile emissions from undamaged and mechanically damaged almonds. *Journal of the Science of Food and Agriculture* **88**: 1363–1368.

BORROR, D.J., TRIPLEHORN, C.A. & JOHNSON, N.F. 1989. *An introduction to the study of insects.* 6^{th} edition. Brooks/Cole, United Stated of America.

BRAGA, M. 2006. The history of pistachios. Available online: http://www.saltednuts.com/articles/the-history-of-pistachios/

CURTIS, C.E., LANDOLT, P.J. & CLARK, J.D. 1985. Disruption of Navel Orangeworm (Lepidoptera: Pyralidae) mating in large-scale plots with synthetic sex pheromone. *Journal of Economic Entomology* **78**: 1425-1430.

DOSTER, M.A. & MICHAILIDES, T.J. 1999. Relationship between shell discoloration of pistachio nuts and incidence of fungal decay and insect infestation. *Plant Disease* **83**: 259-264.

DWA. 1997. Prieska. Available online: http://www.dwa.gov.za/orange/low_orange/prieska.htm

EISNER, T., ZIEGLER, R., MCCORMICK, J.L., EISNER, M., HOEBEKE, E.R. & MEINWALD, J. 1994. Defensive use of an acquired substance (carminic acid) by predaceous insect larvae. *Cellular and Molecular Life Sciences* **50**: 610-615.

FAO. 2007. FAOSTAT data. Available online: http://faostat.fao.org/site/339/default.aspx

FOURIE, D.V. 2007. *Atelocera raptoria* (Hemiptera: Pentatomidae) on pistachio (Anacardiaceae: *Pistacia vera*) in South Africa: Implementing a plant health management strategy. M. Sc. Dissertation, University of the Free State.

GALWAY, K.E. & PURCELL, M.F. 2005. Laboratory life history and field observations of *Poliopaschia lithochlora* (Lower) (Lepidoptera: Pyralidae), a potential biological control agent for *Melaleuca quinquenervia* (Myrtaceae). *Australian Journal of Entomology* **44**: 77–82.

GIANESSI, L. 2009. The benefits of insecticide use: Pistachios. Available online: http://www.croplifefoundation.org/Insecticide_Benefits/Crop_Studies/Combined_Document_Pistachios.pdf

GOOGLE INC. (2010). Google Earth (Version 5.1.3533.1731) [Software]. Available from http://earth.google.com/download-earth.html

HABECK, D.H. & BENNETT, F.D. 1990. *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), a Phycitine new to Florida. *Entomology Circular* **333**: 1-4.

HADDAD, C.R. & LOUW, S.vdM. 2006. Phenology and potential biological control of the stinkbug *Atelocera raptoria* (Hemiptera: Pentatomidae) in pistachio orchards. *African Plant Protection* **12**: 23-27.

HAYES, L.M. 1996. *The biological control of weeds book – A New Zealand guide.* Landcare Research, New Zealand. Available online: http://www.landcareresearch.co.nz/research/biocons/weeds/book.asp

JOHNSON, J.A., VALERO, K.A. & HANNEL, M.M. 1997. Effect of low temperature storage on survival and reproduction of Indian meal moth (Lepidoptera: Pyralidae). *Crop Protection* **16**: 519-523.

JOOSTE, C. 2005. Neutboerdery goed op dreef. *Landbouweekblad*. 1 Julie 2005. Available online: http://www.landbou.com/node/7541?quicktabs_1=0

KHAGHANINIA, S. & POURABAD, R.F. 2009. Investigation on biology of olive leaf worm *Palpita unionalis* Hübner (Lepidoptera: Pyralidae) in constant laboratory conditions. *Munis Entomology & Zoology* **4**: 320-326.

KUENEN, L.P.S., BENTLEY, W., ROWE, H.C. & RIBEIRO, B. 2008. Bait formulations and longevity of navel orangeworm egg traps tested. *California Agriculture* **62**: 36-39.

LESLIE, G.W. 1994. Observations on crop damage and larval populations of the Pyralid borer *Eldana saccharina* in the sugarcane varieties NCo376 AND N11. *Proceedings of the South African Sugar Technologists' Association* **68**: 12-15.

LOBOS, E. & DE CORNELLI, J.O. 1997. Observations on *Cactoblastis cactorum* (Berg) as a pest of cactus pear (*Opuntia ficus-indica*) in Argentina with suggestions on possible control methods. *Journal of the Professional Association for Cactus Development* **2**: 97-102.

MEALS, D.W. & CALTAGIRONE, L.E. 1995. Chapter 53: Navel Orangeworm, pp. 196-198. **In** Nechols, J.R. (ed.) *Biological control in the western United States*. Division of Agriculture and Natural Resources, California.

MICHAILIDES, T.J. & MORGAN, D.P. 2004. Panicle and shoot blight of pistachio: A major threat to the California pistachio industry. Available online: http://www.apsnet.org/online/feature/pistachio/pistachio.pdf

MOHAMED, H.M., KHAN, Z.R., OVERHOLT, W.A. & ELIZABETH, D.K. 2004. Behaviour and biology of *Chilo partellus* (Lepidoptera: Pyralidae) on maize and wild gramineous plants. *International Journal of Tropical Insect Science* **24**: 287–297.

NUSS, K.J., BOND, R.S. & ATKINSON, P.R. 1986. Susceptibility of sugarcane to the borer *Eldana saccharina* Walker and selection for resistance. *Proceedings of the South African Sugar Technologists' Association* **60**: 92-94.

RICE, R.E., BARNETT, W.W. & VAN STEENWYK, R.A. 1996. Chapter 29: Insect and mite pests, pp. 202-205. **In** Micke, W. (ed.) *Almond production manual*. Division of Agriculture and Natural Resources, California.

RICE, R.E., BENTLEY, W.J. & BEEDE, R.H. 1988. *Insect and mite pests of pistachios in California*. University of California, Oakland.

SHELTON, M.D. & DAVIS, D.W. 1994. Navel orangeworm (Lepidoptera: Pyralidae) development in sunburn walnuts. *Journal of Economic Entomology* **87**: 1062-1069.

SHEPHERD, R.C.H. 1990a. Evaluation of *Microthrix inconspicuella*, (Lepidoptera: Pyralidae), a potential biological control agent for *Emex australis* in Australia, carried out in apple orchards in South Africa. *Entomophaga* **35**: 583-587.

SHEPHERD, R.C.H. 1990b. Laboratory tests carried out on *Microthrix inconspicuella* (Lepidoptera: Pyralidae), a biological control agent for *Emex australis* in Australia. *Entomophaga* **35**: 441-447.

SIEGEL, J.P., KUENEN, L.P.S., HIGBEE, B.S., NOBLE, P., GILL, R., YOKOTA, G.Y., KRUGNER, R. & DAANE, K.M. 2008. Postharvest survival of navel orangeworm assessed in pistachios. *California Agriculture* **62**: 30-35.

SIEGEL, J.P., LACEY, L.A., HIGBEE, B.S., NOBLE, P. & FRITTS, J.R. 2006. Effect of application rates and abiotic factors on *Steinernema carpocapsae* for control of overwintering navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in pistachios. *Biological Control* **36**: 324–330.

SIMONSON, S.E., STOHLGREN, T.J., TYLER, L., GREGG, W.P., MUIR, R. & GARRETT, L.J. 2005. Preliminary assessment of the potential impacts and risks of the invasive cactus moth, *Cactoblastis cactorum* Berg, in the U.S. and Mexico. *Final Report to the International Atomic Energy Agency*, April 25.

UC IPM. 2007. UC Pest Management Guidelines: Pistachios. Available online: http://www.ipm.ucdavis.edu/PMG/selectnewpest.pistachios.html

WADE, W.H. 1961. Biology of the navel orangeworm, *Paramyelois transitella* (Walker), on almonds and walnuts in northern California. *Hilgardia* **31**: 129-171.

WANG, S., JOHNSON, J.A., TANG, J. & YIN, X. 2005. Heating condition effects on thermal resistance of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *Journal of Stored Products Research* **41**: 469–478.

W.A. PISTACHIOS. 2005. W.A. Pistachios History. Available online: http://www.wapistachios.com.au/pages/history.html

WILSON, R.L. 1990. Rearing the sunflower moth (Lepidoptera: Pyralidae) for use in field evaluation of sunflower germplasm. *Journal of the Kansas Entomological Society* **63**: 208-210.

ZALOM, F.G., WEAKLEY, C., HENDRICKS, L.C., BENTLEY, W.J., BARNETT, W.W. & CONNELL, J.H. 1984. Cultural management of the navel orangeworm by winter sanitation. *California Agriculture*, March-April: p. 28.

ZIMMERMANN, H.G., MORAN, V.C. & HOFFMANN, J.H. 2000. The renowned cactus moth, *Cactoblastis cactorum*: its natural history and threat to native *Opuntia* floras in Mexico and the United States of America. *Diversity and Distributions* **6**: 259–269.

CHAPTER 2

Influence of orchard row management practices on survival of navel orangeworm

INFLUENCE OF ORCHARD ROW MANAGEMENT PRACTICES ON SURVIVAL OF NAVEL ORANGEWORM

ABSTRACT

Orchard floor management practices, such as cover crops between rows and mulch in rows, can potentially play a role in increasing natural enemies, slowing erosion and improving soil structure, while irrigation practices, such as hydro-cooling increase humidity in the orchards, enhancing growth. During 2008 and 2009, the effect of these orchard row management practices on the survival of the overwintering navel orangeworm (NOW), *Amyelois transitella* (Walker), occurring in mummy nuts left behind after harvest on the pistachio orchard floor at Green Valley Nuts were examined. Emergence cages, placed over sampled mummy nuts, and light traps were placed in orchards of three different pistachio cultivars to monitor for the emergence of adult navel orangeworm. Two of the cultivars (*i.e.* Ariyeh & Shufra) were hydro-cooled and had cover crops between rows and mulch under trees, whilst the third cultivar (*i.e.* Sirora) was in a dry land block with no row cover between trees. Even though the experiment using the emergence cages was run during both years, no moth emergence was recorded for any of the cultivars, suggesting *in situ* factors that enhance mortality rates. Moths were, however, also observed at light traps, providing evidence of adult navel orangeworm presence in the field.

INTRODUCTION

Deploying cover crops as a general management tactic is starting to fulfil an increasingly important role on farms since the tactic decreases erosion, improves soil structure and contributes towards pest management. This opinion is, amongst others, well demonstrated by Phatak & Diaz-Perez (2007) and Bone, Thomson, Ridlan, Cole & Hoffmann (2009), who respectively mention that infestations by insects, nematodes and weeds can be reduced with cover crops, minimizing the reliance on pesticides and that cover crops provide a nutritional resource that can play an important role in increasing parasitoid and predator prevalence.

Several systems are used for orchard floor management. The most popular of these methods include growing cover crop alleys, usually a grass, between the rows of trees, whilst leaving a vegetation-free strip in the tree row. This vegetation free strip provides an area for root growth without weed and grass competition, and the area is often covered with mulch (Fig. 1). Maintenance is minimal once the ground cover between the rows is established (Roper, 1992). Oats (*Avena sativa* L.), a popular alley cover crop, is known to suppress weeds and conserve soil moisture levels (Clark, 2007).

Mulch placed on the soil surface under the trees act as an insulator to aid in conserving moisture by reducing evaporative loss of soil water, as well as by maintaining uniform soil temperature (Williams, 1997). In addition to this, mulch is also very effective in suppressing weed germination and growth, therefore also reducing water use by weeds (Dreistadt & Clark, 2004). These areas must be hoed and turned regularly to allow surface drying and to prevent the growth of moulds. Precautions should also be taken to avoid the overwintering of problem organisms, such as insects and rodents in the mulch layer (Williams, 1997).

Figure 1. Wood chip mulch is placed under trees and rows between trees are planted with oats (Green valley Nuts, 2008 – 2009).

Hydro-cooling is a type of secondary irrigation used in orchards. It is mostly used for temperature control on fruits to prevent postharvest losses (Strand, 1999), but it can also be useful as an overhead irrigation system in orchards that apply water with sprinklers over the trees for tree canopy cooling (Fig. 2). This helps to reduce plant heat stress during warm conditions by lowering plant canopy temperature through evaporative cooling (Van der Gulik, 1995). It is suspected that hydro-cooling, which increases soil moisture levels considerably, should have an effect on the survival of resident organisms.

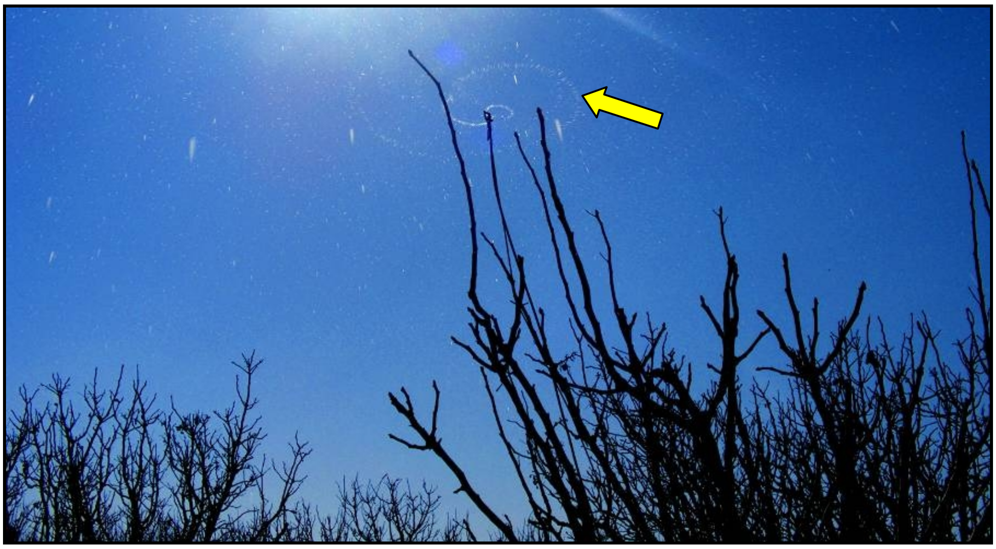

Figure 2. Sprinklers (arrow) in a pistachio tree which gives rise to a management tactic known as hydro-cooling (Green Valley Nuts, 2008 – 2009).

In 2005, reconnaissance monitoring was conducted during August, September and November at Green Valley Nuts (GVN) to determine the incidence of navel orangeworm in the orchards. Results clearly indicated that navel orangeworm was present in all the pistachio cultivars cultivated at GVN.

With this as background the aim of this part of the study was to investigate the influence of orchard row management practices, such as mulch, alley cover crops and hydro-cooling, on the incidence and population dynamics of the navel orangeworm.

MATERIAL AND METHODS

1. Study Site

The experiment was conducted in 2008 and 2009 at Green Valley Nuts (29˚34'56''S 22˚54'35''E) near Prieska in the Northern Cape Province of South Africa. The trail sites consisted of three orchard blocks planted with different pistachio cultivars, *i.e.* Ariyeh, Sirora and Shufra (Fig. 3).

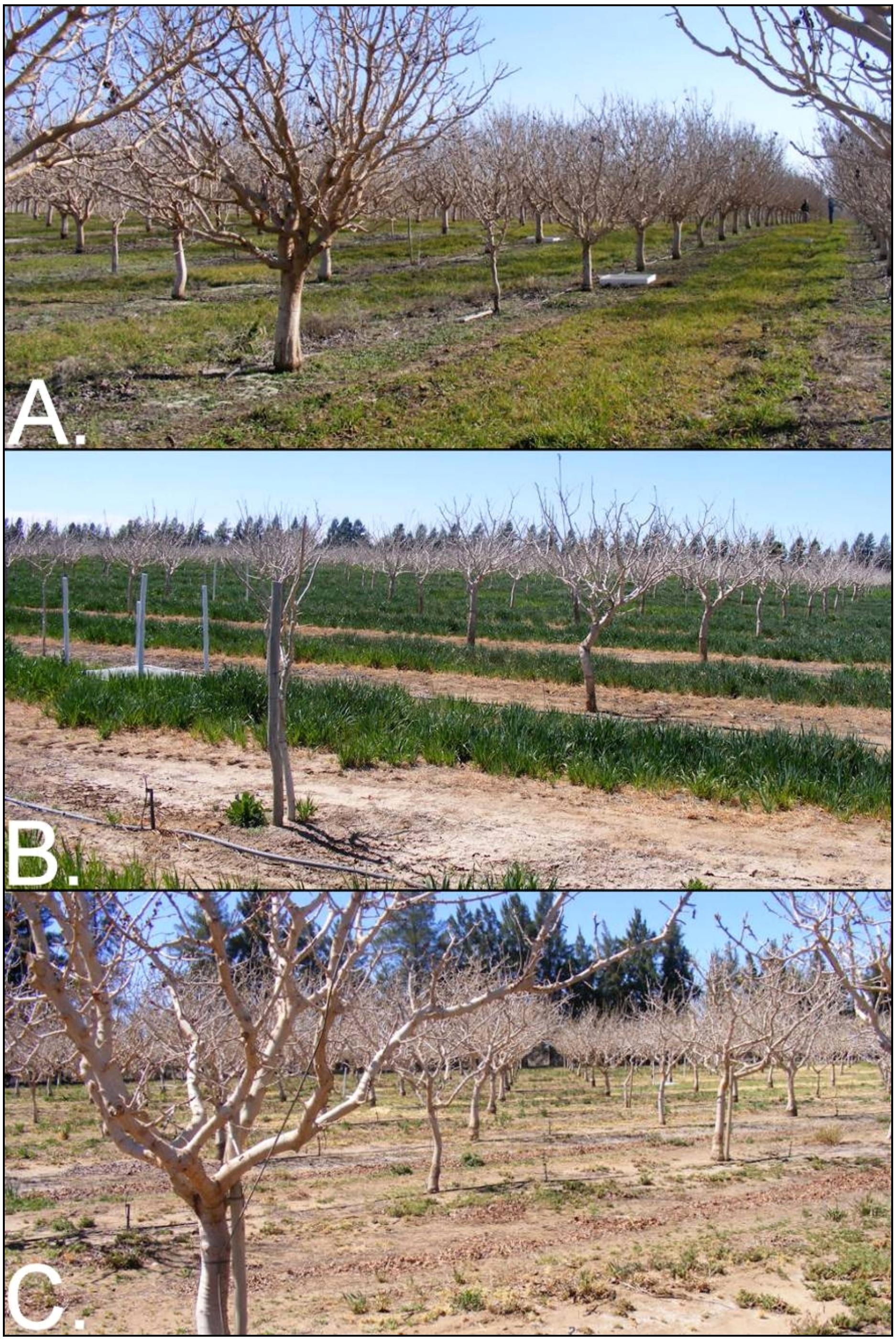

Figure 3. The three orchard blocks at Green Valley Nuts (2008 – 2009). A: Ariyeh. B: Shufra. C: Sirora.

The blocks with the Ariyeh and Shufra cultivars were hydro-cooled, the ground beneath the trees was covered with mulch and the rows between the trees were planted with oats. The Sirora block received normal periodical surface irrigation, and mulch and alley crop between rows were absent. In comparison to the other two blocks mentioned above this represented a dry land condition.

Two types of orchard monitoring approaches were carried out for these three cultivars. The first was an experiment monitoring the effect of inter-tree row management practices on navel orangeworm survival in mummy nuts on the soil surface. The second was the monitoring of navel orangeworm adult occurrence in orchards through the use of light traps.

2. Inter-tree row management

Mummy nuts were sampled in July 2008 and May 2009 from orchards of each of the three different cultivars. In 2008, in the respective Ariyeh and Shufra blocks, mummy nuts that had dropped from the trees were sampled by raking the orchard floor under 12 randomly selected trees, whilst mummy nuts left behind on the trees were hand-sampled off eight randomly selected trees. All mummy nuts sampled for Shufra was from under 20 randomly selected trees, since no nuts could be found on the trees.

All sampled nuts were quantified and placed in emergence cages in the orchard blocks where sampling took place (Fig. 5). Cages for this trial were manufactured from wood, painted white and covered with fine metal mesh (Fig. 4). Five of these cages were placed in the blocks of each of the cultivars. Two treatments were investigated, namely i) placing mummy nuts in the rows of the alley cover crop between trees (three cages per cultivar) and ii) placing mummy nuts in cages in tree rows (two cages per cultivar). The cages were regularly inspected by GVN staff for adult moth emergence.

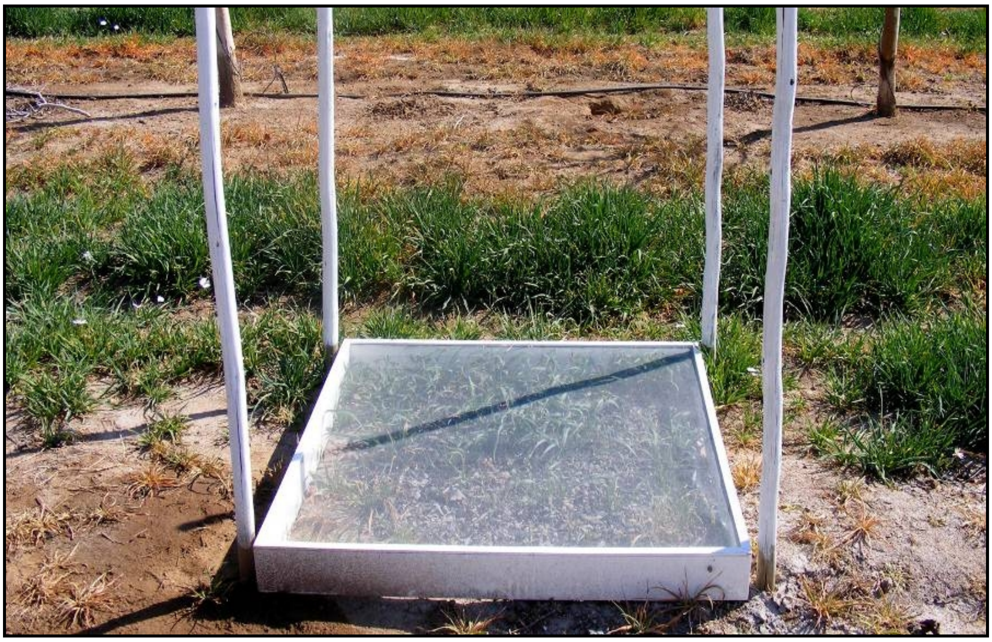

Figure 4. Emergence cages placed in pistachio orchards to monitor navel orangeworm emergence rates from mummy nuts (Green Valley Nuts, 2008 – 2009).

In 2009, the five cages per cultivar were placed in cultivar blocks again, but this time all cages were placed in the tree rows on account of dense cover crop growth in the alleys between rows. During the previous season it was noticed that the growing vegetation lifted the cages from the ground, which could have resulted in the possible escape of moths. In 2009 no mummy nuts were present on the trees and all nuts were sampled from under 20 randomly selected trees for each cultivar and divided between the respective cages.

3. Light trap monitoring

Light traps (Fig. 6) placed in the orchards were also monitored on a regular basis from May to September 2009 for the presence of NOW moths. Four of these light traps were placed in different orchard blocks, indicated by E on the map in figure 5. All moths caught in the containers under the light were collected by GVN staff and sent to the laboratory at the University of the Free State for identification.

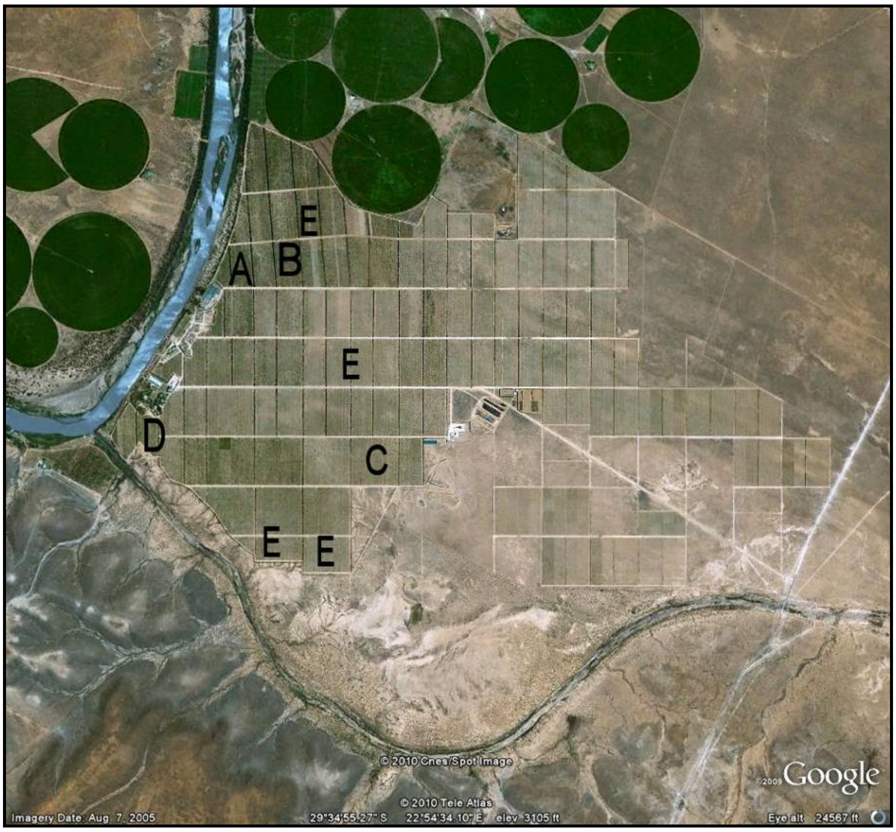

Figure 5. Orchard sites where nuts where sampled and cages were placed (Green Valley Nuts, 2008 – 2009). A: Ariyeh. B: Sirora. C: Shufra (2008). D: Shufra (2009). E: Light traps. (Map from Google Inc., 2010)

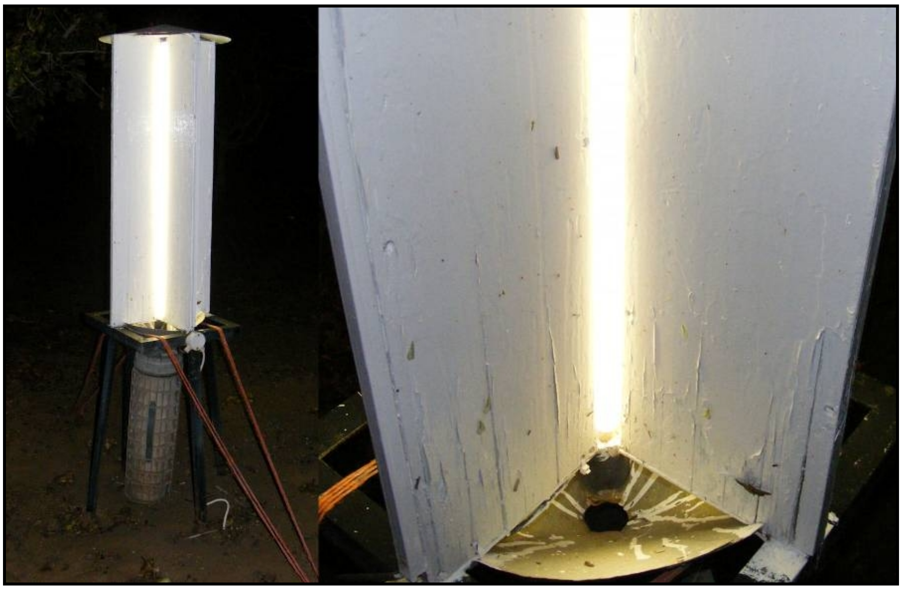

Figure 6. Example of a light trap that was placed in orchards. Close-up photo shows funnel beneath light, which leads to container for collecting insects.

RESULTS AND DISCUSSION

1. Inter-tree row management

No moths emerged in the cages in both years that the experiment was conducted at GVN. Previous studies done on ground management practices in California by Siegel, Kuenen, Higbee, Noble, Gill, Yokota, Krugner & Daane (2008) showed that these procedures reduce NOW emergence, presumably because the humidity in the groundcovers increase mummy rotting that leads to increased NOW mortality.

This effect was, however, not observed during this experiment. As no emerging moths were observed, it can be contemplated that the alley crop and hydro-cooling had a decreasing effect on the navel orangeworm, but the same results were observed in the cultivar with no alley crop and hydro-cooling.

Conditions in the orchards range between the two extremities of very wet at times, due to irrigation, and very hot at times, with summer temperatures in Prieska reaching highs of 35 – 40˚C. This renders the conditions in the cages unsuitable for navel orangeworm survival and can explain why no moths were observed. Shorter monitoring regimes would not have changed anything to this scenario, since no moths whatsoever, alive or as cadavers, were retrieved from the cages.

2. Light trap monitoring

A few NOW moths were sampled at a light trap in September 2009 during one of the orchard visits. This shows that adult navel orangeworm were emerging elsewhere in the environment under natural conditions, albeit that they were not recorded in the cages. It can therefore be assumed that conditions present in the field were more favourable in promoting navel orangeworm survival. GVN staff monitoring the light traps did not record any navel orangeworm individuals and none of the samples forwarded to the UFS laboratory contained any navel orangeworm moths.

According to Wade (1961), NOW moths are not very strong fliers and they probably don't exhibit rapid, strong flight. This could render the method of using light traps to monitor for NOW presence meaningless, especially when considering the prevailing windy conditions at GVN in the evening.

CONCLUSION

Even though the desired results were not obtained with the trial of placing cages in the orchard to demonstrate the effect of orchard floor management practices on the survival of overwintering navel orangeworm, it is strongly suspected that these practices and hydro-cooling may potentially have had an effect on the longevity of the navel orangeworm. No adult emergence was observed in orchards where these practices were implemented. In combination with high temperatures, this could have caused meaningful navel orangeworm mortality and explain the absence of adult detection. This will be discussed in more detail in chapter three. Larvae collected from nuts in orchards for other trials revealed that the navel orangeworm can successfully complete its life cycle under suitable conditions. This will be discussed further in the following chapters.

REFERENCES

BONE, N.J., THOMSON, L.J., RIDLAN, P.M., COLE, P. & HOFFMANN, A.A. 2009. Cover crops in Victorian apple orchards: Effects on production, natural enemies and pests across a season. *Crop Protection* **28**: 675–683.

CLARK, A. 2007. *Managing cover crops profitably*. 3rd edition. Sustainable Agriculture Network, Beltsville.

DREISTADT, S.H. & CLARK, J.K. 2004. *Pests of landscape trees and shrubs: An integrated pest management guide.* ANR Publications, University of California.

GOOGLE INC. (2010). Google Earth (Version 5.1.3533.1731) [Software]. Available from http://earth.google.com/download-earth.html

PHATAK, S.C. & DIAZ-PEREZ, J.C. 2007. Managing pests with cover crops, pp. 25-33. **In** Clark, A. (ed.) *Managing Cover Crops Profitably*. 3rd edition. Sustainable Agriculture Network, Beltsville.

ROPER, T.R. 1992. Orchard-floor management for fruit trees. Bulletin A3562. Available online: http://learningstore.uwex.edu/assets/pdfs/ A3562.pdf

SIEGEL, J.P., KUENEN, L.P.S., HIGBEE, B.S., NOBLE, P., GILL, R., YOKOTA, G.Y., KRUGNER, R. & DAANE, K.M. 2008. Postharvest survival of navel orangeworm assessed in pistachios. *California Agriculture* **62**: 30-35.

STRAND, L.L. 1999. *Integrated pest management for stone fruits*. ANR Publications, University of California.

VAN DER GULIK, T. 1995. Irrigation factsheet: Using irrigation for tree fruit cooling. Available online: http://www.irrigationbc.com/images/clientpdfs/578300-1.pdf

WADE, W.H. 1961. Biology of the navel orangeworm, *Paramyelois transitella* (Walker), on almonds and walnuts in northern California. *Hilgardia* **31**: 129-171.

WILLIAMS, D.J. 1997. Organic mulch. Department of Natural Resources and Environmental Sciences, University of Illinois. Available online: http://web.aces.uiuc.edu/vista/pdf_pubs/mulch.pdf

CHAPTER 3

Winter survival of navel orangeworm in post harvest pistachio mummy nuts

WINTER SURVIVAL OF NAVEL ORANGEWORM IN POST-HARVEST PISTACHIO MUMMY NUTS

ABSTRACT

Larvae of the navel orangeworm, *Amyelois transitella*, causes direct damage to pistachio nut clusters by feeding and boring into individual nuts. In the process the quality of the nuts is reduced and the nuts are rendered more susceptible to fungal infection. After harvest, navel orangeworm (NOW) larvae overwinter inside fallen nuts and nuts left behind on trees. This study focuses on these "mummy" nuts and the inoculum qualities they provide regarding sustenance and shelter for the overwintering larvae. The successful development of these larvae serves as the springboard for orchard infestation during the following season. The prevalence of navel orangeworm in mummy nuts was studied from May to September in both 2008 and 2009 at Green Valley Nuts near Prieska in the Northern Cape Province. The procedure for determining the potential survival of the NOW larvae was estimated by sampling nuts from under trees of three different pistachio cultivars (*i.e.* Ariyeh, Sirora and Shufra) and sorting (as splits and non-splits) and quantifying sampled nuts per cultivar. During 2008 the mummies were placed in containers covered with gauze netting and adults that eclosed were quantified, whilst in 2009 the larvae were quantified by dissecting nuts. In both years NOW was recorded overwintering in these mummy nuts. The highest occurrence of NOW over the two year study period was recorded in nuts from the Sirora cultivar. The results support the assumption that hydro-cooling, alley cover crops and mulch have a suppressing effect on NOW development, causing high mortality rates due to mummy nut decomposition.

INTRODUCTION

The navel orangeworm (NOW), *Amyelois transitella* (Walker) (Pyralidae), is an important insect pest that infests tree nuts (walnuts, almonds and pistachios) in the field (Wang, Johnson, Tang & Yin, 2005). Occasionally NOW might also infest rotting or damaged oranges, lemons and grapefruit at very low levels when compared to nut crops, whilst it has also been recorded as a scavenger in many other mummified fruits, such as figs and pears (Rice, Bentley & Beede, 1988).

The larvae of the navel orangeworm cause direct damage to pistachio nuts by feeding and boring into individual pistachio kernels, leaving behind frass and webbing (Fig. 1). This reduces the quality of the nuts and leads to increased processing costs (Siegel, Kuenen, Higbee, Noble, Gill, Yokota, Krugner & Daane, 2008). Damage can also render the pistachios more susceptible to fungal growth, since it provides an entry point for pathogenic fungi that produce aflatoxins, which in turn render the nuts unmarketable (Mosz, 2002). Elimination or reduction of NOW infestations will therefore have a positive influence both directly, regarding crop damage and indirectly, regarding food sanitation concerns (Siegel *et al*., 2008).

Figure 1. Damage caused to a pistachio kernel by an overwintering navel orangeworm larva.

NOW becomes a primary pest of nuts after hull split and there can be between three and five overlapping generations a year. The insect is active all year round in favourable conditions and there is no diapause (Shelton & Davis, 1994). Larvae shelter (overwinter) and acquire sustenance in nuts, found on the ground and in trees, which are left behind after harvest (Summers & Price, 1964). These left-over nuts, commonly referred to as mummy nuts (Siegel *et al*., 2008) (Fig. 2) constitute an important resource for NOW survival by sustaining NOW population numbers throughout most of the year. These numbers have to reach a certain threshold for the new generation to be reproductively viable and therefore the importance of mummy nuts as such for NOW presence and establishment in orchards cannot be underestimated.

NOW larvae can overwinter in various larval stages or as pupae in mummy nuts. Under local conditions NOW usually enter pistachio nuts from February to April and emerge from August to December when temperatures begin to rise. In this regard Rice *et al*. (1988) reported that with the warmer temperatures in spring in the northern hemisphere, the overwintering stages can complete their development more rapidly to become adult moths. The successful development of these overwintering larvae subsequently serves as a launching pad for orchard infestation during the following season (Rice *et al*., 1988). Mummy nuts are the only known source of sustenance and shelter for the survival of overwintering NOW. Overwintering larvae are not influenced by insecticides and the only method of control is by field sanitation. Sanitation is achieved by removing mummies from trees and tilling fallen nuts into the soil during winter (Siegel, Lacey, Fritts, Higbee & Noble, 2004). This study reports the results of a two year study of NOW monitoring to provide more information for winter sanitation decisions.

Figure 2. Pistachio mummy nuts on orchard floor under trees (A) and on trees (B) (Green Valley Nuts, 2008 – 2009).

MATERIAL AND METHODS

1. Study site

This trail was conducted over a two year period from May to September in both 2008 and 2009 at Green Valley Nuts near Prieska in the Northern Cape Province. Nuts were sampled from orchards of Ariyeh, Sirora and Shufra cultivars (Fig. 3). Ariyeh (Fig. 4) and Shufra (Fig. 5) are hydro-cooled cultivars with a ground cover consisting of oats in the rows between the trees. The orchard with the Sirora cultivar (Fig. 6) was non hydro-cooled without an alley cover crop.

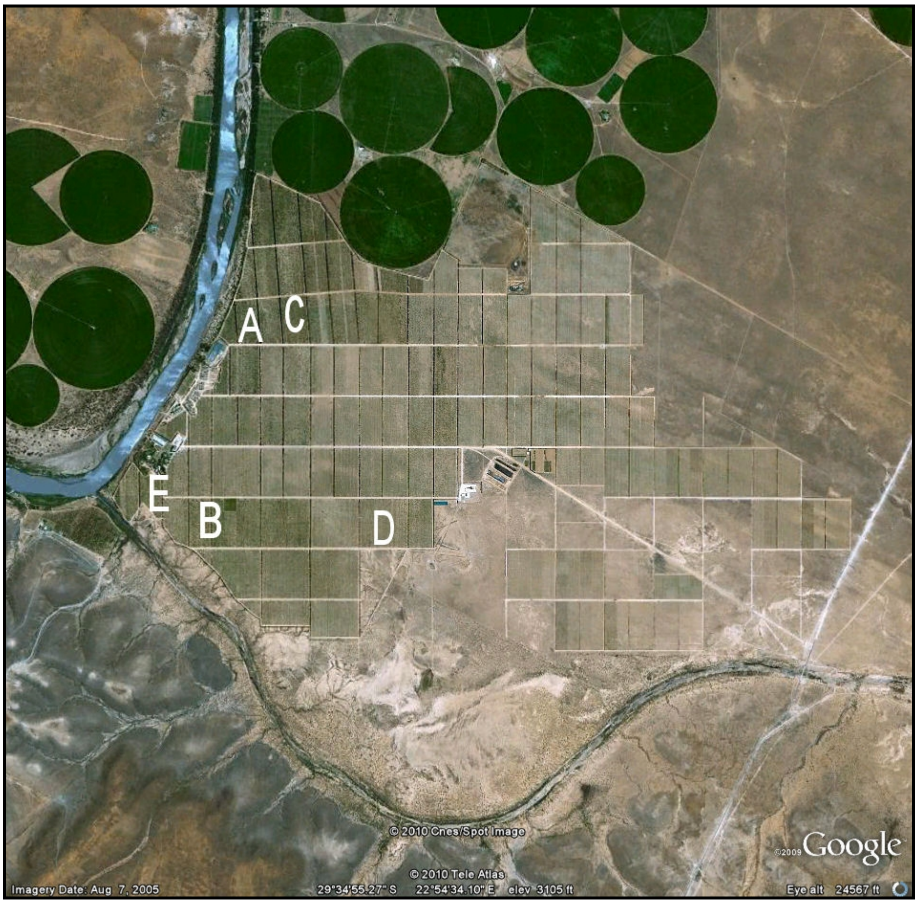

Figure 3. Orchard sampling locations at Green Valley Nuts. A: Ariyeh (2008). B: Ariyeh (2009). C: Sirora. D: Shufra (2008). E: Shufra (2009). (Map from Google Inc., 2010)

Figure 4. Ariyeh orchard at Green Valley Nuts, with ground cover between trees and hydro-cooling (2008 – 2009).

Figure 5. Shufra orchard at Green Valley Nuts, with dense ground cover between trees on account of hydro-cooling (2008 – 2009).

Figure 6. Sirora orchard at Green Valley Nuts with no ground cover between trees and no hydro-cooling (2008 – 2009).

2. Sampling of mummies under trees

Mummy nuts were sampled once a month from under trees. These were raked together in a two meter radius of the tree stem from under ten randomly selected trees for each cultivar (Fig. 7). All nuts were placed in plastic bags and taken back to the UFS laboratory for sorting and quantifying.

Figure 7. Sampling of pistachio mummy nuts under trees at Green Valley Nuts (2008 – 2009).

3. Sampling of mummies on trees

If mummies were present on trees in the respective blocks, they were also sampled. Mummies were sampled on ten randomly selected trees of Ariyeh and Sirora during July and only from Ariyeh in August during 2008. No mummies were found on Shufra trees. In 2009 none of the cultivars had any mummies present on the trees and no tree sampling was conducted.

4. Sorting of nuts and data recording

Nut samples from under the trees were sorted as split, non-split and blank nuts, and quantified. All nuts sampled in 2008 were placed in round plastic containers covered with gauze and maintained at 20-25°C in an insectarium (Fig. 8). The containers were checked daily for adult emergence. All adult moths that emerged were removed from the containers and their numbers and dates of emergence recorded.

Figure 8. Containers with pistachio mummy nuts stored at 20-25ºC in an insectarium (above). Nuts in a container (below).

In 2009, after the nuts were sorted and quantified, the split nuts were opened and examined for the presence of overwintering larvae or pupae. The number of specimens found was recorded in terms of cultivar and condition of the nuts.

5. Nut samples from processing plant

A nut sample from 20 random blocks was also obtained from the pistachio nut processing plant at Green valley Nuts. This replaced and represented tree mummy nut infestation in 2009 and was conducted in order to compensate for the absence of nuts on the trees from the preceding years' harvest. The nut samples from the processing plant were examined for NOW damage (Fig. 9).

Figure 9. Pistachio nut sample from the Green Valley Nuts processing plant (2009). Insert: Nut damaged by NOW.

6. Orchard monitoring

Random orchards were monitored on a regular basis by GVN staff from March to September 2009 for NOW presence. This was conducted by randomly sampling nuts on a non-fixed basis in the larger orchard and dissecting the nuts for NOW presence.

RESULTS AND DISCUSSION

1. Pistachio mummy nut occurrence in orchards during 2008 and 2009

During the two year sampling period for pistachio mummy nuts, the majority of nuts were sampled from the Ariyeh cultivar (Table 1). Furthermore, the percentage of split nuts for each cultivar was calculated to determine the true number of nuts available to overwintering navel orangeworm (NOW), since blank and closed-shell nuts cannot be infested (Siegel *et al*., 2008).

For nuts sampled on the orchard floor, the Sirora cultivar had the most split nuts sampled over this time (34.93%), while the Shufra cultivar had the lowest number of split nuts (19.77%). 33.04% split nuts were recorded for Ariyeh (Table 1).

Table 1. Percentage of split, unsplit and blank nuts sampled under trees in 2008 and 2009 for three cultivars at Green Valley Nuts.

Cultivar	Nut total	Percentage of nuts (%)		
		Split	Unsplit	Blank
Ariyeh	41246	33.04	58.83	8.13
Shufra	25670	19.77	77.53	2.70
Sirora	27328	34.93	60.82	4.24

2. Occurrence of navel orangeworm in pistachio mummy nuts during 2008

<u>*Overall navel orangeworm infestation for each cultivar*</u>: There was no significant difference (df=12; MSE=0.1400567; $P>0.05$) between the mean percentages of infestations for the different cultivars. As shown in Table 2, Ariyeh had the highest

overall infestation of NOW at 0.602%, followed by Sirora at 0.268% and Shufra at 0.204%. The percentage of split nuts available for infestation during 2008 were as follow: Ariyeh = 35.61%; Sirora = 33.03% and Shufra = 20.64%. It can be seen that the percentage of NOW infestation shadowed the decline in split nut availability for infestation. The infestation percentages were based on the number of adults emerging from the sampled mummy nuts kept in containers. Even though infestations were low, these results do show the presence of NOW on all the cultivars and because of this the assumption can be made that infestation is present throughout all of the nut-bearing orchards on the farm.

Monthly navel orangeworm infestation for each cultivar: When considering NOW infestation for each month during the monitoring period, it can be seen that the number of NOW adults emerging from the nuts were not very high (Table 2). Emergence mostly consisted of only a few individuals recorded in the thousands of nuts. The highest infestation was recorded in June (1.45% NOW) and July (0.75% NOW) in Ariyeh. This emergence in the beginning of the winter is due to more favourable conditions after the nuts were removed from the field and placed in containers at a higher temperature. Shufra and Sirora also had NOW emergence from June, but at lower numbers than Ariyeh.

Overall navel orangeworm infestation per month: There was no significant difference (df=10; MSE=0.1762533; P>0.05) between the means of NOW infestations for the different months that sampling was conducted in 2008 (Table 3). Out of a total of 67089 nuts sampled for the three cultivars, 129 NOW adults emerged. This equates to an overall NOW infestation of 0.51% for 2008.

Table 2. Monthly percentage of navel orangeworm infestation based on emerging moths from mummies sampled under trees at Green Valley Nuts in 2008.

Cultivar	Month	Total number of mummies	% Split nuts	Total number of NOW	% NOW infestation
Ariyeh	May	4056	52.05	8	0.38
	June	17152	28.91	72	1.45
	July	9265	43.03	30	0.75
	August	2385	29.22	3	0.43
	September	1872	24.84	0	0
	Total	**34370**	**35.61**	**113**	**0.602**
Shufra	May	2466	16.75	0	0
	June	4764	26.85	2	0.16
	July	7048	20.43	0	0
	August	2455	21.63	2	0.38
	September	2376	17.55	2	0.48
	Total	**19109**	**20.64**	**6**	**0.204**
Sirora	May	2188	32.31	0	0
	June	2357	36.15	2	0.23
	July	4480	33.57	2	0.13
	August	2301	34.64	2	0.25
	September	1924	28.48	4	0.73
	Total	**13250**	**33.03**	**10**	**0.27**

Table 3. Overall navel orangeworm infestation based on emerging moths from mummies sampled under trees of all cultivars at Green Valley Nuts in 2008. Means of navel orangeworm infestation followed by the same letter are not significantly different at $P \leq 0.05$.

Month	Total number of mummies	% Split nuts	Total number of NOW	% NOW infestation
May	8710	37.01	8	0.25a
June	24273	29.2	76	1.07a
July	20793	33.35	32	0.46a
August	7141	28.36	7	0.35a
September	6172	23.17	6	0.42a
Total	**67089**	**30.22**	**129**	**0.51**

3. Occurrence of navel orangeworm in pistachio mummy nuts during 2009

Overall navel orangeworm infestation for each cultivar: When comparing nut samples from 2009 with 2008, it can be seen in Table 1 that there was a decrease in the total number of mummies and split nuts, with the exception of the Sirora cultivar. This was the effect of a poor harvest year in 2009, resulting in fewer mummies left behind in the orchard during the winter. It caused a noticeable decrease in NOW infestation and no NOW larvae were found in the Shufra and Ariyeh cultivars (Table 4). NOW infestation percentages throughout 2009 were calculated by recording larvae found during the dissecting of mummy nuts.

There was a significant difference (df=12; MSE=0.1762933; $P<0.05$) between the mean percentages of infestations on Sirora compared to Ariyeh and Shufra. Sirora was the only cultivar with a higher NOW infestation (1.96%) and more mummy nuts in comparison to the previous year (Table 3 & 4).

Table 4. Monthly percentage of navel orangeworm infestation based on larvae found in mummies sampled under trees at Green Valley Nuts in 2009.

Cultivar	Month	Total number of mummies	% Split nuts	Total number of NOW	% NOW infestation
Ariyeh	May	836	26.44	0	0
	June	772	40.03	0	0
	July	2397	9.64	0	0
	August	1383	26.54	0	0
	September	1128	24.65	0	0
	Total	**6516**	**25.46**	**0**	**0**
Shufra	May	1285	17.98	0	0
	June	653	16.54	0	0
	July	2311	9.17	0	0
	August	1159	22.00	0	0
	September	1153	16.39	0	0
	Total	**6561**	**16.42**	**0**	**0**
Sirora	May	3074	34.16	28	2.67
	June	2256	31.29	20	2.83
	July	3776	41.42	23	1.47
	August	2438	39.25	15	1.57
	September	2534	34.02	11	1.28
	Total	**14078**	**36.03**	**97**	**1.96**

When comparing Table 3 & 5, it can be seen that the total NOW infestation for 2009 (1.31%) was higher than the previous year (0.51%), even though two of the cultivars showed no signs of NOW infestation in 2009. Pistachios are alternate bearing and produce a heavy crop in one year followed by a light crop the following year (Burks, Higbee, Brandl & Mackey, 2008). 2009 was a light crop year compared to 2008. According to Burks *et al*. (2008), it can be expected that there will be heavier infestations in a light crop following a heavy crop year. Sirora, the only cultivar in which NOW was recorded in 2009, had the highest number of split mummy nuts (36.03%) available on the ground (Table 4), providing more sustenance for overwintering larvae. It's also the cultivar that had no hydro-cooling and alley crops, rendering conditions more favourable for NOW survival.

Monthly navel orangeworm infestation for each cultivar: The percentage of infestation on Sirora split nuts was the highest in June (2.83 %) (Table 4). After June infestation levels declined, eventually reaching only 1.28% in September. This was as a result of rising temperatures, because individuals go into the next stage of development when conditions get more favourable and the first moths already emerged in August. Sirora had an overall NOW infestation of 1.96%, considerably higher than the previous year that only had a 0.27% infestation. This increase in NOW population numbers can be a sign that NOW are beginning to get more established in certain orchards with more favourable conditions, such as Sirora.

Overall navel orangeworm infestation during each month: There was no significant difference (df=10; MSE=1.4268; P>0.05) between the means of NOW infestations for the different months (Table 5). A total of 97 NOW larvae were recorded out of 27155 nuts sampled for the three cultivars, but all the larvae were recorded infesting only a single cultivar. This figure provides an overall NOW infestation of 1.31% for 2009.

The trees of nut-bearing age at GVN were during the study period in the minority and actual harvesting has only been conducted for the last four years. Even though infestations were low in the pistachio orchards during 2008 and 2009, a steady increase can be expected over time which will have to be considered in future orchard management planning. These results therefore send out an early warning signal so that proactive NOW management tactics can be implemented.

Table 5. Navel orangeworm infestation based on larvae recorded in mummies sampled under trees of all cultivars at Green valley Nuts in 2009. Means of navel orangeworm infestation followed by the same letter are not significantly different at P≤0.05.

Month	Total number of mummies	% Split nuts	Total number of NOW	% NOW infestation
May	5195	28.91	28	1.86a
June	3681	30.51	20	1.78a
July	8484	23.66	23	1.15a
August	4980	31.71	15	0.95a
September	4815	27.6	11	0.83a
Total	27155	28.48	97	1.31

4. Total navel orangeworm infestations of mummy nuts during 2008 and 2009

There was a significant difference (df=27; MSE=0.4443441; P<0.05) between the mean percentages of infestations of Sirora compared to Ariyeh and Shufra. The NOW infestation of Sirora over two years was the highest of all three cultivars (1.116%). Ariyeh had the second highest infestation (0.301%), followed by Shufra with the lowest infestation (0.102%) (Fig. 10).

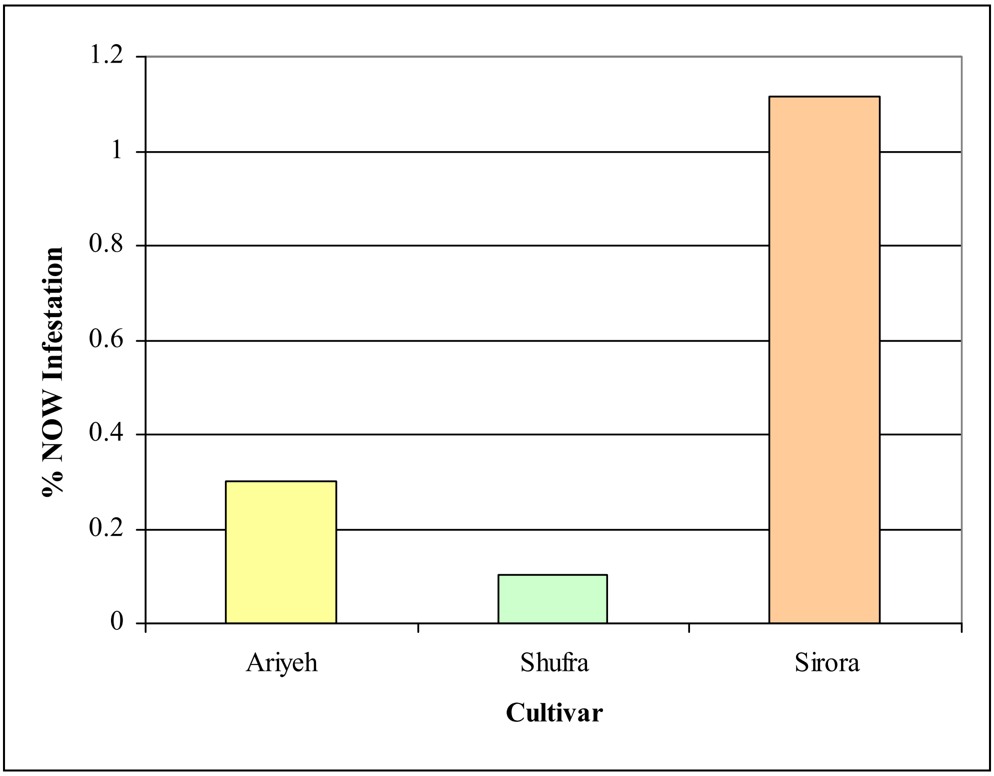

Figure 10. Total navel orangeworm infestation of all cultivars at green Valley Nuts in 2008 and 2009.

There was no significant difference (df=25; MSE=0.6868033; P>0.05) between the means of NOW infestations for the different months (Table 6). A total of 226 NOW was recorded infesting 94244 mummy nuts sampled for the three cultivars in 2008 and 2009. This provides a total NOW infestation of 0.8% over a period of two years.

From these results it can be seen that the combination of hydro-cooling, alley crops and mulch most probably exert a suppressing effect on NOW development and survival. Sirora, the orchard with no hydro-cooling and alley-crops, had a higher NOW infestation of mummies sampled from the ground than the other two orchards. This can be ascribed to hydro-cooling that cause higher humidity which increases the decomposition of mummies on the ground, leading to increased NOW mortality. According to Sibbett & Van Steenwyk (1993), NOW survival is low in mummy nuts remaining in a weedy cover throughout winter. Alley crops retain moisture in the orchard floor environment, thereby contributing towards the rotting process of the mummy nuts prone on the surface.

Table 6. Navel orangeworm infestation recorded in mummies sampled under trees of all cultivars at Green Valley Nuts in 2008 and 2009. Means of navel orangeworm infestation followed by the same letter are not significantly different at P≤0.05.

Month	**Total number of mummies**	**% Split nuts**	**Total number of NOW**	**% NOW**
May	13905	34.04	36	0.76a
June	27954	29.38	96	1.17a
July	29277	30.54	55	0.62a
August	12121	29.73	22	0.61a
September	10987	25.11	17	0.62a
Total	94244	29.98	226	0.8

Mulch can have a similar decreasing effect on NOW by maintaining moist conditions that increase mummy decomposition, but only when the nuts are buried underneath the mulch. The nuts that fall from the trees after the mulch has been placed in orchards will not decompose so fast, leading to the survival of NOW from infested nuts. This makes it necessary to regularly turn the mulch in order to make sure no nuts remain on the surface.

5. Mummy samples from trees

In 2008 a sufficient number of mummy nuts were obtained from Ariyeh and Sirora trees. These nuts had a NOW infestation of 1.65% and 1.93% respectively. In 2009 none of the cultivars had any mummies present on the trees and nuts were obtained from the processing plant to represent NOW presence of nuts on trees. From these samples Ariyeh had the highest NOW infestation (10.34%), Sirora 6.4% and Shufra 1.43%. When comparing the infestation percentages of the mummy nuts on the trees with those on the orchard floor, it can be seen that when mummy nuts are present on the trees it seems to have a higher infestation with NOW than mummy nuts lying on ground. This can be because mummies on the ground have a higher decaying tempo, which leads to more navel orangeworm mortality.

6. Orchard monitoring

From nuts randomly sampled in orchards across the GVN estate, there was a significant difference (df=12; MSE=15.50244; P<0.05) between the mean percentages of infestations of Sirora compared to Ariyeh and Shufra. Sirora again had the highest NOW presence at 7.77%, Ariyeh 2.87% and Shufra 1.19% (Fig. 11), indicating that the cultivar without hydro-cooling and alley crops once again had the highest NOW presence. The sampling of these mummy nuts probably reiterate that hydro-cooling and alley crops have a suppressing effect on NOW.

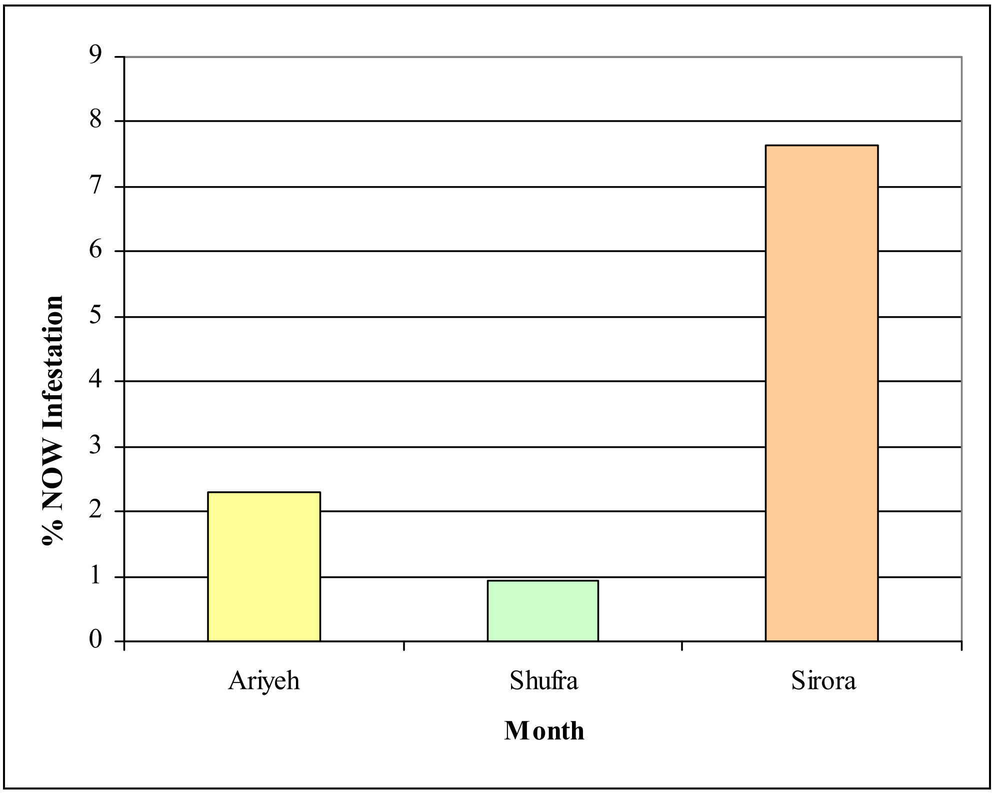

Figure 11. Total navel orangeworm infestation from random orchard monitoring of all cultivars at Green Valley Nuts in 2008 and 2009.

There was no significant difference (df=10; MSE=19.10917; P>0.05) between the means of NOW infestations for the different months that random orchard monitoring was conducted (Table 7). The highest NOW occurrence when all cultivar data was pooled was recorded during April (8.5%), after which the NOW incidence decreased until it reached 1.86% in September (Fig. 12). The presence of NOW in the nuts emanating from these samples reflects a more realistic degree of infestation that can be found in pistachio mummy nuts under field conditions. Generally it would seem that the number of larvae recorded in mummy nuts starts decreasing from August onwards when temperatures start rising and NOW development shifts to the next stage of the life cycle.

Table 7. General orchard monitoring at Green Valley Nuts 2009.

Cultivar	Month	Total number of mummies	Total number of NOW	% NOW infestation
Ariyeh	March	150	0	0
	April	750	47	6.27
	May	540	6	1.11
	August	1440	54	3.75
	September	990	4	0.40
	Total	**3870**	**111**	**2.87**
Shufra	March	120	0	0
	April	900	26	2.89
	May	570	1	0.18
	August	1380	21	1.52
	September	1140	1	0.09
	Total	**4110**	**49**	**1.19**
Sirora	March	270	0	0
	April	600	98	16.33
	May	480	51	10.63
	August	1140	70	6.14
	September	960	49	5.10
	Total	**3450**	**268**	**7.77**

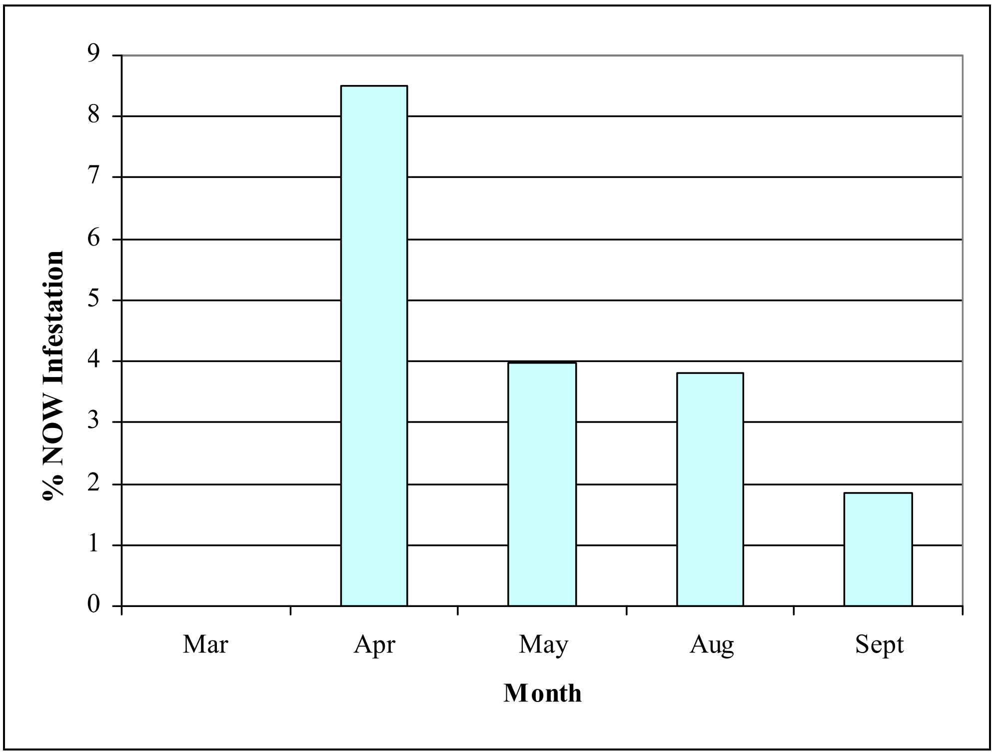

Figure 12. Total navel orangeworm infestation for all cultivars, recorded during random orchard monitoring at Green Valley Nuts during 2009.

CONCLUSION

At GVN mummy nuts were recorded to be a source of shelter for overwintering navel orangeworm (NOW) throughout the winter months. The infestation percentage ranged from very low in Shufra, a pistachio orchard with alley crops and hydro-cooling, to a higher infestation in Sirora, an orchard without alley crops and hydro-cooling. This study therefore provides information regarding the potential effect that alley crops and hydro-cooling can have on the survival of overwintering NOW in pistachio orchards in particular and other nut orchards in general. When mummy nuts are present on trees, it is recorded to have a higher infestation with NOW than mummy nuts lying on ground. This can be because mummies on the ground have a higher decaying tempo, which leads to more navel orangeworm mortality. It is therefore important in post harvest sanitation practices to not only remove mummy nuts from the soil surface, but to remove nuts from trees as well. Orchard row management practices such as alley cropping can keep NOW infestations in mummy nuts on the ground at low densities, but it does not seem to annihilate the population completely since it still provides an overwintering site for enough NOW to survive through the winter to give rise to a new generation in spring.

REFERENCE

BURKS, C.S., HIGBEE, B.S., BRANDL, D.G. & MACKEY, B.E. 2008. Sampling and pheromone trapping for comparison of abundance of *Amyelois transitella* in almonds and pistachios. *Entomologia Experimentalis et Applicata* **129**: 66–76.

GOOGLE INC. (2010). Google Earth (Version 5.1.3533.1731) [Software]. Available from http://earth.google.com/download-earth.html

MOSZ, N. 2002. Pistachio timeline. Available online: http://pestdata.ncsu.edu/cropTimelines/pdf/CApistachio.pdf

RICE, R.E., BENTLEY, W.J. & BEEDE, R.H. 1988. *Insect and mite pests of pistachios in California.* University of California, Oakland.

SIBBETT, G.S. & VAN STEENWYK, R.A. 1993. Shredding “mummy” walnuts is key to destroying navel orangeworm in winter. *California Agriculture* **47**: 26-28.

SIEGEL, J.P., KUENEN, L.P.S., HIGBEE, B.S., NOBLE, P., GILL, R., YOKOTA, G.Y., KRUGNER, R. & DAANE, K.M. 2008. Postharvest survival of navel orangeworm assessed in pistachios. *California Agriculture* **62**: 30-35.

SIEGEL, J., LACEY, L.A., FRITTS, J.R., HIGBEE, B.S. & NOBLE, P. 2004. Use of steinernematid nematodes for post harvest control of navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in fallen pistachios. *Biological Control* **30**: 410–417.

SHELTON, M.D. & DAVIS, D.W. 1994. Navel Orangeworm (Lepidoptera: Pyralidae) Development in Sunburned Walnuts. *Journal of Economic Entomology*. **87**: 1062-1069.

SUMMERS, F.M. & PRICE, D.W. 1964. Control of navel orangeworm. *California Agriculture* **18**: 14-16.

WANG, S., JOHNSON, J.A., TANG, J. & YIN, X. 2005. Heating condition effects on thermal resistance of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *Journal of Stored Products Research* **41**: 469–478.

CHAPTER 4

Life cycle of
the navel orangeworm,
***Amyelois transitella* (Pyralidae)**

<u>LIFE CYCLE OF THE NAVEL ORANGEWORM, *AMYELOIS TRANSITELLA* (PYRALIDAE)</u>

ABSTRACT

The navel orangeworm, *Amyelois transitella* (Walker), is an important pest of pistachios, almonds and walnuts. The pistachio industry has only relatively recently been initiated in South Africa at Green Valley Nuts in the Northern Cape Province. Research was conducted to observe the life cycle and behaviour of South African populations of the pest under laboratory conditions. Live larvae were dissected from mummy nuts from the field and maintained on pistachios until adult emergence. In the lab, moths were reluctant to mate under laboratory conditions and ultimately only two female moths laid 124 eggs in total. Egg incubation lasted two to three days before larval eclosion. Larvae went through five instars, each of which lasted between 7 - 15 days. Adult eclosion from pupae occurred after 8 - 14 days. Mortality was recorded to be the highest in the pupal stage. The complete life cycle duration of the navel orangeworm ranged from 50 to 84 days under labortory conditions. Only a single life cycle which gave rise to a next generation was tracked.

INTRODUCTION

The navel orangeworm (NOW), *Amyelois transitella* (Walker), is an important pest of pistachios and other tree nut crops such as almonds and walnuts (Rice, Bentley & Beede, 1988). NOW is indigenous to the southwestern United Sates and Mexico, and the moth was first described in 1915 from specimens collected in Mexico (Wade, 1961; Rice, Barnett & Van Steenwyk, 1996).

The development of a pistachio industry in South Africa is a relatively recent phenomenon. By 2007, approximately 1000 ha of pistachio trees have been planted at Green Valley Nuts in the Northern Cape Province. NOW was recorded in the orchards and although it is kept under control through field sanitation strategies and chemical application, it has the potential to establish itself as a serious primary pest.

Wade (1961) published the biology of NOW when this species showed signs of becoming a nut pest in northern California. He conducted investigations to determine the habitat requirements and ecological factors that influence NOW behaviour and abundance. In addition Finney & Brinkman (1967) conducted research in California on the rearing of navel orangeworm in the laboratory to develop methodology and equipment to multiply NOW colonies in large numbers for biological control research.

Husseiny & Madsen (1964) reported that NOW can complete their life cycle in only 40 days under favourable conditions. Furthermore, these authors reported that NOW larvae apparently show no tendency to undergo a diapause phase, but their development will slow down in unfavourable conditions.

The current study reports on the life cycle of the navel orangeworm in South Africa in an attempt to better understand the behaviour and survival of this pest. Over the years numerous studies have been conducted on the development of NOW, but these studies were mostly conducted in California under northern hemisphere conditions (*viz* Wade, 1961; Husseiny *et al.*, 1964; Finney *et al.*, 1967; Engle & Barnes, 1983; Seaman & Barnes, 1984; Girling & Cardé, 2006; Parra-Pedrazzoli & Leal, 2006). This study is the first of its kind that deals with the life cycle of southern hemisphere NOW populations. Even though the life-cycle information was generated under laboratory conditions, the information will still contribute towards a better understanding of NOW biology, which in turn will be important in the development of improved management programmes for NOW on nut crops.

MATERIAL AND METHODS

Observations on NOW biology were conducted in the laboratory at the University of the Free State in Bloemfontein, South Africa. The study was carried out from October 2009 to January 2010, with a room temperature of 28ºC during this period.

Larvae to initiate a colony were obtained from infested mummy nuts collected at orchards at Green Valley Nuts from May to August 2009. These larvae were maintained on pistachio kernel pieces in Petri dishes at room temperature until pupation and subsequent eclosion of the first adults during October.

On a daily basis the newly emerged moths were carefully removed from Petri dishes with a soft forcep to avoid injury. All the moths were placed in a larger white plastic container covered with gauze to allow ventilation and provided with a cotton ball soaked in a 10% honey solution (Fig. 1). Female moths oviposited on paper towelling placed in the base of the container. The total number of eggs laid was counted, the date noted and orange coloured fertilized eggs were transferred to Petri dishes containing moist paper towel on the base.

Newly hatched larvae were transferred with a brush to Petri dishes with moist paper towel and crushed pistachio nuts. At first, about 10 larvae were placed together in a Petri dish. These larvae were monitored closely for the onset of moulting to determine instars. After they reached second instar, the larvae were removed and placed in separate Petri dishes at a ratio of one larva per dish and monitored through the instar stages up until pupation (Fig. 2). Close scrutiny was necessary here since the larvae become cannibalistic if kept together in the close quarters of the Petri dishes.

Figure 1. Navel orangeworm moths in a container with paper towelling and diluted honey feeding source. Insert shows the container lid with gauze covering.

Pupae were carefully removed from the loose silken cocoons because the moths had trouble emerging from the cocoons under laboratory conditions. The pupae were placed in Petri dishes until adult eclosion. Data on duration of each stage in the life cycle were recorded for one generation. All the containers harbouring adults, pupae and eggs were also placed under a light during the day with the hope that extended light cycles would enhance development. Paper towelling placed in the Petri dishes was also moistened daily to maintain a relatively high humidity level.

Figure 2. A navel orangeworm larva on moistened paper towel in a Petri dish with pistachio nut kernels as food resource.

RESULTS AND DISCUSSION

1. Life cycle

The complete navel orangeworm life cycle from egg to adult stage under unnatural conditions in the laboratory took an average of 50 to 84 days to complete (Fig. 3). The majority of individuals completed their life cycle in approximately 55 days. Figure 4 shows the number of individuals that progressed from one stage of the life cycle to the next. The colony was established from 124 eggs that ultimately yielded 78 larvae, 69 pupae and 36 moths. The mortality rate was calculated for eggs, larvae and pupae that did not develop to the next stage in the life cycle (Table 1). Pupae were recorded to depict the highest mortality at 48% and approximately 19% of the moths that did emerge were incapable of proper maturation and subsequent reproduction due to deformities (Fig. 5). The reason(s) for these deformities are unclear, but it could be possible that conditions in the container were not sufficient to allow proper wingspread and sclerotization following eclosion from the pupae. This phenomenon demonstrates a degree of sensitivity immediately after adult eclosion and is probably compensated for by secluded hatching shelters under natural conditions. A life cycle breakdown per life stage that provides more detail is provided below.

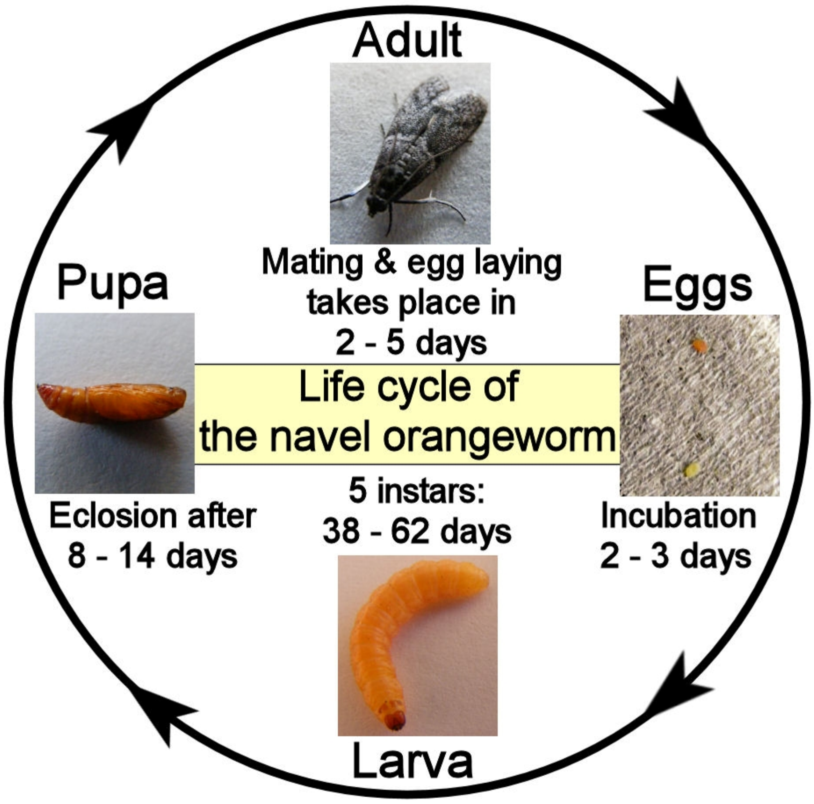

Figure 3. The life cycle of the navel orangeworm under laboratory conditions.

Table 1. Duration and mortality rate of navel orangeworm life cycle stages under laboratory conditions.

Stage in life cycle	Duration (days)	Average duration	Mortality (%)
Eggs	2-3	2	37
Larvae	38-62	57	12
Pupae	8-14	10	48
Adults	2-5	3	

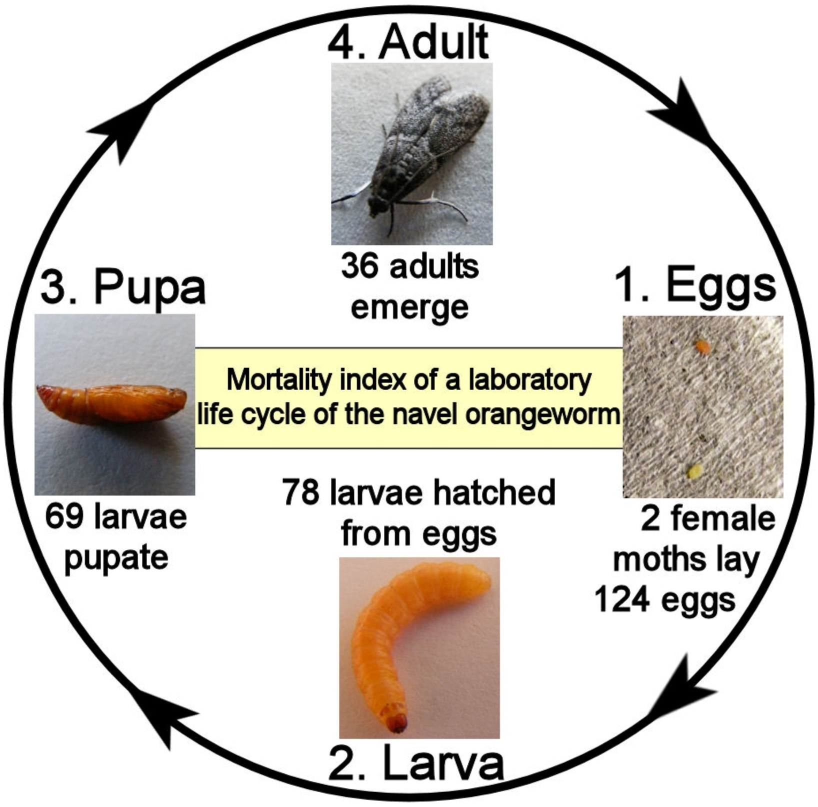

Figure 4. Mortality index of a laboratory life cycle of the navel orangeworm, indicating the numbers of individuals during each stage as the life cycle progressed (Month and year – Month and year).

Figure 5. Deformed post-eclosion navel orangeworm moth.

All NOW collected in the field to initiate the colony were recorded to overwinter as 3rd or 4th instar larvae. No pupae were recorded during this sampling period from May to August, demonstrating that NOW prefers to overwinter in the larval stage.

Other studies report that the duration of NOW life cycle ranges from 54 to 90 days (Wade, 1961) and from 35 to 65 days (Husseiny *et al.*, 1964), under conditions more or less similar to the present study. The outcome of the present study is therefore reasonably accurate and more or less in agreement. Exactly how similar or different the life cycle will be under natural southern hemisphere conditions remain unanswered and will be the focus of future research.

2. Eggs

The oval eggs are a creamy white in colour when oviposited and darken to a reddish orange colour after a few hours (Fig. 6). Unfertilized eggs stay creamy white in colour. During this study a total of 124 eggs were laid, of which 78 hatched. The period of incubation lasted between 2 to 3 days (Table 1). The incubation period of the eggs are determined by temperature, as temperatures too low or high can cause failing in the hatching of eggs (Wade, 1961).

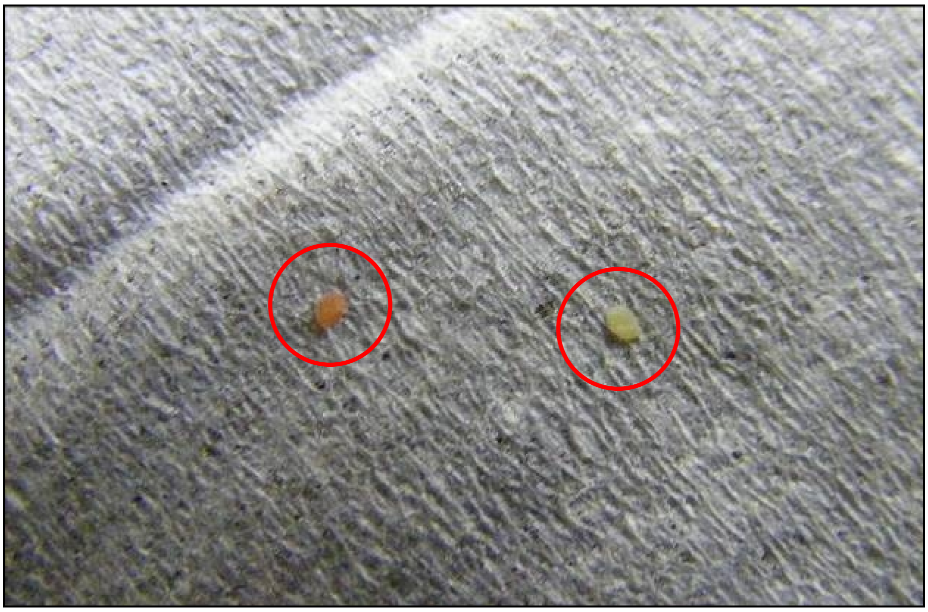

Figure 6. Navel orangeworm eggs, showing a fertilized egg on the left and an unfertilized one on the right.

3. Larvae

The first instar of the newly hatched larvae is a creamy orange at first and darkens to a bright orange as instars progressed. According to Wade (1961), the colour of the larvae is influenced by the food they eat. It was observed that the intestinal tract of larvae feeding on necrotic pistachio kernels will appear black (Fig. 7).

Figure 7. A navel orangeworm larva recorded feeding on a necrotic pistachio kernel. The black alimentary canal is clearly visible.

From the first instar onwards the larvae move around actively in search of food (*e.g.* Fig. 8). Most mortality of larvae occurred in the first and second instar. Each instar period lasted from 7 to 15 days and the total larval period ranged from 38 to 62 days (Table 1), after which 69 of the larvae spun a loose cocoon (Fig. 9) and pupated. The number of instars usually vary from five to seven (Wade, 1961), although in this study there was only five instars.

4. Pupae

Newly formed pupae are yellow-brown in colour, but start to darken after the second day until they reach a dark brown to brownish black colour by the time the adults start to emerge (Fig. 10). Pupae on days six and seven almost resemble the younger two day old pupae, but can be distinguished by having darker sclerotized eyespots when compared to younger pupae.

The average pupal period ranged from 8 to 14 days (Table 1). Pupae were removed from the cocoons, since it was found that the laboratory reared moths have trouble emerging. Some moths would become entangled with their cocoon, causing damage to the fragile wings. This would probably not be a problem under natural conditions, as the larvae would be able to spin proper cocoons from which they can escape. The confined space available makes this difficult for larvae under laboratory conditions. The cocoons are very delicate and removing the pupae would not have an influence on the life cycle. Only 36 moths emerged from the pupae. Seven of these moths were malformed and were unable to reproduce. They were removed and not used further in the study.

Figure 8. A newly hatched navel orangeworm larva moving around between crushed pistachio kernels.

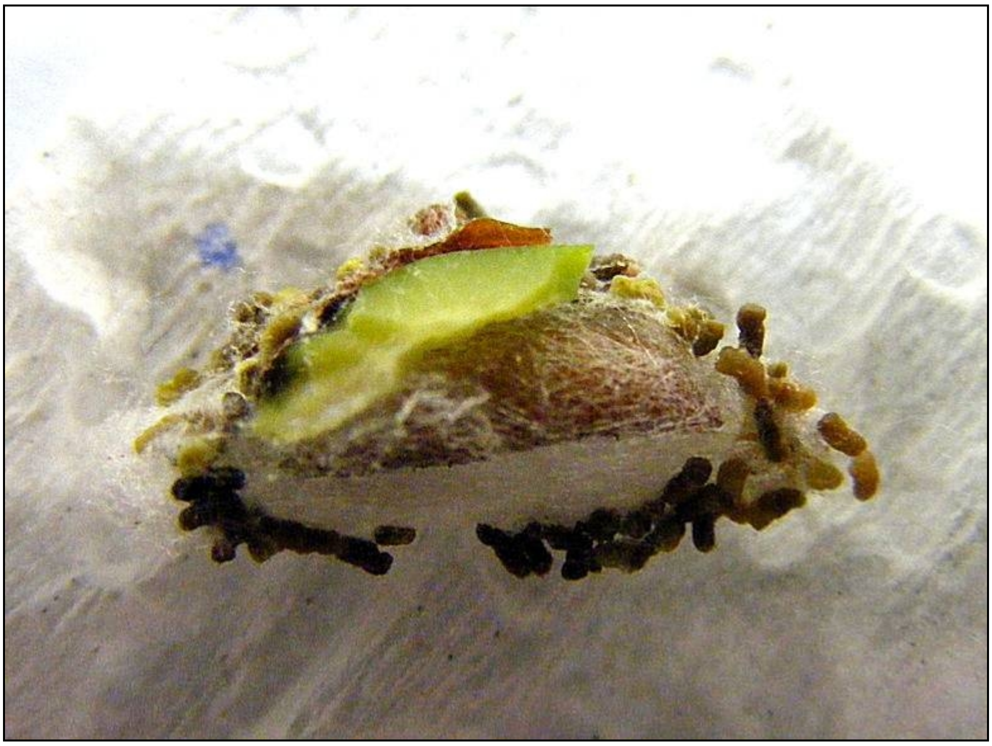

Figure 9. A navel orangeworm pupa in a cocoon. It is surrounded by frass and a piece of pistachio nut kernel.

Figure 10. Colour transformation of navel orangeworm pupae over 10 days.

5. Adults

The moths are greyish in colour with transverse zig-zig banding and black markings on the folded forewings. Hind wings are uniformly light brown in colour (Fig. 11). Females are slightly larger than males and females were more abundant, with a male to female sex ratio of 1:3 in this specific laboratory trial. Average longevity of the moths ranged from 2 to 5 days (Table 1). According to Wade (1961), the average life span of adults range from 11 to 12 days, depending on conditions. Adults have a tendency to live longest at 25°C with a 75% relative humidity. Higher temperatures cause adults to perish sooner.

In this study it was found that the moths were reluctant to mate under laboratory conditions. Once placed inside the container, they would remain inactive after they settled on the bottom or sides of the container. According to Wade (1961), copulation usually took place within the first two days after emergence and none after four days, even if the moths live longer than four days. Mating would also occur more frequently in larger cages and the use of larger cages might therefore favour an increase in this incidence.

Figure 11. A newly emerged female navel orangeworm moth. The uniformly lighter coloured hind wings are visible under the forewings.

The moths are nocturnal and show almost no activity during the day. It was observed that adult eclosion always occurred in the late afternoon. Courtship behaviour and copulation wasn't observed and is believed that this took place in the early morning hours when no observations were made. According to observations made by Parra-Pedrazzoli & Leal (2006), copulation took place between 7 AM and 8:30 AM PST, during the last hour of the scotophase and the first 30 minutes of the photophase. Finney *et al.* (1967) found that navel orangeworm mating rarely occurs under lighted up conditions and that turning on a strong light disrupts oviposition.

Some of the adults that died were removed from the container and kept separate in a clean Petri dish. An interesting observation was made after about a month (in January 2010) when larvae were discovered in the Petri dish feeding on the remains of these dead moths (Fig. 12). Some larvae had already developed as far as 4th instar. These larvae must have hatched from fertilized eggs that were never oviposited. It indicates that even if only two females laid eggs, other females did mate and develop fertilized eggs in their ovaria, but failed to lay eggs before they died.

Figure 12. Navel orangeworm larvae that hatched from and survived on female cadavers.

In the process of tracking the navel orangeworm life cycle it was found that even though female moths were not very keen to lay eggs, it was possible to hatch the eggs and nurse the larvae through to pupal stage in order to complete the life cycle. According to Wade (1961), mating under laboratory conditions is very sporadic, while mating outdoors are more frequent. Therefore it might be more successful to do further studies on the navel orangeworm life cycle under conditions more similar to the field.

CONCLUSION

The complete life cycle and a single generation of the navel orangeworm were successfully reared on pistachio kernels in the laboratory. These studies revealed that under laboratory conditions at room temperature of 28°C the life cycle can be completed in 50 to 84 days. With this as background it is postulated that under optimal conditions from September onwards the navel orangeworm can complete up to four generations during a nut development cycle. This equates to rapid population increase in an orchard with probable large scale damage ramifications.

REFERENCES

ENGLE, C.E. & BARNES, M.M. 1983. Developmental threshold temperature and heat unit accumulation required for egg hatch of navel orangeworm (Lepidoptera: Pyralidae). *Environmental Entomology* **12**: 1215-1217.

FINNEY, G.L. & BRINKMAN, D. 1967. Rearing the navel orangeworm in the laboratory. *Journal of Economic Entomology* **60**: 1109-1111.

GIRLING, R.D. & CARDÉ, R.T. 2006. Analysis of the courtship behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), with a commentary on methods for the analysis of sequences of behavioral transitions. *Journal of Insect Behavior* **19**: 4497-4520.

HUSSEINY, M.M. & MADSEN, H.F. 1964. Sterilization of the navel orangeworm, *Paramyelois transitella* (Walker), by gamma radiation (Lepidoptera: Phycitidae). *Hilgardia* **36**: 113-137.

PARRA-PEDRAZZOLI, A.L. & LEAL, W.S. 2006 Sexual behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *Neotropical Entomology* **35**: 769-774.

RICE, R.E., BENTLEY, W.J. & BEEDE, R.H. 1988. *Insect and mite pests of pistachios in California*. University of California, Oakland.

RICE, R.E., BARNETT, W.W. & VAN STEENWYK, R.A. 1996. Chapter 29: Insect and mite pests, pp. 202 - 205. **In** Micke, W. (ed.) *Almond production manual* Division of Agriculture and Natural Resources, California.

SEAMAN, W.S. & BARNES, M.M. 1984. Thermal summation for the development of the navel orangeworm in almond (Lepidoptera: Pyralidae). *Environmental Entomology* **13**: 81-85.

WADE, W.H. 1961. Biology of the navel orangeworm, *Paramyelois transitella* (Walker), on almonds and walnuts in northern California. *Hilgardia* **31**: 131-171.

CHAPTER 5

General discussion

GENERAL DISCUSSION

1. Summary

1.1. Green Valley Nuts (GVN), located near Prieska in the Northern Cape Province, is the first commercial pistachio farm to be established in South Africa. By 2007, approximately 1000ha of pistachio trees have been planted there. Even though the navel orangeworm populations are kept under control through field sanitation and chemical application, it has the potential to establish itself as a serious primary pest.

1.2. The navel orangeworm is endemic species to southwestern United States and Mexico, first recorded infesting rotting navel oranges in Arizona during 1921. This pest causes great damage to mature almonds, walnuts and pistachios. Pistachio nuts are infested by navel orangeworm larvae during and after hull split occurs. The larvae cause direct damage by feeding and boring into individual kernels, while contaminating the nuts with frass and webbing. Furthermore, the damaged nuts are also more susceptible to aflatoxin contamination. Reduction of navel orangeworm infestations will therefore have a positive influence regarding crop damage and food sanitation concerns.

1.3. The navel orangeworm overwinters in various larval stages or as pupae in mummy nuts remaining behind in the orchard. The overwintering stages complete development when the temperature starts to rise. There are between three and five overlapping generations a year, with no diapause in the life cycle. Mummy nuts are the only known source of shelter and sustenance for immature overwintering navel orangeworm, and these nuts are an important resource for navel orangeworm survival throughout most of the year.

1.4. The use of orchard row management practices helps to fulfill an important role in orchards by decreasing erosion, improving soil structure and contributing towards pest management. Orchard floor management practices that are used at GVN include cover crop alleys and mulch under trees. Hydro-cooling, an irrigation system that reduces plant heat stress during warm times of the year, was also present in some of the orchards. An experiment using emergence cages in the field was run during 2008 and 2009. No adult emergence was observed for any of the cultivars, but it is strongly suspected that these practices could have had an effect on navel orangeworm survival in mummy nuts.

1.5. Orchard monitoring was conducted in the winter seasons of both 2008 and 2009. This monitoring involved the collecting of mummy nuts left behind in the orchard after harvest. Mummy nuts were collected from the orchard floor and the trees. It was found that the pistachio cultivar Sirora had the highest navel orangeworm infestation in mummies sampled from the soil surface, when compared to the other two cultivars, Ariyeh and Shufra. Sirora was the only cultivar that had no hydro-cooling and alley crops, thus rendering conditions more favourable for navel orangeworm survival. The other two cultivars experienced hydro-cooling, alley crops and mulch, which can possibly have a suppressing effect on navel orangeworm development and survival. Orchard row management practices seem to keep navel orangeworm populations in mummy nuts on the ground at low densities, but it does not eliminate the population completely. Enough navel orangeworm are able to overwinter in mummy nuts to give rise to a viable new generation in spring.

1.6. Mummy nuts collected from trees were found to have higher navel orangeworm infestations than nuts on the ground. This can be because nuts lying on the ground are more susceptible to decay due to higher humidity, leading to increased navel orangeworm mortality.

1.7. Overall NOW incidence was low in the pistachio orchards at GVN. However, across the farm the trees reaching nut-bearing age are in the minority and actual harvesting has only been conducted for the last four years. Therefore NOW infestation in this context has a completely different meaning. In this regard infestation as such is important, irrespective of levels of infestation, since a steady increase can be expected over time which will have to be considered in future orchard management planning. What these results therefore contribute, is that it sends out an early warning signal so that proactive NOW management tactics can implemented.

1.8. One generation of navel orangeworm was successfully reared on pistachio kernels in the laboratory. It was recorded that the life cycle can be completed in 50 to 84 days under laboratory conditions at 28˚C. Under optimal conditions from September onwards the navel orangeworm can therefore probably complete up to four generations during a nut development cycle. This may lead to a rapid increase of populations in orchards, with probable large scale damage ramifications.

2. Recommendations

Concentrating solely on a chemical control program will not be efficient for navel orangeworm eradication in pistachio (and other nut) orchards. The most effective method for the management of the navel orangeworm is to rather concentrate on a combination of cultural control practices, supplemented with an extremely diligent insecticide spraying programme. Early harvest and regular and accurate sanitation practices, especially the removal of mummy nuts and other orchard debris that act as nutrition and refugia resources for overwintering navel orangeworm, are strongly recommended. It is also important in post harvest sanitation practices to not only remove mummy nuts from the ground surface, but to remove nuts from trees as well. This waste material must be burned and not dumped.

3. Future research

Mummy nuts were confirmed to be an important source of nutrition for navel orangeworm. Orchard row management practices revealed the likelihood of having an impact on navel orangeworm survival and a regular monitoring programme will be required to resolve this issue. However, more research under variable orchards conditions are required to establish a reliable model for navel orangeworm management in South Africa.

Previous studies conducted on the life cycle of the navel orangeworm were mostly carried out in California under northern hemisphere conditions. More information pertaining to the life cycle of navel orangeworm populations under natural South African conditions will be necessary in the development of improved management programs for local nut crops.