ATELOCERA RAPTORIA (HEMIPTERA: PENTATOMIDAE) ON PISTACHIO (ANACARDIACEAE: *PISTACIA VERA*) IN SOUTH AFRICA: IMPLEMENTING A PLANT HEALTH MANAGEMENT STRATEGY

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Submitted in fulfillment of the requirements for the degree

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DECEMBER 2007

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Acknowledgements

I would like to thank the following persons and institutions for their invaluable support and assistance throughout the course of this study, without which, it would not have been possible.

- Prof. Schalk vd M. Louw for his tremendous patience, supervision, guidance and support throughout the better part of my studies.

- My parents, Joop and Valerie Fourie, for the many years of support, encouragement and trust.

- The staff of Green Valley Nuts, especially Anton and Kevin, for the invaluable assistance during the studies and for their hospitality.

- Also, my two brothers, Philip and Johan, and my little sister Leana, for always being there.

- All the personnel, colleagues and friends from the Department of Zoology and Entomology for the support, guidance and encouragement provided over the past years.

- The National Research Foundation (NRF) for the financial support provided over the course of my post-graduate studies.

CHAPTER 1

The impact of stinkbugs as primary pests in nut production systems

1. Introduction

Stinkbugs (Hemiptera: Pentatomidae) possess opisthognathous, piercing-sucking mouthparts and the majority of the family are phytophagous sapsuckers (Bevis, 1964). The Pentatomidae is one of the largest families within the Hemiptera and Panizzi, McPherson, James, Javahery, & McPherson (2000) state that of the estimated 36 000 plus species already described within the order, more than 4000 belong to the family Pentatomidae. In turn, the sub-family Pentatominae is the largest within the family and all of it's members are plant-feeders. Stinkbugs are easily recognized by their round or ovoid shape and five-segmented antennae. Stinkbugs are aptly named, as they are the most common of the true bugs to produce a disagreeable odor by means of scent glands that open in the region of the metacoxae. (Borror, Triplehorn, & Johnson, 1992; Panizzi *et al.,* 2000).

Most stinkbugs that are of economic importance belong to the sub-families Esiddinae and Pentatominae. However, the economic importance of these insects varies greatly from species to species and even within a species, depending on the plant that is under attack. Among the Pentatominae, *Nezara viridula* (Linnaeus) is perhaps the most notorious, since it is considered to be a major pest on a wide range of crops. (Panizzi *et al.,* 2000).

The focus of this study is the management of a stinkbug species on pistachio nuts in South Africa. South Africa has only fairly recently become a producer and exporter of various nut products. These include cashew nuts, coconuts, pecan nuts and macadamia nuts (DAIS, 2003). It wasn't until recently (1993) that pistachio orchards were established near Prieska in the Northern Cape Province (Haddad & Louw, 2006). Although it is a definite future prospect, there is no South African pistachio export market to date. In this review, which provides a background to stinkbug – nut crop interaction in a global perspective, the emphasis will be on three nut crops, *i.e.* macadamia, pecan, and pistachio. All three these crops are known to be significantly impacted by stink bug pests.

2. Macadamia

2.1. History

According to Annecke & Moran (1982), the macadamia, otherwise known as the Queensland nut (*Macadamia ternifolia* (Proteaceae)), originated in eastern Australia. Australian botanists discovered and named the macadamia nut in 1843, but it was only 125 years later, when it was successfully cultivated in Hawaii, that it actually became established as a tree crop. The first large-scale plantings were at Keauhou and Kona, where 7000 trees were established. Research on the cultivation of macadamias were undertaken by the Hawaii Agricultural Experiment Station (HAES) and the first processing factory in the country was established in 1931 (ARC, 1993). Subsequently, large plantations of macadamia have been established in the USA, Hawaii, Australia, Kenya and South Africa.

It is not known exactly when macadamia became established in South Africa, but officially the first seeds were imported by the Citrus and Subtropical Fruit Research Institute (CSFRI) in 1931. Most of the macadamia research in South Africa was conducted during the early 60's by the CSFRI and as a result, the industry is now well established in the country (ARC, 1993).

2.2. International production and export

Five countries are primarily responsible for most of the world's macadamia production and exportation. The top four producers during 2001/2002 were Australia (34 300 tons), United States (24 494 tons), South Africa (11 000 tons) and Guatemala (9 360 tons), with other key producers being Costa Rica and Kenya. During 2001/2002, total world production equaled 118 688 tons (Anon, 2002). However, Hawaii has also become a major producer of macadamias, producing an estimated total of 27 200 tons during the 2005/2006 season, making it one of the largest current producers of macadamia in the world (NASS, 2006).

Over the last few years world macadamia production and export figures fluctuated considerably, dropping significantly during 2002/2003, but beginning to rise again during 2003/2004. Especially in Australia, Guatemala and South Africa large production increases were observed. The reason for increases were the high market value of macadamia nuts and the need to diversify sources of agricultural income in developing countries (Anon., 2004). This led to an increase in plantings and production. Australia was expected to reach a maximum of 37 000 tons during 2003/2004 and more than 38 500 tons during 2004/2005. However, actual figures showed that Australia was the largest producer and exporter of macadamia and exported 28 000 tons during the 2003/2004 season. Production of Kenya and Guatemala was estimated at 6 500 and 10 290 tons respectively during 2003/2004.

In South Africa, specifically, production climbed steadily, reaching 16 000 tons in 2003/2004. Furthermore, South African plantings are expected to increase by 15 – 20% annually, which will result in production of more than 30 000 tons in 2010, which will make South Africa one of the largest producers of macadamia in the world. Overall the main reason for these dramatic increases in production are the commercial maturation of the orchards, mainly in developing countries (Anon., 2004).

As mentioned previously, Australia is the largest producer and exporter of macadamia and exported 28 000 tons during the 2003/2004 season. During 2003/2004 South Africa was the second largest exporter of macadamia nuts in the world, exporting 96% of it's total production (16 000 tons). The United States is a notable exporter of macadamia, but also the leading importer. Kenya exported approximately 6000 tons during 2003/2004 and Guatemala approximately 10 000 tons (Anon., 2004).

It thus becomes clear that macadamia production and export is a booming trade and very important agricultural industry in the world, especially in developing countries like South Africa, Kenya, Guatemala and Hawaii.

2.3. Pentatomidae pests of macadamia

2.3.1. Hawaii

According to Kawate & Tarutani (2006), one of the most important insect pests of macadamia cultivated in Hawaii is the southern green stinkbug (SGS) (*Nezara viridula*). However, the southern green stinkbug cannot survive on macadamia nuts alone and requires an alternate host as well. Moreover, there are differences in stinkbug infestations between orchard locations, but these differences can vary with environmental conditions. Kawate & Tarutani (2006) states that in the normally wetter growing areas, stinkbug damage has become serious in the last three years. Also, rains in late 2001 and early 2002 were followed by a return to drought-like conditions in the normally dry Kona growing areas and increased insect infestations. In 2002-2003, the Hawaii Agricultural Statistics Service reported unusually high levels of stinkbug damage in Kona, Ka'u and Kohala. These findings were also confirmed by Sugano & Mau (2002).

When feeding, the stink bug inserts its hollow needle-like mouthpart stylets into the nut and injects saliva straight into the nut kernel. Consequently, enzymes in the saliva liquefy the tissue around the tip of the mouthparts allowing the bug to consume the liquid. This feeding activity causes pitting of the kernel. SGS feeding on nuts that are not fully sized often results in premature abscission. Peak damage is normally observed from July through September but usually takes place earlier in the year. Damage to the kernels are cumulative and can be observed year-round (Sugano & Mau, 2002; Kawate & Tarutani, 2006).

Feeding by the stink bugs can cause premature nut drop and kernel damage. During the 2004-2005 growing season, 3.7% of the delivered crop was rejected by processors because of damage attributed to stinkbug feeding, representing a loss of $1.6 million and comprising 23.4% of all losses. During the 2001 season, losses of only $1 million, representing 18.9% of total losses,

was recorded. This indicates a clear upward curve where SGS damage is concerned (Sugano & Mau, 2002; Kawate & Tarutani, 2006)

During the 2005/2006 season, crop losses were estimated at 10.8 million British pounds or 16% of the total crop. Immature nuts ranked as the highest cause of losses at nearly 28%, followed by moldy or rotten nuts and losses due too stink bug feeding with 20% each (NASS, 2006).

2.3.2. Australia

As mentioned earlier, Australia is currently the largest producer and exporter of macadamias in the world. However, although Australia possesses several important macadamia pests, with two of the major pests being hemipteran, no species belonging to the family Pentatomidae are currently registered as pests on macadamia in Australia (Ironside, 1973; Swaine, Ironside & Corcoran, 1991).

2.3.3. United States

Very little research has been done concerning pentatomid pests in the United States. This seems to indicate that stink bugs are not considered a key-pest in the US. However, Malo & Campbell (2006) state that SGS can cause considerable damage at times by injuring very young fruit when the shell is still soft.

2.3.4. South Africa

As recently as 1982, it was reported by Annecke & Moran (1982), that insects were not seen as an important consideration in macadamia cultivation in South Africa and only short mention is made of the SGS as a potential pest.

However, barely a decade later, the situation changed dramatically, with various authors reporting heavy losses due to stink bug feeding activity. Currently, stinkbugs are the most important pests on macadamias in South

Africa, with damage being caused by an extended stinkbug complex consisting of at least 20 species (Froneman & De Villiers, 1993; van den Berg, 1995; van den Berg, Steyn & Greenland, 1999; DAIS, 2003). According to Froneman & De Villiers (1993), the most important species attacking macadamia in South Africa is the two-spotted stinkbug (*Bathycoelia rodhaini* Schouteden), the green vegetable stinkbug (*Nezara viridula*), the coconut stinkbug (*Pseudotheraptus wayi* Brown), the small green stinkbug (*Nezara* sp.), the yellow-edged stinkbug *(Nezara pallidoconspersa* Stål), and the yellow-spotted stinkbug (*Bathycoelia* sp.) These findings are also confirmed by the Directorate of Agricultural Information Services (DAIS, 2003).

When untreated, damage resulting from stinkbugs can result up to 80% of the entire crop lost. Overall damage is caused when the stinkbugs suck the sap from the flowers and young fruit. Under South African climatic conditions there are usually four stinkbug generations per year, with each generation causing a different type of damage to the nuts (Froneman & De Villiers, 1993; DAIS, 2003). Froneman & De Villiers (1993) & DAIS (2003) report that the first generation (Spring) occurs from August to September, during and after flowering. This generation can cause extensive flower and/or fruit drop of small macadamia fruit resulting in large losses.

The second generation occurs during the summer months (around December), and damage occurs during fruit development or just before the fruit reaches mature size. Once the fruit matures, they usually remain on the tree even after stinkbug feeding has occurred. However, when harvesting, these nuts will have large, sunken lesions on the kernels (Froneman & De Villiers, 1993; DAIS, 2003).

The third generation, or the autumn generation (February to March), is normally the largest of all the generations. Feeding will occur on the nuts before and during harvest. Although feeding causes lesions on the nut kernel, no fruit drop occurs as was the case during the summer and spring generations. The size of the lesions depends on the type of stinkbug. The coconut-, two-spotted-, yellow-spotted-, and spotted stinkbugs are capable of

inflicting damage late in the season because of their longer mouthparts. Less trouble is experienced from other stinkbugs during autumn (Froneman & De Villiers, 1993; DAIS, 2003).

The fourth and last generation stinkbugs (winter) usually does not cause problems because most nuts have already been harvested. Also, the stinkbugs are not very active during the colder season. Thus, the damage evident at the end of the season (stung nut kernels) is mostly inflicted from December up until harvest. Unfortunately, the hardness of the shell does not limit stinkbug feeding. Nuts must therefore be protected against stinkbugs throughout the year from flowering until harvest (Froneman & De Villiers, 1993; DAIS, 2003).

In a study conducted by van den Berg *et al.* (1999) on macadamia nuts in the Mpumalanga lowveld of South Africa, 4458 specimens of Hemiptera and 20 different pentatomid species were collected in an unsprayed orchard and two commercial macadamia orchards, both within 20 km of Nelspruit. It was found that the pentatomids *Bathycoelia natalicola* Schouteden (Two-spotted stinkbug) and *A. raptoria* (Powdery stinkbug) represented the highest percentage of all hemipteran specimens collected, representing 44.6% and 25.5% respectively. Other pentatomids collected represented only 7% in total.

It is therefore clear that stinkbugs, especially *N. viridula* and *B. natalicola*, appear to be serious pests on macadamia in especially South Africa, causing significant crop losses if not properly managed.

3. Pecan nuts

3.1. History

The pecan nut (*Carya illinoensis*: Juglandaceae) was originally indigenous to North America, where it grows wild in the states along the Gulf of Mexico and around the Great Lakes. The first pecan trees were imported into South Africa during the late 1800's by a Natalian nurseryman called Wilkinson. The first

grafted trees were imported in 1912 and initial plantings were made in the sub-tropical regions around Nelspruit. Currently, the southern lowveld of South Africa is the biggest production area in the country. Other important production areas include Tzaneen, Louis Trichardt, Kwazulu-Natal, the Vaalharts irrigation scheme, the middleveld around Pretoria and some parts along the Orange river (Oosthuizen, 1991).

3.2. International production and export

Currently, the largest producer of pecan nuts in the world is the United States. It is estimated that the US produces up to 75% of the world's pecan production per annum. In 2003, the United States produced in excess of 252 million pounds (114 545 metric tons), with an estimated value of $263 million per year. Also, unlike other tree nut production orchards that are mostly concentrated within specific regions, pecan production in the U.S. is dispersed throughout the South and South-West of the country (Hadjigeorgalis, Lilywhite & Herrera, 2005). The second largest producer of pecan nuts in the world is Mexico, producing an estimated average of 34 000 tons during the 2003/2004 season (Trejo & Hernandez, 2003). This constitutes around 20% of the world's pecan production. Other countries that are minor pecan producers include Australia, Israel, Peru and South Africa (Hadjigeorgalis *et al.,* 2005).

3.3. Pentatomidae pests of macadamia

3.3.1. United States

There are many insects that feed on the leaves, nuts and branches of pecan trees, reducing the tree's productive yield and potential. Only a few species of Pentatomidae are considered pests on pecan in the US (Table 1).

As is the case on macadamia nuts, these sucking insects feed on the developing nut kernels and cause an injury known as black pit. This may cause immature nuts to be aborted, resulting in large losses. Feeding occurring on mature nuts after the shells have hardened results in brown or

black spots on the kernels. These kernels will then also taste bitter (Knutson & Ree, 1997; Anon, 2000; Ree, 2003).

As adults, these bugs over-winter under fallen leaves and in other sheltered places on the ground. Adults lay eggs on many crops and weeds, where populations increase during the summer. Normally, pecans are only attacked by adult bugs, when these fly in from surrounding cover crops and weeds. Fields of soybeans, other legumes and sorghum may be the host-plant sources of the adults that fly to pecans. Infestations are usually greatest from September throughout the growing season. Several authors mention that the implementation of trap crops may aid in the management of these stinkbugs (Knutson & Ree, 1997; Anon, 2000; Ree, 2003).

Although stinkbugs are considered to be pests on pecan in the US, they do not appear to be one of the major pest groups. Of the five species mentioned by Ree (2003), only *N. viridula* (southern green stinkbug) and *Euschistus servus* (Brown stinkbug) seems to be of much importance (Ree, 1993; Anon, 2000). Not much research has thus far been conducted concerning stinkbugs on pecan, although a nationwide survey to determine the abundance and spread of two stinkbugs in pecan orchards, *i.e. N. viridula* and *E. servus*, is currently being conducted by the Texas Cooperative Extension in conjunction with Texas A&M University.

Table 1: Pentatomidae pests occurring on pecan nuts in the United States. Adapted from Ree, 2003.

Euschistus servus (Say)	Brown stink bug
Chlorochroa ligata (Say)	Conchuela stink bug
Euschistus tristigmus (Say)	Dusky stink bug
Acrosternum hilare (Say)	Green stink bug
Nezara viridula (Linnaeus)	Southern green stink bug

3.3.2. South Africa

In a study conducted by Joubert & Neethling (1994), it was stated that widespread damage to pecans grown in South Africa is caused annually by stinkbugs. It not only appears as if damage varies from location to location, but also between the different cultivars. Stinkbugs and other hemipterans cause damage to pecan kernels in more than one way. When nuts are attacked in the early water stage of development (before the shells harden), it often results in the premature dropping of nuts during the summer. The nuts may also be attacked during the later stages of development (after the shells harden), and this may cause dark-brown to black lesions on the kernels themselves. These lesions then also render the kernels useless in terms of commercial use (Joubert & Neethling, 1994).

During the study, Joubert & Neethling (1994) sampled 896 specimens belonging to the family Pentatomidae. Of all these, the powdery stinkbug, *Atelocera raptoria* represented the vast majority (86%) of all the specimens collected. The second most numerous species collected was the coconut stinkbug (*Pseudotheraptus wayi*), representing only 6%. Only 2.2% of all the pentatomids collected were found to be *N. viridula.* According to Joubert & Neethling (1994), the fact that such low numbers of *N. viridula* were recorded, seemed to indicate that it was unlikely for this species to be responsible for most of the kernel damage found on the nuts, as is the case in the US.

N. viridula is also named as a pest of pecan in South Africa by Annecke & Moran (1982), although little attention was given to this stinkbug. Annecke & Moran (1982) also states that the southern green stinkbug is not a serious pest in Southern Africa.

It is also mentioned by Joubert & Neethling (1994), that the most abundant species*, A. raptoria*, seemed to make little or no contribution towards kernel damage on the pecan nuts. It was concluded that a less abundant species, *P. wayi,* was probably responsible for the lesions found in the kernels due to the length of their mouthparts which were long enough to pierce even the

hardened shells of mature pecans. It was also strongly suggested at the time that the pecan industry of South Africa could benefit substantially from the development and implementation of control programs against selected stinkbugs on pecan nuts.

In this context neither the pecan cultivation manual released by the Agricultural Research Counsel (ARC, 1992), nor the manual for cultivating subtropical crops (DAIS, 2003) even mention any hemipteran pests on pecan in South Africa. The only sucking insect mentioned in these sources is the yellow pecan aphid and no mention is made of either of the stinkbugs *N. viridula* or *P. wayi*.

As the subtropical growers manual (DAIS, 2003) appears to be the most recent publication available on pecan production and pests, it would seem that stinkbugs do not play such a major role as pests of pecan nuts in South Africa. Moreover, stinkbugs did not develop into such a major problem as Joubert & Neethling (1993) feared would be the case.

4. Pistachio nuts

4.1 History

According to Janick (2002), pistachio is native to Western Asia, and has been widely cultivated since ancient times in the Middle East. There are more than a dozen species belonging to the genus , *Pistacia* (Anacardiaceae) , but only *P. vera* yields commercially acceptable edible fruits. In more recent times, pistachio has been cultivated all over the world. However, in India and Afghanistan, nuts are still generally obtained from wild trees. Furthermore, the orchard cultivars of Iran, Turkey, and Italy differ little from wild pistachio populations. These cultivars were generally selected due to their large nut size. Also, an increasing number of pistachio orchards are still being established in the interior valleys of California, producing larger, better-flavoured nuts (Janick, 2002). Pistachio nut orchards have only recently been established in South Africa. Approximately 1000 hectares were planted at

Green Valley Nuts (GVN), a division of the Industrial Development Corporation, in the Northern Cape Province in 1993 (Louw, 2003; Haddad & Louw, 2006)).

4.2. International production and export

According to the United Nations' Food and Agriculture Organization (FAO), the top five pistachio producers in the calendar year 2002 were Iran at 300,000 metric tons (53% share of the world's production), followed by the United States (136,000 tons, 24% share), Syria (53,000 tons, 9% share), Turkey (40,000 tons, 7% share), and China (26,000 tons, 5% share). From 1963 to 2002, reported world pistachio production increased 2,264%, from 24,000 tons to 571,150 tons. (Anon, 2003).

In the export market, the US is the second largest exporter of pistachios in the world. During the 2002/2003 season, pistachio exports reached $99 million in the US alone. According to the Global Trade Atlas, Iran is still the leading exporter of pistachios, exporting over three times as much as its nearest competitor, the United States. This constitutes an almost $300 million industry in Iran (Anon, 2003). This industry is still in it's infancy in South Africa, and no significant export has been made thus far. Internationally, pistachio exports contributes $488 million of the world nut export market (Anon, 2003)

Clearly, this is a major industry in many countries, and a very important economical commodity for many smaller countries such as Iran, Greece, Syria, and Turkey.

4.3. Pentatomidae pests of pistachio

4.3.1. California

In the United States pistachio is almost exclusively cultivated in the state of California and most of the research on pistachio pests and diseases in this state is conducted by the University of California. Although many invertebrates

are registered as pests on pistachio in the US, only three species of Pentatomidae seem to be of much importance. These are the redshouldered stink bug (*Thyanta pallidovirens*), the green plant bug (*Chlorochroa uhleri*), and the green soldier bug (*Acrosternum hilare*) (Bentley, Beede & Daane*,* 2004). As is the case on pecan nuts, the stink bugs often complete their life-cycles in weeds or crop fields in close vicinity of the pistachio orchards, with the adults flying in from these sites. (Knutson & Ree, 1997; Anon, 2000; Ree, 2003). Another stink bug, the rough stink bug (*Brochymena quadripustulata*), is also common in pistachio orchards throughout the year. However, this stink bug is a beneficial predator and does not damage pistachio trees or nuts.

As is the case on both pecan and macadamia nuts, feeding on the nuts by these stink bugs cause kernel necrosis or pitting. This necrosis usually occurs after shell hardening has taken place. Usually, kernel damage is not obvious externally, but inside the nut, the nutmeat is darkened, often developing sunken lesions and has an off-flavour. During the early season, stinkbugs may cause epicarp lesions, but damaging populations are usually not observed until later the season. Also, stinkbugs are capable of transmitting some diseases like stigmatomycosis and shoot blight. (Bentley *et al.,* 2004).

4.3.2. Iran

Since Iran is the biggest producer and exporter of pistachio nuts in the world, pests capable of causing damage to the nuts are especially important. According to Mehrnejad (2001), there are several pests on pistachio in Iran that are considered widespread pistachio primary - pests. This group of pests is almost always found throughout the main pistachio-producing areas and usually induce significant damage on pistachio yields by attacking pistachio leaves, fruits, and branches.

Among this group of pests, several hemipteran species have been reported. These bugs cause significant damage by attacking young or adult pistachio fruits during the growing season and feeding directly on the pistachio kernel. The stink bugs, *including species such as Acrosternum heegeri* Fieber,

Acrosternum millieri Mulsant & Rey, *Apodiphus amygdalei* Germar, *Brachynema germari* Kolenati and *Brachynema segetum* Germari, which all belong to the family Pentatomidae, are abundant and serious pests of pistachio nuts in the pistachio plantation areas of the country (Mehrnejad, 2001). All of the above mentioned species are also generalist polyphagous feeders, occurring on a wide range of plants.

Damage occurs throughout the season, from early spring up until the time of harvest. Mehrnejad (2001) mentions that very young and immature nuts are attacked by the life stages of the bugs in the spring, causing epicarp lesions followed by nut drop. In this regard piercing of the soft-shelled pistachios by the stylets of stink and other sucking bugs is reported to cause necrotic lesions on the hull (epicarp and mesocarp). However, damage to the nuts usually begins to decline as the shells harden. In spite of this, epicarp lesions caused by stink bug feeding are considered as one of the most important problems in pistachio orchards in Iran. As shells mature and the kernel begins filling in midsummer, the stink bugs feed on the developing kernel through the shell, causing kernel necrosis or deformity up to harvest time. Kernel necrosis symptoms usually caused by pentatomid bugs can be identified as the indented brown to black spots on the kernel surface. (Mehrnejad, 2001). Mehrjenad (2001) also states that because of their wide host-range, adult dispersal habits and diverse habitats, this group of pistachio pests are very difficult to control.

4.3.3. Turkey

In Turkey, the cultivated pistachio areas has expanded greatly. Consequently, new areas possessing depauperate soils or that are otherwise less appropriate have been used to establish pistachio orchards. This has opened a niche for some new pests and diseases that were previously of minor importance or were not considered pests at all. Some native non-pest species became major pests on the newly established pistachio orchards (Yanik & Yücel, 1999).

During a study conducted by Yanik & Yücel (1999) on six different orchards situated in the Sanliurfa province of Turkey, 19 injurious species of invertebrates have been recorded. However, only one species belonging to the family Pentatomidae is mentioned. The species, *Dolycoris baccarum* (L), is named as the only stinkbug species found on pistachio in Turkey. Adults of this species have previously caused great damage to pistachio orchards in the Bozovo region. As was the case regarding stink bug damage in California and Iran, injury to the nuts is caused when adults feed on the immature green nuts, and on the panicle bases of these nuts. This causes fruit drop and the production of dark sticky exudates, followed by fruit drying (Yanik & Yücel, 1999).

4.3.4. South Africa

As mentioned earlier, the pistachio industry is still in it's infancy in South Africa, rendering these plantations the ideal habitat for translocated and endemic, naturally occurring pests. According to Louw (2003), the newly planted orchards in the harsh environment of Prieska, where Green Valley Nuts (GVN) is situated, provides a kind of "green oasis", which eventually results in the establishment of pest organisms in the orchards. Consequently, an in-depth insect bio-monitoring survey was conducted in the pistachio orchards, whereby potential pests and beneficial insects were identified, whilst seasonal distribution of all invertebrate species and also their trophic structure was determined.

Louw (2003), identified the Hemiptera as a group possessing potential pest species on pistachio. In particular, pentatomid bugs were found to be abundant in the orchards. Overall, 16 species of Pentatomidae were sampled and of these, *Atelocera raptoria* Germar dominated. Eventually Haddad & Louw (2006) were able to state that the Hemiptera are a key group of pests, with several species found in the pistachio canopies during sampling. Of these, only one species, the powdery stink-bug, *Atelocera raptoria*, occurred in sufficient densities to pose a major threat to production.

Atelocera raptoria is endemic to South Africa and has been reported on various other crops. Understanding the management of this species in terms of the environmental conditions where pistachio is cultivated in South Africa, is the thrust of this dissertation and will be treated in more detail in the following chapters.

5. References

Agricultural Research Council (ARC), 1993. *The cultivation of macadamia*. Institute for Tropical and Sub-tropical crops, Pretoria. 66 pp.

Annecke, D. P. & Moran, V. C., 1982. *Insects and ,mites of cultivated plants in South Africa.* Butterworth & Co, Pretoria. 383 pp.

Anonymous, 2000. *Pecan pest management handbook*. University of Georgia Entomology. Retrieved from http://www.gaipm.org/pecan/scout/html/stink.html

Anonymous, 2002. *Situation and outlook for macadamia nuts* - World Horticultural trade & U.S. Export Opportunities. Retrieved from http://www.fas.usda.gov/htp/Hort_Circular/2002/02-04/Stats/MAC.pdf

Anonymous, 2003. *World pistachio situation & outlook*. World Horticultural trade & U.S. Export Opportunities. Retrieved from http://www.fas.usda.gov/htp/Hort_Circular/2004/

Anonymous, 2004. *Macadamia situation and outlook in selected countries*. World Horticultural Trade & U.S. Export Opportunities. Retrieved from http://www.fas.usda.gov/htp/Hort_Circular/2004/

ARC, Institute for Tropical and Subtropical Crops. 1992. *The cultivation of pecan*. 54 pp

Bentley, W. J., Beede, R. H. & Daane, K. M. 2004. *UC IPM, Pest Management Guidelines: Pistachio.* University of California, Agriculture & Natural Resources. 28 pp.

Bevis, B. 1964. *The Insects of Southern Africa*. Nelson Publishers. 186pp.

Borror, D. J., Triplehorn, C. A. & Johnson, N. F. 1992. *An introduction to the study of insects*. Sixth Edition. Saunders Publishing, New York. 874 pp.

Directorate Agricultural Information Services (DAIS). 2003. *Cultivating Subtropical Crops*. Pretoria. 153 pp.

Froneman, I. J. & De Villiers, E. A. 1993. Stinkbugs, pp. 49 – 52. In: Anonymous (*Editors*) *The cultivation of macadamia.* Agricultural Research Council, Nelspruit.

Haddad, C. R. & Louw, S. vd M. 2006. Phenology and potential biological control of the stink-bug *Atelocera raptoria* Germar (Hemiptera: Pentatomidae) in pistachio orchards. *African Plant Protection* **12:** 23 – 27.

Hadjigeorgalis, E., Lillywhite, J. M. & Herrera, E. 2005. *International trade in pecans.* Cooperative Extension Service of College of Agriculture and Home Economics. New Mexico State University. Retrieved from http://cahe.nmsu.edu:16080/pubs/_z/Z-503.pdf

Janick, J. 2002. *Fruit & nut crops.* Tropical Horticulture, Purdue University. Retrieved from http://www.hort.purdue.edu/newcrop/tropical/lecture_34/fruits_nuts_R.html

Joubert, P. H. & Neethling, C. 1994. Kernel damage to pecan nuts in the Nelspruit area of South Africa. *Journal of the South African Society of Horticultural Science* **4:** 42–44

Ironside, D. A. 1973. Insect pests of macadamia. *Queensland Agricultural Journal* **99(5) :**241-250

Knutson, A. & Ree, B. 1997. *Managing insect and mite pests of commercial pecans in Texas.* Texas Agricultural Extension Service. Retrieved from http://insects.tamu.edu/extension/bulletins/b-1238a.html.

Kawate, M. K. & Tarutani, C. M. 2006. *Pest management strategic plan for macadamia nut production in Hawaii*. Macadamia nut PMSP. Workshop Summary. Honolulu.

Louw, S. vd M. 2003. *Pistachio Entomology at IDC – Green Valley Nuts: Present knowledge and future focus.* Report to Industrial Development Corporation, Green Valley Nuts, Prieska. 27 pp.

Malo, S. E. & Campbell, C. W. 2006. *The macadamia*. University of Florida IFAS extension. Retrieved from http://edis.ifas.ufl.edu/pdffiles/MG/MG05200.pdf

Mehrnejad, M. R. 2001. The current status of pistachio pests in Iran. *Cahiers Options Mediterraneenes* **56:** 315 – 322.

National Agricultural Statistics Service (NASS), 2006. *Hawaii macadamia nuts, final season estimates*. USDA, Hawaii field office. Retrieved from http://www.nass.usda.gov/hi/fruit/mac-fin.pdf.

Oosthuizen, J. H. 1991. Origin and production areas of the pecan nut. pp A1. In: Anonymous (*Editors*) *The cultivation of pecan*. ARC, Institute for Tropical and Subtropical Crops, Nelspruit.

Panizzi, A. R., McPherson, J. E., James, D. G., Javahery, M. & McPherson, M. 2000. Stink Bugs (Pentatomidae), pp. 421 - 474. In: Panizzi, A.R & Schaefer, C.W. *(Editors). Heteroptera of economic importance*. CRC Press, London.

Ree, B. 1993. Trap cropping for stink bugs get good results. *Pecan South* 25: 21 – 22. (Original article not seen).

Ree, B. 2003. *Pecan insect pests*. Pecan Kernel. Texas Cooperative Extension, Texas A&M University System. Retrieved from http://pecankernel.tamu.edu/pecan_insects/pests/index.html#pecan

Sugano, J. S. & Mau, R. F. L. 2002. *Crop profile for macadamia nut in Hawaii*. Retrieved from http://www.ipmcenters.org/cropprofiles/docs/HImacadamia.html

Swaine, G., Ironside, D.A. and R.J. Corcoran (1991). *Insect pests of fruit and vegetables.* Second edition. Queensland Department of Primary Industries Information Series Q191018, 126 pp.

Trejo, S. & Hernandez, G. 2003. *Mexico tree nuts.* Annual report. USDA Foreign Agricultural Service. Retrieved from http://www.fas.usda.gov/gainfiles/200308/145985812.pdf

Van den Berg, M. A. 1995. *Pests attacking macadamia in South Africa*. The 6th Conference of the Australasian Council on Tree and Nut Crops, Lismore, Australia.

Van den Berg, M. A., Steyn, W. P. & Greenland, J. 1999. Hemiptera occurring on macadamia in the Mpumulanga Lowveld of South Africa. *African Plant Protection* **5:** 89–92.

Yanik, E. & Yücel, A. 1999. *The pistachio (P. vera L.) pests, their population development and damage state in Sanliurfa province*. GREMPA seminar on pistachios and almonds, Turkey.

CHAPTER 2

The general bio-ecology of *Atelocera raptoria* (Hemiptera: Pentatomidae)

1. Introduction

1.1. Aim

To successfully manage *Atelocera raptoria* (Germar) by means of the implementation of plant health management strategies including degree-day models, the broader biological and ecological structure of the species must be understood. This is of primary importance, as almost no investigation in this regard has previously been conducted. Above-mentioned management practices include the construction and implementation of a degree-day model, pesticide bio-assessments and possible parasitoid applications. The life-cycle and number of instars, temperature thresholds and optimal developmental temperature and developmental time of instars should also be determined. Also, the presence, if any, of parasitoids attacking the egg packages had to be examined. During this study, these issues were addressed as fully as possible.

1.2 *Atelocera raptoria* as a pest in South Africa

In South Africa pistachio is attacked by a variety of insect pests which damage the leaves, nut-clusters or stems. Amongst these, various sap-sucking insects have been recorded. Of these, in turn, stinkbugs, (Hemiptera: Pentatomidae), appears to be the most abundant and most damaging. These insects usually attack the nut-clusters, penetrating the nut husks with their piercing-sucking mouthparts, causing necrosis or premature dropping of the nuts. Necrosis usually renders the kernels unusable for the commercial market. The study species, *Atelocera raptoria* (Germar), appears to be a key pest on Pistachio (Louw, 2003; Haddad, Louw & Dippenaar - Schoeman, 2004). This species also appears to be endemic to South Africa and has not been recorded as a plant pest anywhere else.

Limited literature on specifically *A. raptoria* is currently available. Also, this species has only been reported to be of economical importance on a few crops, primarily nuts. Joubert & Neethling (1994) first reported *A. raptoria* from

pecan nut orchards near Nelspruit. In this regard, they presumed that it was unlikely for *A. raptoria* to cause significant kernel damage to the nuts, despite the fact that this pentatomid was abundant during the entire monitoring period of the study. By inducing forced feeding of adult *A. raptoria*, it was found that feeding by this species may indeed cause premature dropping of nuts during the early stages of development, but also that the lesions caused by *A. raptoria* feeding were so small, that it was unlikely they would be observed on harvested nuts.

Van den Berg (1998) also reported *A. raptoria* to be abundant in avocado orchards at Nelspruit. During this study more than 20 species (602 individuals) of Hemiptera were sampled, of which the most abundant was *A. raptoria* which made up 42% of the total sample. Van den Berg (1998) also stated that *A. raptoria*, and the Coconut stinkbug, *Pseudotheraptus wayi* (Brown), are amongst the most important hemipteran species found on avocado in South Africa.

Based on these studies, *A. raptoria* was subsequently recognized as an important species of the sucking bug pest complex on avocado in South Africa. During a study conducted by Bruwer (2004), he stated that the sucking bug complex on avocado in South Africa causes massive economical losses and consists of at least six species, one of which is the powdery stinkbug, *A. raptoria.* Damage to the crops would include the dropping of flowers and fruit, the latter causing fruit lesions. Bruwer (2004) also stated that chemical control of this sucking bug complex was a necessity in many areas. Consequently, several “soft” pesticides were evaluated as part of an integrated pest management strategy for the management of the problem.

A. raptoria was also found to be abundant on macadamia nuts in the Mphumalanga lowveld district of South Africa. In a study conducted by Van den Berg, Steyn & Greenland (1999), 4458 specimens and 20 different pentatomid species were sampled from an unsprayed and untreated orchard as well as two treated commercial macadamia orchards, in the Nelspruit district. High numbers of *A. raptoria* were sampled during this study, with the

species *Bathycoeliae natalicola* and *A. raptoria* constituting 44.6% and 25.5% of the samples respectively. These authors also reported that *A. raptoria* adults were sampled throughout the year, with peak numbers recorded from August to April. It was also observed that *A. raptoria* feeds on the bark of branches and stems, and presumably also the nuts. These diverse feeding sites on the trees could account for the almost constant presence of the species throughout the year. When compared to previous studies conducted on macadamia in Mpumalanga (Bruwer, 1992), where *A. raptoria* is not even mentioned in the survey conducted in the Levuba area, it would appear that *A. raptoria* has a sporadic occurrence in the area (Van den Berg *et al.,* 1999).

It was only in recent years that *A. raptoria* was identified as a pest on pistachio nuts. This is due to the fact that the pistachio industry is still in its infancy in South Africa, with the first commercial orchards only being established at Green Valley Nuts (GVN), near Prieska in the Northern Cape Province in 1993 (Haddad & Louw, 2006). Following on this extensive insect biomonitoring programs were initiated on site at GVN to determine a reference framework for potential insect pests present in the pistachio orchards (Louw, 2003). Louw (2003) mentions that since pistachio can be regarded as a new crop in South Africa, a number of biological threats become a reality. These include the translocation of existing pests and high levels of susceptibility to diseases and pests which occur naturally in the area. This prediction would appear to hold true for *A. raptoria*.

The broad-based insect biomonitoring survey in the pistachio orchards culminated in the construction of a potential pest database for future use in pest management programs at GVN. Monitoring continued from 2000 until 2003 and covered 17 orchards situated at GVN and Remhoogte, a nearby farm, which were chosen to address pistachio cultivar, tree age, and soil type differences (Louw, 2003). The database revealed that a Pentatomidae stinkbug species complex also occurs within the GVN orchards, especially during the nut forming, nut fill and nut ripening periods. Adult pentatomid peaks were strongly evident during the latter half of the pistachio growth season. It was determined that these sap-sucking insects, with their piercing-

sucking mouthparts, feed on the individual nuts in the nut clusters, causing necrotic blackening, senescence and eventually the abortion of nuts. As was the case on macadamia nuts in the lowveld (Van den Berg *et al.,* 1999), *A. raptoria* also feeds on other plant parts, such as leaf petioles and stem bark.

Louw (2003), reports that 16 species of Pentatomidae stinkbugs were recorded on Pistachio at GVN. Of these, *A. raptoria* was numerically dominant in the orchards and made up a pertinent component of the wider bug species complex responsible for causing nut necrosis and desiccation. Haddad & Louw (2006) reiterate this point by reporting that Hemiptera emerged as a key group of pests on pistachio, with several species occurring in the tree canopies. They also stated that of these species, only one, *A. raptoria*, occurred in sufficient numbers to pose a major threat to production at the present time. It was also stated by these authors that the geographical, climatic and host plant influences differ greatly between the lowveld area of Mpumalanga, where *A. raptoria* was found in previous studies (Joubert & Neethling 1994; Van den Berg 1998; Van den Berg *et al.* 1999; Bruwer 2004), and the Prieska district, indicating that *A. raptoria* is a highly adaptable polyphagous species.

During their study Haddad & Louw (2006) sampled 170 pistachio trees of differing cultivars during a 21 month survey. During this study the *A. raptoria* populations typically remained low during the winter months and early spring, and increased slowly during late spring, with a sudden and sharp increase during summer.

During the study conducted on macadamia nuts in the lowveld (Van den Berg *et al.,* 1999), where most *A. raptoria* adults were collected from August to April, it was implied that this species is far more abundant from early spring to late summer. In comparison and as expected, more distinct occurrence peaks are evident on pistachio (Louw, 2003; Haddad & Louw, 2006), with overall seasonal abundance patterns differing markedly from that on macadamia in the Mpumalanga Lowveld (Van den Berg *et al*., 1999). In concurrence with this, Haddad & Louw (2006) state that on pistachio, with the pronounced

seasonal climatic fluctuations of a considerably more arid region and the deciduous nature of the trees, the distinct evidence of troughs and peaks of *A. raptoria* can be explained. In the subtropical climate of Mpumalanga, *A. raptoria* populations were much more stable throughout the year, with comparably smaller peaks in January and April. The latter phenomenon can also be attributed to the evergreen phenology of macadamia trees that provides foliage for feeding throughout the year.

Variable seasonality of stinkbugs is verified by Panizzi (1997) who states that when food plants become scarce and abiotic factors (e.g. temperature & photoperiod) becomes unfavourable, adult stinkbugs usually show different over-wintering survival strategies. In more temperate zones, adults will overwinter in deciduous woods, in above-ground habitats, underneath dead leaves, and elsewhere. In more tropical regions (such as the Mpumalanga lowveld), in direct contrast, some species will breed continuously.

It thus becomes clear that *A. raptoria* is present throughout the year, but with distinct regional differences. In more temperate regions, such as Prieska in the Northern Cape Province, only low numbers are evident during the winter months, with individuals sheltering under bark and prone objects inside orchards and inside grass tussocks in natural veld outside orchards (Louw, 2003). In contrast, in more tropical regions, where temperature fluctuations and food availability are not as pronounced, *A. raptoria* may be found throughout the year in relatively large numbers.

2. Material and Methods

2.1. Study area

All material used in determining the bio-ecology of *Atelocera raptoria* was sampled at the Green Valley Nuts (GVN) estate (22°56'41"S, 29°35'11"E) in the Prieska district, Northern Cape Province, South Africa (Fig. 1). The climate in the area is semi-arid, with very hot summers (occasionally exceeding 40 °C), very cold winters (night temperatures often falling below –5 °C) and low

rainfall, averaging between 200 and 300 mm per annum. (Haddad & Dippenaar-Schoeman, 2006).

The farm is wedged between the banks of the Brak and Orange rivers. The Orange River forms the western border and the Brak River the southern border. The farm bordering on the permanent flowing Orange River allows irrigation of the orchards all year round. As mentioned previously, the area in which this farm is situated is arid and the successful cultivation of Pistachio would not have been possible without the river as a water source.

The farm Green Valley Nuts is approximately 1450 ha in size and is comprised of approximately 1080 ha of cultivated land (Fig. 2). The entire cultivated area is planted with pistachio nut (*Pistacia vera,* Anacardiaceae) of different cultivars.

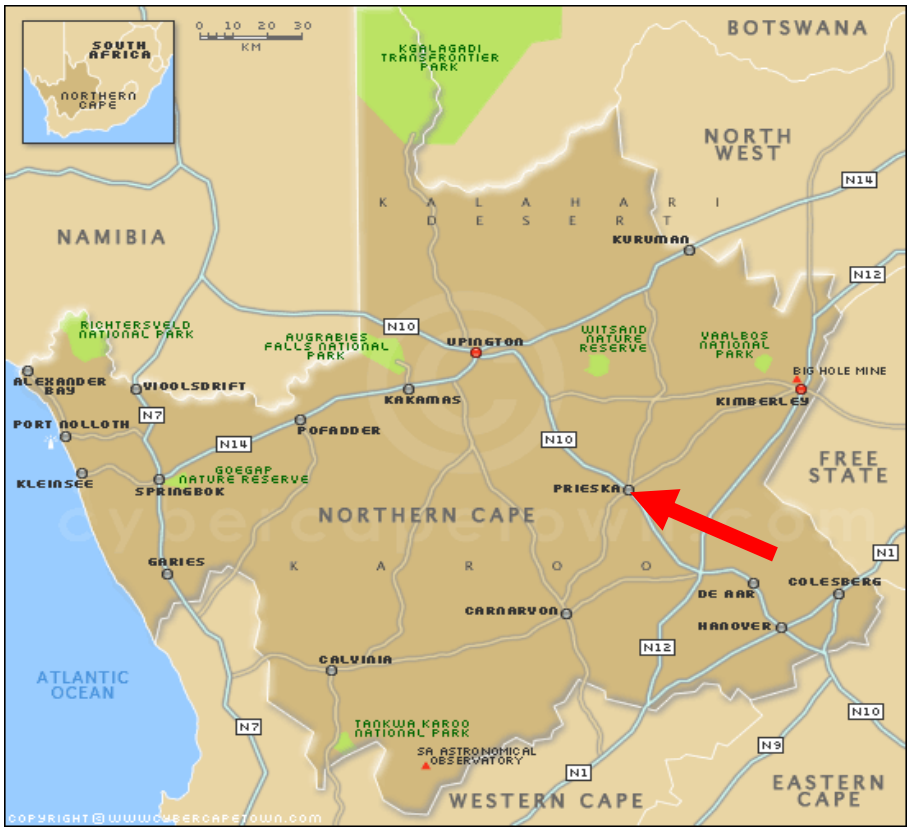


Figure 1: Map of the Northern Cape province, South Africa (Arrow indicates Prieska town).

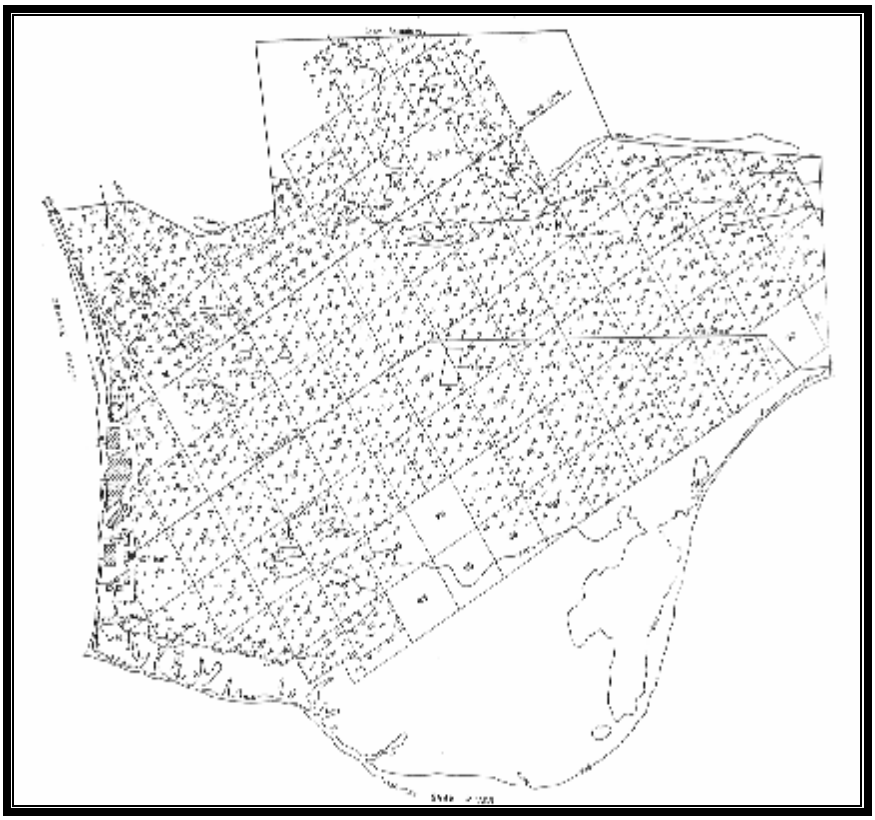


Figure 2: Map of Green Valley Nuts (GVN).

2.2 Influence of fluctuating temperatures on the hatching rate, parasitism and temperature tolerance of *Atelocera raptoria*

2.2.1 Experimental setup

In total, 168 *A. raptoria* egg packages were used in this experiment. The egg packages were sampled from the pistachio trees at GVN by making leaf cutlets around the packages, without touching the eggs themselves. This ensured that the eggs were minimally disturbed (Fig. 3).

Figure 3: Sampled *Atelocera raptoria* egg packages on pistachio leaves.

Sampling of egg packages took place in January 2004. Eggs were sampled from blocks 19 and 45 and initially stored at low temperatures to inhibit development. In the laboratory, egg packages were removed from the low temperature conditions and randomly divided into seven groups of 24. To insure a random effect, no attention was paid to number of eggs per package, visual condition or date of sampling. This was followed by random placement of two groups per plastic, screw top container supplied with moist tissue paper on the bottom. This was necessary to protect the eggs from dehydration and desiccation which would skew the results. Containers were tightly sealed to prevent moisture loss.

To determine the effect of fluctuating temperatures on the hatching success of A. raptoria and related life-cycle events, seven fridges (breeding cabinets) set at varying temperatures were used. The temperatures ranged from 10°C to 40°C with 5°C differences between fridges. Inside each fridge 12 containers, which each contained two egg packages, were placed (Table 1). The temperatures of the different fridges remained constant throughout the study.

Table 1: Distribution of containers with *Atelocera raptoria* egg packages in fridges of fluctuating temperatures.

Fridge temperature	Number of plastic containers	Number of egg packages in fridge	Approximate number of eggs in fridge
10°C	12	24	336
15°C	12	24	336
20°C	12	24	336
25°C	12	24	336
30°C	12	24	336
35°C	12	24	336
40°C	12	24	336

Daily visual inspection (at noon) off all containers was carried out to check for the eclosion of either *A. raptoria* nymphs and/or egg parasites. When eclosion from egg packages had occurred, the success rate was noted and the particular container relocated to prepared terrariums to monitor further development of the nymphs.. Containers with parasitized egg packages were left in fridges to allow opportunity for maximum hatching and to contain hatched parasitoid individuals properly. The wasp parasitoid was identified as *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). This parasitoid will be more thoroughly discussed in Chapter 3. All hatching dates were noted relative to date of sampling. The fluctuating temperature trials were terminated when it became apparent that no further development of the eggs were taking place.

2.2.2. Determination of *A. raptoria* egg status by visual inspection

After completion of the fluctuating temperature trials, all hatched and unhatched egg packages were removed from the containers and examined under a dissection microscope. The total number of eggs in each package was counted and the status of each egg determined. As a working tool it was attempted to categorize egg status by means of visual inspection only. The categories that were decided upon had to encompass the entire range of

events related to egg eclosion success. The following categories were chosen:

1. Nymphal mortality, which relates to successful development, but that first instar nymph mortality had occurred inside the egg and no eclosion took place.
2. Successful hatching, which relates to successful eclosion of first instar nymphs.
3. Parasite mortality, which relates to parasitized eggs and mortality of parasitoids inside eggs and no eclosion took place.
4. Successful parasitism, which relates to successful eclosion of adult parasitoids
5. Empty, which relates to the absence of first instar nymph or parasitoid development based on visual inspection.

2.2.3. Effect of temperature on parasitoid loads in *Atelocera raptoria* egg packages

Further from the above-mentioned visual inspection of egg status, the impact of egg parasitoids as a possible biological control tactic affecting successful *A. raptoria* egg eclosion was given special attention. Parasitoid loads present in all egg packages were determined and the successful eclosion of these determined. Comparisons were done between the percentage of parasitized eggs and the percentage normal hatching eggs and their respective mortality rates. All this was conducted across the different temperature regimes.

2.2.4. Effect of temperature on the hatching succession of *Atelocera raptoria* eggs

It is presumed that higher temperatures should accelerate the rate of development from oviposition to eclosion. By noting and comparing the times of eclosion of same-age egg packages at fluctuating temperatures, *A. raptoria* (and egg parasitoid) hatching succession could be determined.

2.3 Using Dyar's Rule to determine the number off *Atelocera raptoria* nymphal instars

When determining the life-cycle of an organism, an analysis index is required. To conduct this on *A. raptoria*, nymphs of varying size and instar, as well as adults, were sampled. To aid in this, Dyar's rule was used as a measuring index (Dyar, 1890). All material was sampled by hand on Pistachio trees at GVN. These were randomly sampled, paying no attention to size or stage of development and placed in 70% ethanol to kill and preserve the material. A wide range of 407 individuals were sampled and preserved in this manner. This material was placed inside a Petri dish and sorted into six hypothetical instar groups according to size and appearance using a dissection microscope. Each group was placed inside separate plastic containers and the respective individuals morphometrically measured using two different approaches and the results plotted.

2.3.1 Method 1: Measurement of specimens using total length

This method involved the measurement of the total length of the specimens by using an ocular micrometer accurate up to 1/10 of a millimeter (100 λm). The ocular was placed onto a standard dissection microscope, which was then used to examine each individual separately. The body of each individual was measured from the tip of the head to the tip of the abdomen (Fig. 4A). This was done on each of the 407 specimens in the 6 groups, accurate up to one nanometer. The length and specimen number was recorded on a datasheet

2.3.2 Method 2: Measurement of specimens using pronotum width

This method was conducted by measuring the width of the pronotum at the wider points of the structure (Fig. 4B). The same technique was followed as with the first method. Again, all 407 specimens were examined and the pronotum width of each individual was recorded.

(A) **(B)**

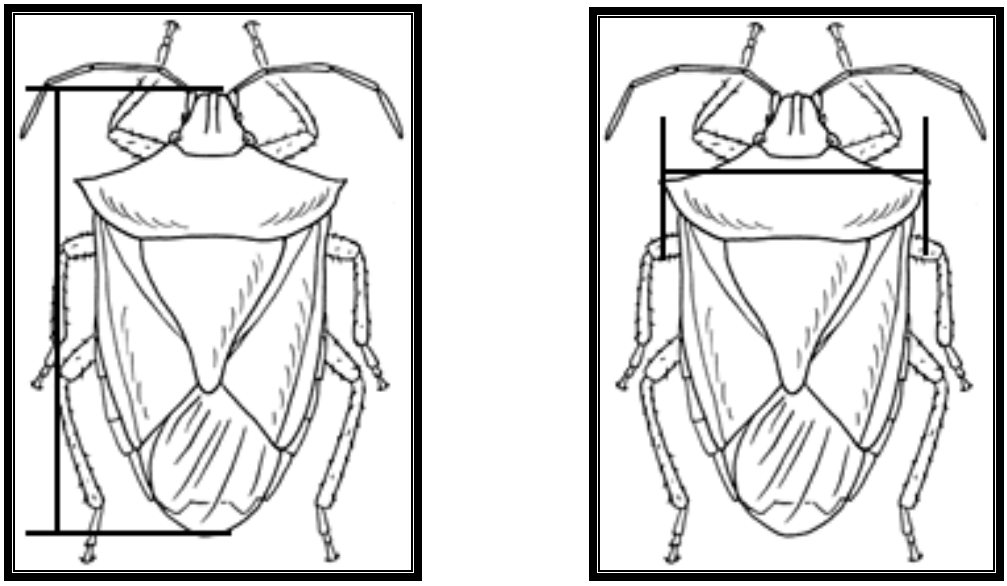


Figure 4: Pentatomidae sketches illustrating the total body length (A) and the pronotum apex width (B) of an individual.

2.3.3 Evaluation of the different methodologies

Two methods were used to determine which would produce the most significant and most accurate results. The pronotum is a solid structure, which is heavily sclerotized. The abdomen is softer, especially when permeated with ethanol, and is subject to swelling or deformation. While the pronotum width should remain fairly constant, even when preserved, the soft abdomen does not. This can have a significant effect on the consistency of the measurements.

2.3.4 Constructing a Dyar's Rule Analysis Index Model for *Atelocera raptoria*

All collected data was grouped according to the method used. Data was then sorted from smallest to largest value, independent of theoretical instar groups. This was done for both the pronotum width and total body length measuring methods. This data was then entered into Microsoft Excel® and the resultant

identical values stacked on top on each other. From this, in turn, a graph was formed where instar tendency can clearly be depicted.

2.3.5 Determination of statistical significance between instar differences

To determine the significance between the different proposed instar pronotum widths, as well as the different total body lengths, a One-way Analysis of Variance (ANOVA) was conducted using GraphPad InStat©, version 3.00. Further to this a Tukey-Kramer Multiple Comparisons Test was also conducted.

3. Results and Discussion

3.1. Determining status of eggs by using visual inspection

To determine the temperature thresholds of hatching or parasitism, visual inspection regarding egg status was done and status categories determined.

1. **Nymphal mortality**

   This category shows that development took place, but that nymphs died inside the eggs. This could be identified by determining the color of the egg. Such eggs would have a reddish hue, which is the color of the unborn embryos (developing first instar nymphs), which could clearly be seen through the egg's translucent chorion. Such eggs would still be sealed, with the pseudoperculum, which occurs in all members of the Pentatomidae, intact. (Fig. 5).

Figure 5: *Atelocera raptoria* egg packages with unborn nymphs still inside the chorion. Nymphs with a reddish hue can be discerned through the translucent chorion.

2. Successful Hatching

This can be seen when the eggs are successfully broken open and healthy first instar nymphs congregate close by. The empty eggs appear pure white, with the pseudoperculum conspicuously flapped open (Fig. 6).

To assist the embryo to break open the operculum an egg-burster ('Eisprenger'), a highly sclerotized portion of the embryonic cuticle, present in Pentatomidae, Coreidae and possibly other families, is used (Miller, 1956). This apparatus can possess various shapes and in the Pentatomidae it is commonly anchor-shaped or has the form of a T with a short, conical spur at one or both ends of the shaft portion (Miller, 1956). This egg-bursting apparatus also occurs in closely related species such as *Nezara viridula.* Here the egg-burster is a tri-furcate, chitinous apparatus and an integral part of the embryonic cuticle with an apical spine responsible for the rupturing of the thick chorion. This allows the pseudoperculum or cap to be lifted and the nymph to escape (Culligan, 1975).

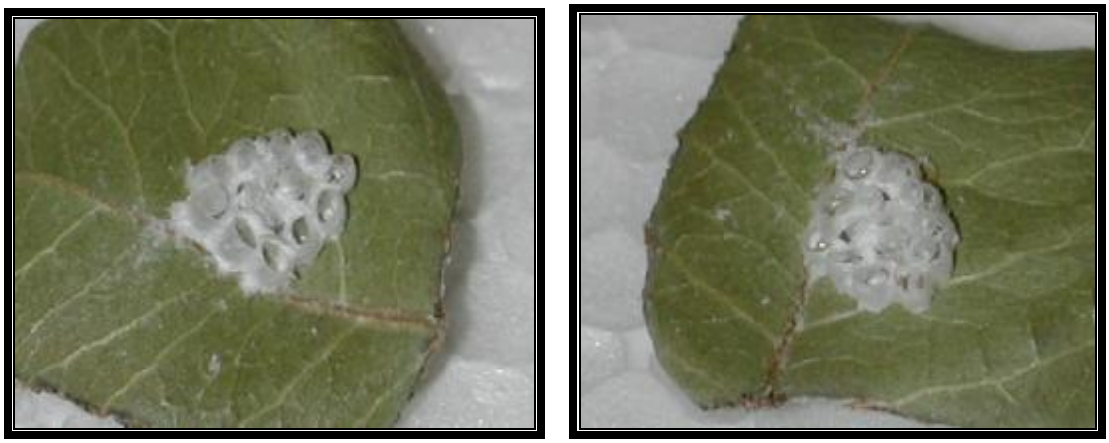


Figure 6: Empty egg chorions of successfully hatched Atelocera raptoria nymphs. Open pseudopercula can be identified clearly.

3. Parasite mortality

This category is for eggs that are parasitized, but where the parasitoid wasps died in the egg. In this case parasitoid larvae kill the inhabiting nymphs and then die for some or other reason prior to emergence. These eggs are conspicuously black in color due to the parasitoid larvae or adults which can be observed through the translucent chorion of the egg (Fig. 7).

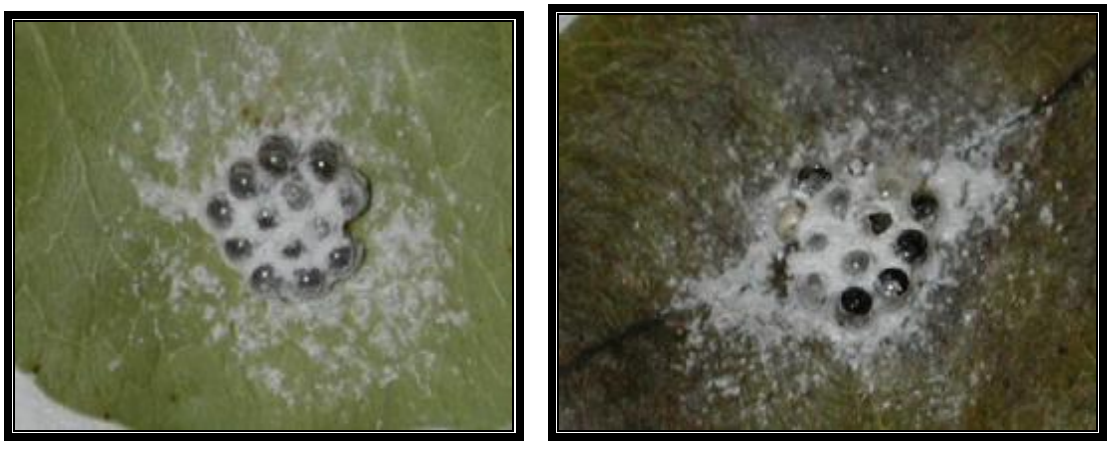


Figure 7: Parasitized eggs of *Atelocera raptoria*. Black wasps can be seen through egg wall.

4. Successful parasitism

These are eggs that were parasitized and the parasitoids hatched successfully. Here the parasitoid female oviposits inside the *A. raptoria* egg, the developing parasitoid larvae consume the egg content, progress through the life stages to adulthood and hatch successfully. Parasitized eggs appear to be similar to successfully hatched eggs, except for the exit opening. Adult wasps chew their way out of the egg causing the exit hole to appear diagnostically spiral-like. A clear difference between the perfectly circular flap-like pseudoperculum when *A. raptoria* have hatched and the spiral-like opening caused by the parasitoid wasps can therefore be identified (Fig. 8).

Figure 8: Empty *Atelocera raptoria* eggs that have been parasitized by a wasp parasitoid. Spiral-like exit openings can be identified.

5. Empty

These are eggs that showed no development whatsoever. Neither parasitoids nor nymphs could be observed inside the eggs. These eggs show a yellow hue and shrivel up (Fig. 9A). There is therefore a clear difference between empty eggs and normal, healthy eggs. (Fig. 9B).

(A) **(B)**

Figure 9: (A) An undeveloped (empty) egg package and (B) a healthy egg package.

Using these five egg package identification categories, all egg packages were counted and grouped accordingly. The number of eggs per package were also determined. For this part of the study 168 egg packages were examined and of these, only 8 packages (4.8%) did not contain 14 eggs. No deviation greater than 1 egg per package was noted, with a maximum of 15 and a minimum of 13 eggs per package.

The number of eggs per batch in *A. raptoria* may be meaningful in their identification in orchards and also provides clues regarding the life-strategy of this particular pentatomid species. The latter point is discussed further by considering the batch sizes of another, related species, *Nezara viridula*.

N. viridula, the southern green stinkbug, is a cosmopolitan pest of agricultural and horticultural crops feeding on fruits, pods, and mature seeds of the plants, thereby reducing crop yield and quality (Miller, Rose & Fakunaga, 1965). It is a major pest on various types of nuts, including pecan, (*Carya illinoinensis)*, macadamia, (*Macadamia integrifolia)* and pistachio. Historically, *N. viridula* has a worldwide distribution, occurring throughout the tropical and the subtropical regions of Europe, Asia, Africa and the Americas (Lethierry & Severin, 1893). It is highly polyphagous species, attacking both monocots and

dicots. And as many as 145 host plants, within 32 plant families, have been recorded. (Panizzi, McPherson, James, Javahery & McPherson, 2000).

A comparison of the egg batch sizes of *A. raptoria* and *N. viridula*, shows that those of *N. viridula* are much more variable than those of *A. raptoria.* According to Culligan (1975) it was found that in *N. viridula* the number of eggs per batch varied from one to 107, with an average of 55. He also stated that eggs are rarely laid singly, but that if it did happen, these were frequently sterile. Clearly the batch size variability of *N. viridula* therefore differs from the more constant and smaller batch size of *A. raptoria*, which probably reflects different life strategies for the two species. In this regard it could be argued that *A. raptoria* probably follows a kind of k-strategy, where low reproductive rates combined with low mortality rates ensure offspring survival. *N. viridula*, in contrast, is probably more r-strategy orientated with it's comparatively high reproduction rate.

3.2. Effect of temperature on parasitoid loads in *Atelocera raptoria* egg packages

At 10°C little or no development occurred. Of the trial packages, only 12 eggs showed successful emergence of parasitoids. These comprised approximately 4% of the total eggs. It appeared as if these eggs were empty and that no development whatsoever had occurred. No successful hatchings of *A. raptoria* or nymph mortalities could be observed either (Fig. 10).

At 15°C a higher hatching rate was observed.. High parasitoid loads were observed, with 33% of the trial showing successful parasitism and 9% showing parasite mortalities. Only 1% and 3% showed successful *A. raptoria* hatching and nymphal mortality respectfully. More than half of the eggs (52%) were empty, however, and showed no signs of development (Fig. 11).

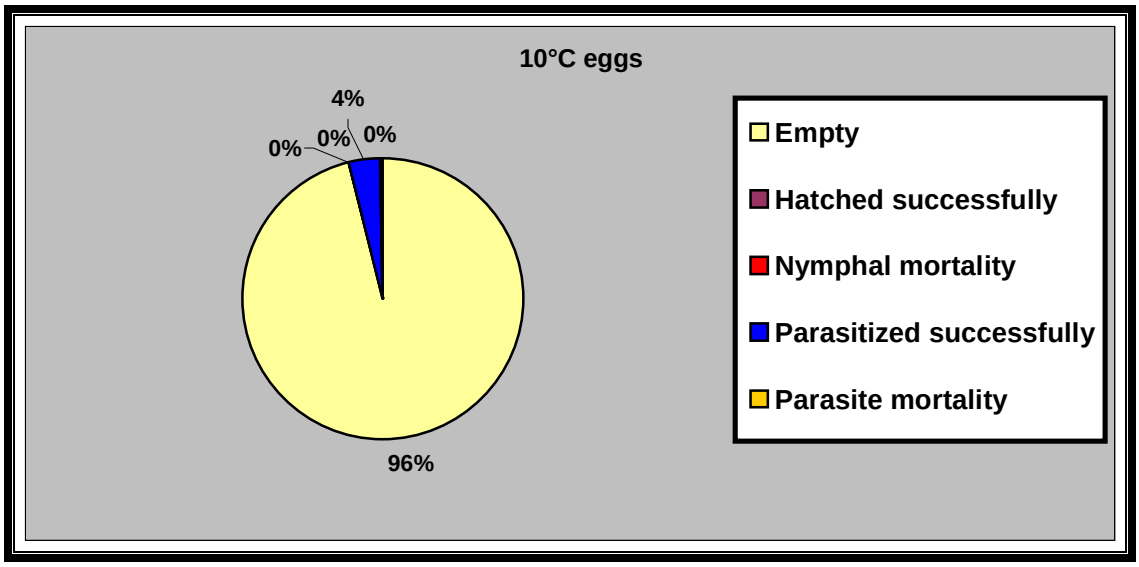


Figure 10: Hatching status of *Atelocera raptoria* eggs at 10°C.

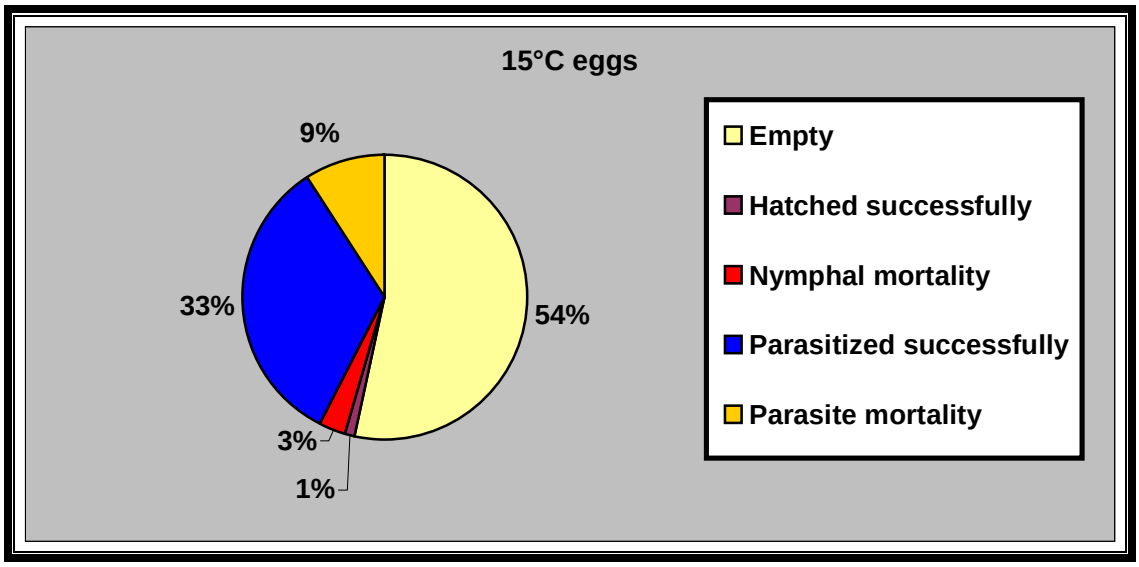


Figure 11: Hatching status of *Atelocera raptoria* eggs at 15°C.

At 20°C a still greater hatching activity could be observed. A temperature of 20°C was regarded to fall within the mid-range temperatures at which the organisms should be benefited best. Here, high successful parasitoid loads (44%) were detected, as well as a further 17% unsuccessful parasitism. Again, very little *A. raptoria* hatching occurred, with only 5% successful

hatching and 3% mortality in the eggs. A relatively high (31%) empty egg percentage was also noted (Fig. 12).

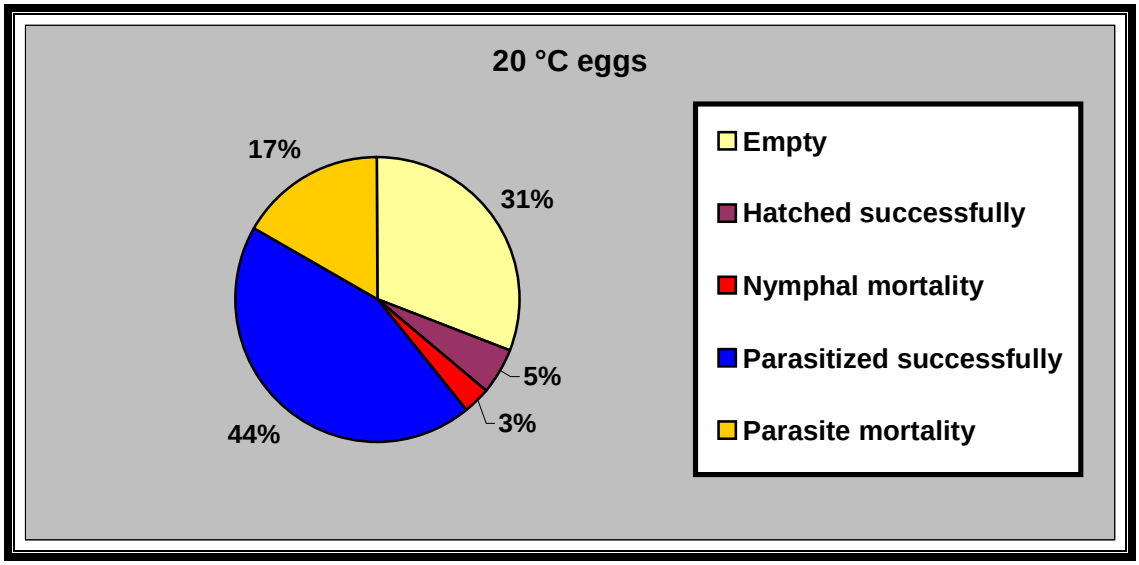


Figure 12: Hatching status of *Atelocera raptoria* eggs at 20°C.

The 25°C was regarded as the perfect mid-range temperature, since it falls exactly between the extremes of 10°C and 40°C. Almost all the trial eggs showed development activity. The highest successful hatching rates of *A. raptoria* were recorded at this temperature, with 12% of the eggs producing healthy nymphs. Only approximately 4% of the total number of eggs did not hatch successfully. The highest parasitoid loads were also detected here, with 51% showing successful parasitoid emergence. Also, considering the number of eggs that were parasitized (69%), mortality rates (18% of load) were considerably lower than those of previous temperatures. Only 15% of the eggs showed no development and remained empty (Fig. 13).

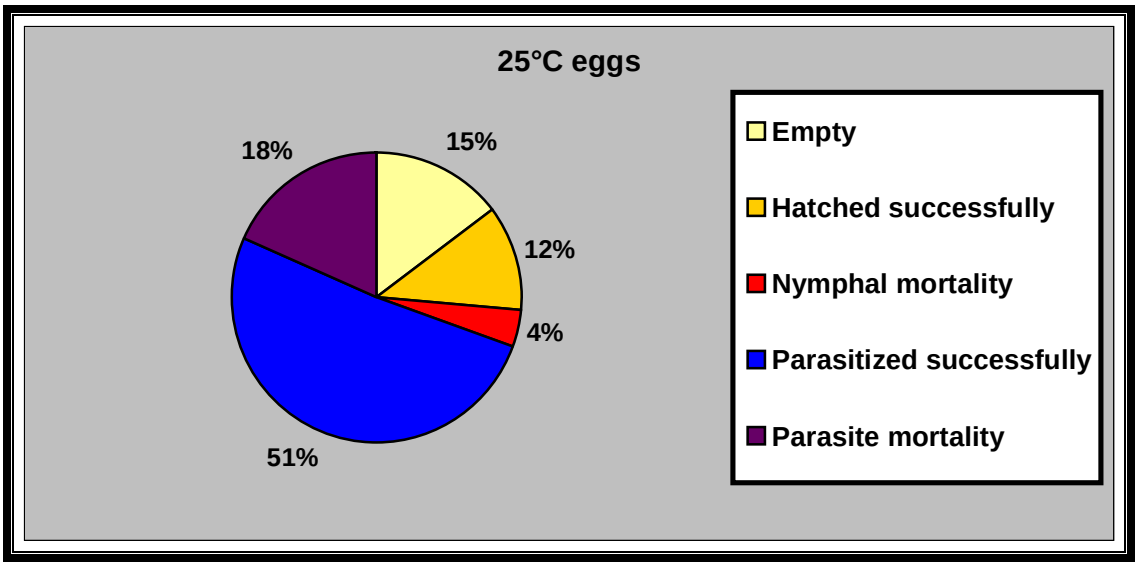


Figure 13: Hatching status of *Atelocera raptoria* eggs at 25°C.

At 30°C almost all of the eggs still showed development and this temperature can therefore still be regarded as mid-range. Only 15% of the eggs showed no development. Again, a high (68%) parasitoid load was observed. However, parasitoid mortality rates were considerably higher, with 29% of the load dying and 39% emerging successfully. A relatively high number (12%) of the eggs hatched successfully (Fig. 14).

At 35°C almost all of the eggs developed and only a very low percentage (5%) showed no development. Also, high parasitoid loads (60%) were again detected, but parasitoid mortality rates showed a dramatic increase, with 46% of the individuals dying inside the eggs and 14% emerging successfully. Very high *A. raptoria* nymph mortality also occurred, with 28% dying inside the eggs prior to eclosion and only 7% producing healthy individuals (Fig. 15). At 35°C it would therefore appear that immature development of the particular species was becoming severely influenced.

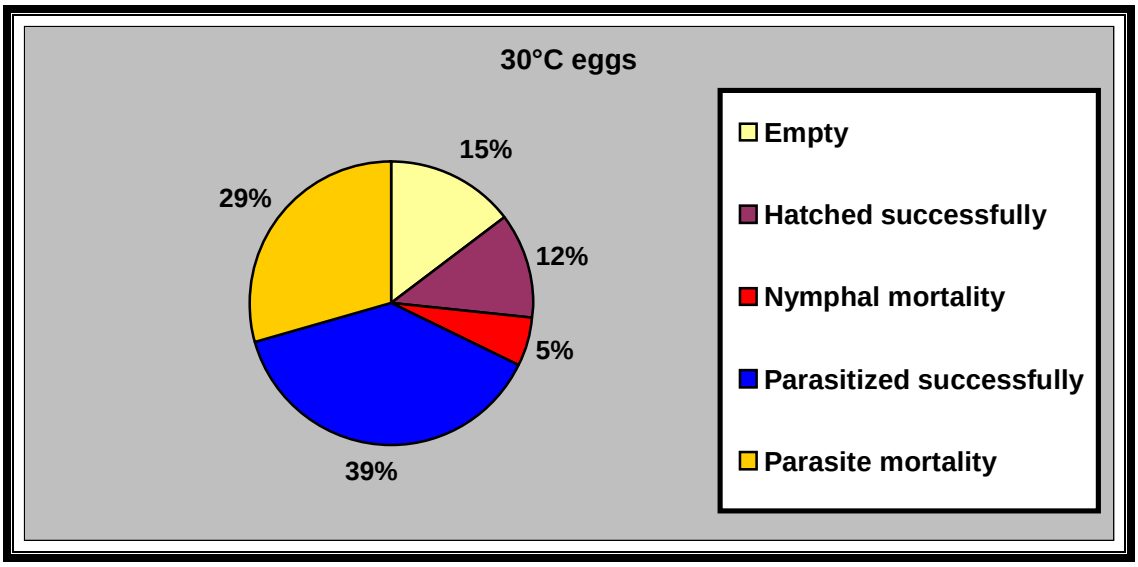


Figure 14: Hatching status of *Atelocera raptoria* eggs at 30°C.

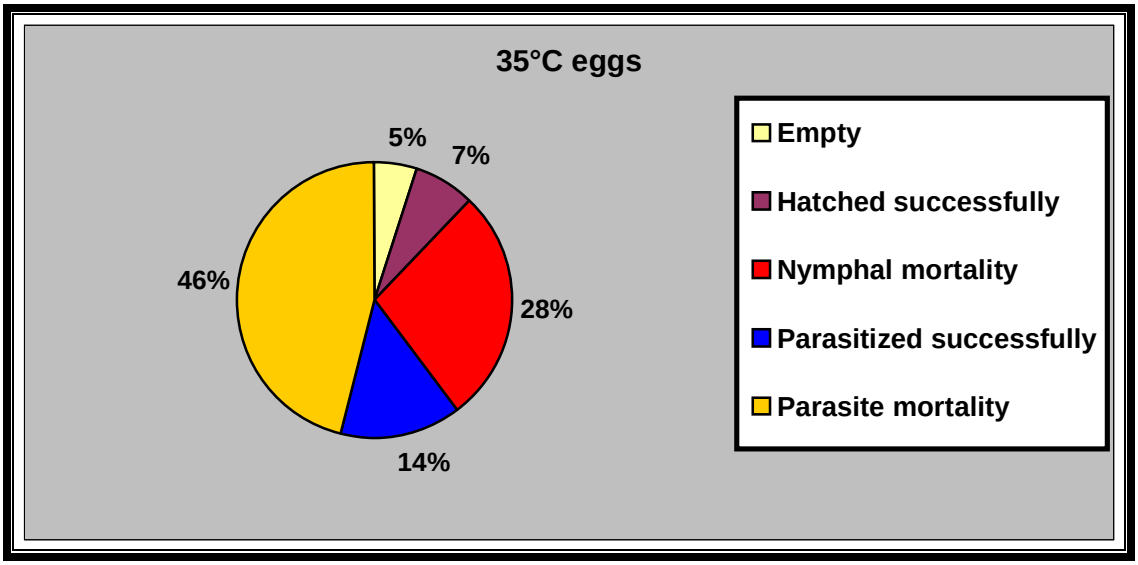


Figure 15: Hatching status of *Atelocera raptoria* eggs at 35°C.

At 40°C low success rates were observed. Here 34% of the *A. raptoria* nymphs died inside the eggs. Similarly, low (9%) parasitoid loads were detected, all of which died prior to emergence (Fig. 16). The majority of the eggs (57%) were empty, reflecting no development whatsoever.

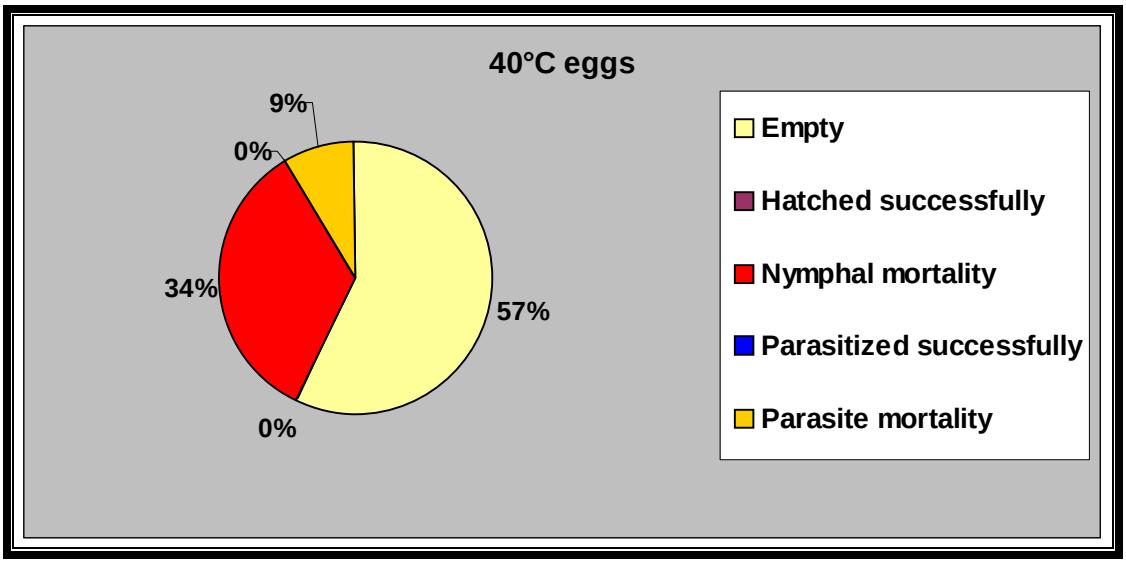


Figure 16: Hatching status of *Atelocera raptoria* eggs at 40°C.

From Table 1 it becomes clear that parasitism and *A. raptoria* hatching values are benefited most at the mid-range temperatures (20°C - 30°C)(Table 1) and that this range represents the optimum circumstances for life-cycle development of both the particular species. Empty egg numbers are much greater at very low (10°C) or very high (40°C) temperatures. With an increase in temperature beyond 30°C, development still took place in the eggs, but nymphal mortality increased probably due to a combination of too high heat units and desiccation. Overall it would therefore appear that at very low temperatures there is no development, and at very high temperature development is retarded.

Table 2: Summary of hatching status of *Atelocera raptoria* eggs at fluctuating temperatures. (Values expressed as a factor of 100%)

Temperature	Empty	Hatching successful	Nymphal mortality	Parasitized successful	Parasite mortality
10°C	96	0	0	4	0
15°C	54	1	3	33	9
20°C	31	5	3	44	17
25°C	15	12	4	51	18
30°C	15	12	5	39	29
35°C	5	7	28	14	46
40°C	57	0	34	0	9

3.3. Effect of temperature on the hatching succession of *Atelocera raptoria* eggs

Since insects are poikilothermic organisms, temperature plays a major role in their growth and development (Johnson, Bessin & Townsend, 1998). Plants and invertebrates, such as insects and nematodes, require a certain amount of heat to develop from one point in their life cycle to another. This measure of heat is known as physiological time (Anon, 2003).

During this study, fluctuating temperature trials showed that nymphal development occurred at a faster rate at higher temperatures. As mentioned, eggs exposed to 40°C showed no development, whereas eggs maintained at 35°C were the first to show activity, with development taking place within day 2 to day 3. Eggs maintained at 30°C, where development occurred up to day 4, closely followed this. Eggs maintained at the mid-range temperature of 25°C show varied results, with development taking place on days 4, 6 and 9. In general, this reflects a trend whereby the earliest development occurred at the higher temperatures (Fig. 17).

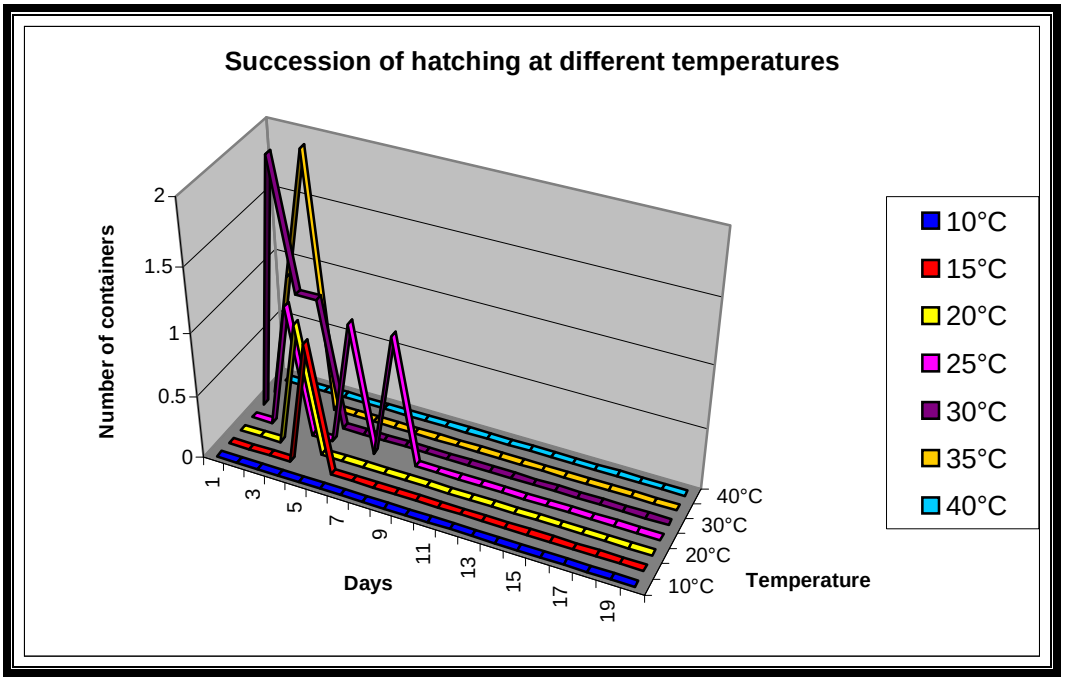


Figure 17: *Atelocera raptoria* nymph development succession at fluctuating temperatures.

3.4. Using Dyar's Rule to determine the number off *Atelocera raptoria* nymphal instars

Understanding the life-cycle of *A. raptoria* forms the basis of understanding its population dynamics. This is of great relevance towards determining any timed management practices, such as degree-day models (see Chapter 7).

3.4.1. Constructing a Dyar's Rule Analysis Index Model for *Atelocera raptoria*

The number of instars was determined by modifying and applying Dyar's Rule to compile a analysis index. This was carried out by using two different measuring methods. The first method measured the total body length of the individuals (Fig. 18) and the second the pronotum width (Fig. 19).

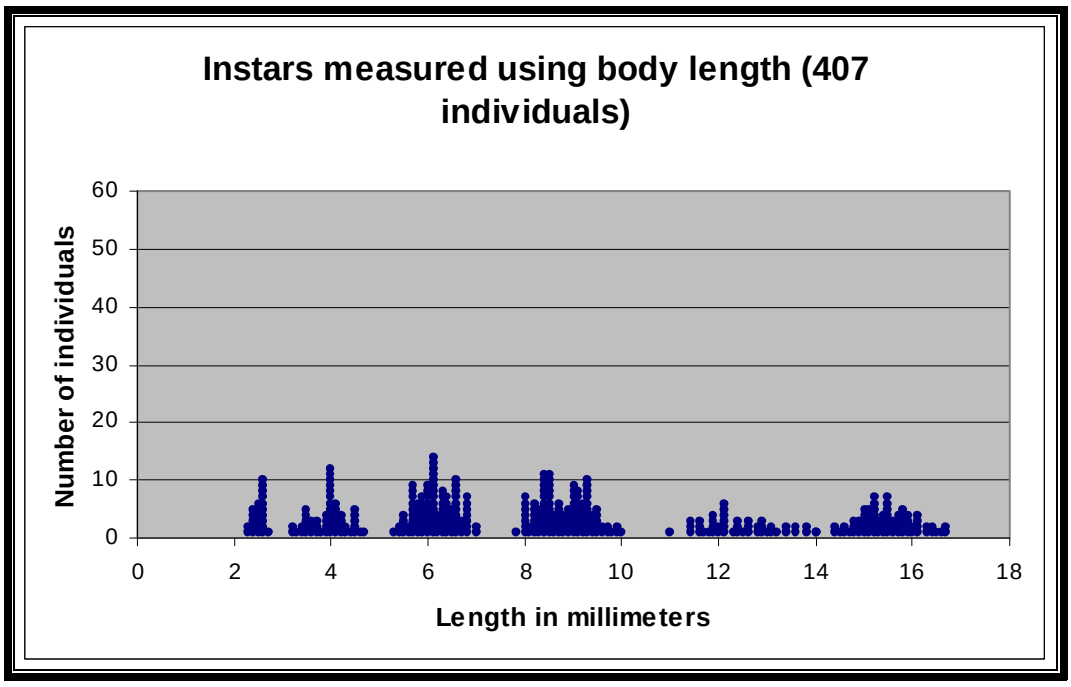


Figure 18: Analysis index for *Atelocera raptoria* of the number of nymphal instars up to (and including) adult eclosion, based on total body length measurements.

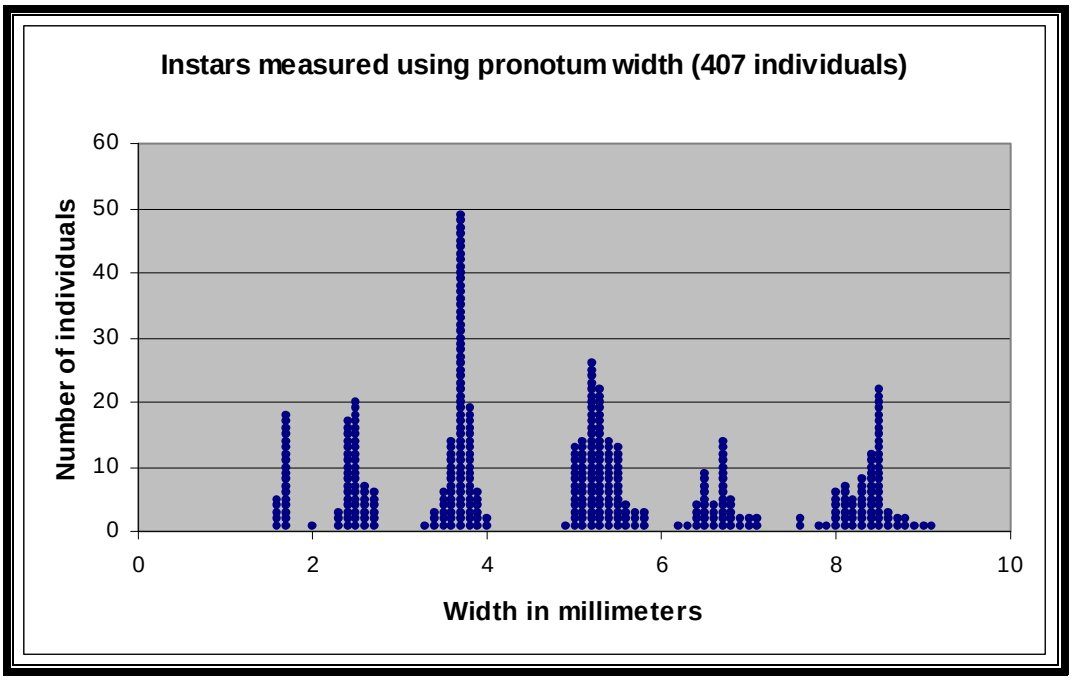


Figure 19: Analysis index for *Atelocera raptoria* of the number of nymphal instars up to (and including) adult eclosion, based on pronotal width measurements.

3.4.2. Measurement of total body length

Instars can be determined by considering at the peak values of each measurement group. There is no set value determining each instar, but there is a considerable difference in length between different instars. Thus, an instar average trend can be determined. From this five nymphal instars can be identified, with a last group showing the adults (Fig. 18). The overall expression of the groups depicting instars, using this method, is one of fuzziness.

3.4.3. Measurement of pronotum width

As was the case with the total body length measurement index, five nymphal instars plus the adult stage can be distinguished (Fig. 19). However, the impression is that measurement of pronotum width, instead of total body

length, provides much more accurate results. This is due to the fact that the pronotum is a more heavily sclerotized, fixed structure, with less possibility of swelling and deformation, than the abdomen. Applying Dyar's Rule for determining number of nymphal instars on the basis of pronotum width provides better fixed values and a larger degree of confidence.

The same individuals were used in both measurement approaches, yet by measuring the pronotum instead of the total body length, better results were obtained due to the fact that more individuals in the same instar group produced exactly the same values. By measuring total body length, the instar groups were much less defined, with values even overlapping in some cases and hence the fuzziness problem mentioned previously. No value overlap occur as a result of pronotal width measurements (Fig. 19).

3.4.4. Determination of statistical significance between instar differences

(A) Total body length index

A One-way Analysis of Variance (ANOVA) showed very significant variation ($P < 0.0001$) between body-length measurements of various nymphal instars. Variation among column means were also greater than expected. Further to this, by implementing the Tukey - Kramer Multiple Comparisons Test it was determined that should the value of q be greater than 4.047, then the P value is less than 0.05. All instar comparisons were above 4.047.

(B) Pronotum width index

The ANOVA for the pronotum width index showed the same results as the total body length index (i.e. $P < 0.0001$). Also, q values were universally above 4.047, which indicate a P value of less than 0.05. It can therefore be confidently stated that *A. raptoria* develops through five nymphal instars before adult eclosion. This correlates with instar numbers of most hemipterans and serves as verification. According to Miller (1956), hemipterans usually pass through five or six instars and at each moult, some modification, slight in the early instars, but greater in succeeding ones, takes place in the external appearance of the individuals. This also appears to be the case for *A. raptoria* (Fig. 20).

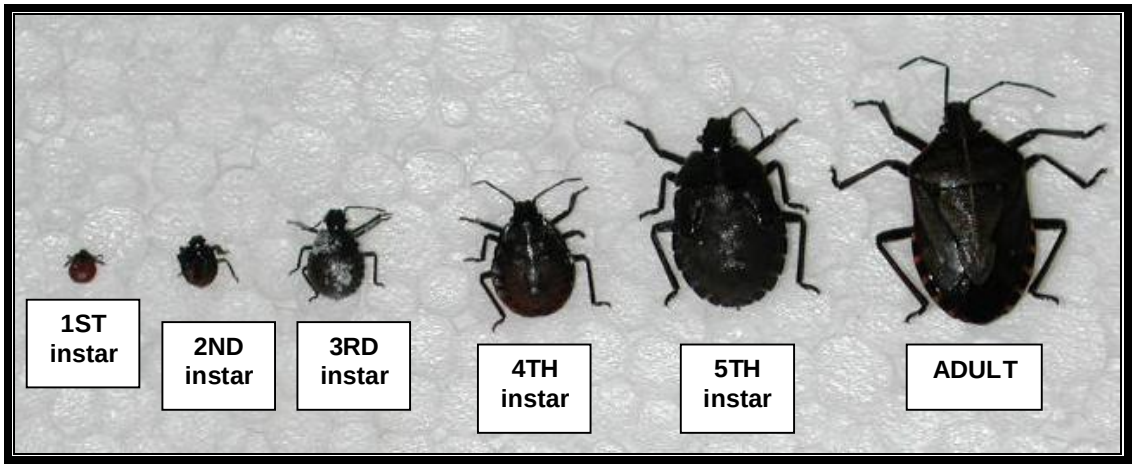


Figure 20: The five nymphal instars and an adult stage of *Atelocera raptoria.*

4. Conclusion

This study provided valuable information on to the life history of *A. raptoria*. Previously, the number of nymphal instars, as well as the differentiation in egg status and the possibility of their visual identification, were unknown. In addition, the general temperature preferences for optimal hatching of the eggs provides valuable information regarding possible seasonal peaks of this species. It would appear that successful hatching occurs at a much higher rate during warmer temperatures (25°C to 35°C), which implies that adult and nymphal peaks will in all probability occur during the warmer months of the year, i.e. from late spring until summer. This period coincides with nut harvesting at GVN. This supports a previous study by Haddad & Louw (2006) which reports that the populations of *A. raptoria* typically remain low during the winter months and early spring, and increase slowly during late spring, with a sudden and sharp increase in summer. The presence of a possible biological control agent in the form of an egg parasitoid, is also confirmed. Relatively high parasitism rates could be seen in some of the trial egg packages, showing great promise as a possible non-chemical management tactic. This parasitoid, as well as it's history and possible application, will be more thoroughly discussed in Chapter 3.

5. References

Anonymous, 2003. *About degree-days.* Statewide IPM Program, Agriculture and Natural Resources, University of California. Retrieved from Http://www.ipm.ucdavis.edu/WEATHER

Bruwer, I. J. 1992. *The influence of various hemipteran species on macadamia and some factors which can limit nut damage*. PhD thesis, University of Stellenbosch. 184pp. (Original article not seen.)

Bruwer, I. J. 2004. Eko-vriendelike insekdoders vir die beheer van suigbesies in avokadoboorde. *South African Avocado Growers' Association Yearbook* **27:** 40-44.

Culligan, G. A. 1975. *Structure and physiology of the egg of Nezara virudula (L.) (Pentatomidae) with notes on oviposition and hatching.* M.Sc.dissertation, University of Pretoria, Pretoria. 104 pp.

Dyar, H. G. 1890. The number of moults of lepidopterous larvae. *Psyche, A Journal of Entomology, Cambridge Entomological club*. **5:** 420-422. (Original article not seen).

Haddad, C.R. & Dippenaar - Schoeman, A.S. 2006. Epigeic spiders (Araneae) in pistachio orchards in South Africa. *African Plant Protection* **12:** 12 – 22.

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

Haddad, C.R., Louw, S. vd M. & Dippenaar - Schoeman, A.S. 2004. An assessment of the biological control potential of *Heliophanus pistaciae* (Araneae: Salticidae) on *Nysius natalensis* (Hemiptera: Lygaeidae), a pest of pistachio nuts. *Biological Control* **31:** 83-90.

Johnson, D., Bessin, R. & Townsend, L. 1998. *Predicting insect development using degree days.* University of Kentucky Entomology. Retrieved from Http://www.uky.edu/Agriculture/entomology

Joubert, P. H. & Neethling, C. 1994. Kernel damage to pecan nuts in the Nelspruit area of South Africa. *Journal of the South African Society of Horticultural Science* **4:** 42-44.

Lethierry, L. & Severin, G. 1893. *Catelogue Général des Hémiptéres. Tomo I. Hétéroptéres Pentatomidae*. F. Hayez, Académie Royale de Belgique, Bruxelles, Belgium. (Original article not seen).

Louw, S. vd M. 2003. *Pistachio Entomology at IDC – Green Valley Nuts: Present knowledge and future focus.* Report to Industrial Development Corporation, Green Valley Nuts, Prieska. 27 pp.

Miller, N.C.E. 1956. *The Biology of the Heteroptera.* Leonard Hill [Books] Limited, London.

Miller, L.A., Rose, H.A. & Fukunaga, E.T. 1965. The effects of damage by the green vegetable bug, *Nezara viridula* (L.) on yield and quality of soybeans. *Journal of the Australian Entomological Society* **16:** 421 - 426 (Original article not seen).

Panizzi, A. R. 1997. Wild hosts of pentatomids: Ecological significance and role in their pest status on crops. *Annual Review of Entomology* **42:** 99 - 122.

Panizzi, A.R., McPherson, J.E., James, D.G., Javahery, M. & McPherson, R.M. 2000. Stink Bugs (Pentatomidae), pp 421 -474 **In:** Schaefer, C.W. & Panizzi, A.R. (Editors). *Heteroptera of Economic Importance.* CRC press, London.

Van den Berg, M. A. 1998. Studies on Hemiptera. *South African Avocado Growers' Association Yearbook* **21:** 78-79

Van den Berg, M. A., Steyn, W. P. & Greenland, J. 1999. Hemiptera occurring on macadamia in the Mpumulanga Lowveld of South Africa. *African Plant Protection* **5:** 89–92.

CHAPTER 3

Trissolcus basalis (Hymenoptera: Scelionidae): History, ecology and possible application as bio-control agent against *Atelocera raptoria*

1. Introduction

1.1. Aim

As stated in Chapter 2, the presence of the egg-parasitoid, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), was discovered in sampled egg packages during the trials. This wasp has not previously been recorded parasitizing *A. raptoria* eggs, and its promise as a possible biological control agent seemed obvious. All parasitized egg packages were parasitized by the natural, existing *T. basalis* population occurring in the Prieska district, where the orchards are situated. The aim of this study was to determine the overall success of this parasitoid, and to investigate the possibility of population augmentation as a future biological control measure.

1.2. History of *Trissolcus basalis* as a bio-control agent

Trissolcus basalis has been employed by many countries, specifically for the control of the southern green stinkbug (SGS) *Nezara viridula* (Hemiptera: Pentatomidae), as a bio-control agent for many years. Especially in Australia, Hawaii and California, the implementation and establishment of *T. basalis* has been well-documented.

Basically, *T. basalis* is an egg-parasitoid that oviposits inside the eggs of the natural host, *N. viridula L*).. According to Johnson, Follett, Taylor & Jones (2005), *T. basalis* females lays one egg inside each host stink bug egg, often parasitizing up to 100% of eggs in an egg mass. The parasitoid larvae will feed and develop inside the host egg until ready to emerge as a new adult, usually about 15 days from oviposition in *N. viridula* egg batches at 25°C (Johnson *et al.,* 2005)

T. basalis, is a specialist parasitoid on pentatomids. It parasitizes several economically important stinkbugs. These include *Euschistus servus* (Say), *N. viridula* (Linnaeus), *Piezodorus guildinii* (Westwood), *Plautia stali* (Scott), *Thyanta perditor* (F.), *Agrosternum* spp., *Dichelops melacanthus* (Dallas), as

well as *Eurygaster maura* (L) (Scutelleridae) (Panizzi, McPherson, James, Javahery & McPherson, 2000). No previous record of *T. basalis* parasitizing *A. raptoria* could be traced in the literature.

T. basalis disperses and reproduces well, it parasitizes other stinkbug species when its preferred host is not available, and in North America it is not known to be subject to hyper-parasitism or pathogens.. *T. basalis* has a high female to male ratio (up to 5:1) which increases its effectiveness as a parasitoid in field trials (Weedon, Shelton, Li & Hoffmann, 2004).

Van den Bosch & Messenger (1973) name the several characteristics as important for a biological agent to be effective. These include full adaptability to target species, vigorousness, good searching powers, and the ability to distribute their progeny successfully. It would appear that *T. basalis* possess most of these characteristics, which may explain its success in so many countries across the world.

1.2.1. Australia

N. viridula is widely considered as a very important cosmopolitan pest on various crops (Todd, 1989; Clarke, 1990). Also, numerous attempts at the biological control of the SGS had been made all over the world (Loch & Walter, 1999; Meats & Castillo Pando, 2002). Loch & Walter (1999) also reported that the most important and widely spread natural enemy of the SGS, is the egg parasitoid *Trissolcus basalis* (Wollaston)

T. basalis was first imported from Egypt into Australia in 1933 by the Western Australian Department of Agriculture (WADA). Since then, *N. viridula* egg parasitoids, principally *T. basalis*, were sporadically imported from several countries and released, augmenting existing *T. basalis* populations in Australia for almost half a century (Clarke, 1990). Clarke (1990) also reported that because of the reputed success of the wasp to control *N. viridula* in Australia, it had also been exported to many other countries around the world. Australia was also the first country to deploy *T. basalis* as a biological control measure against *N. viridula.* However, although *T. basalis* is reputedly very

successful in controlling the SGS in Australia, several authors do not agree with this statement.

Clarke (1990), reported that most of the success rate attributed to *T. basalis* concerning the control of *N. viridula*, is based on visual observations in the decline of *N. viridula* populations, not on collected data. The two principal assumptions made about this project are that *T. basalis* is the egg parasitoid species responsible for decreasing SGS population levels and that SGSs are not controlled by other factors (Clarke, 1990). He also stated that some important areas, especially those concerning identification of bio-control agents and post-release evaluation, were far from adequate. According to Loch (2000), high numbers of *N. viridula*, both nymphs and mating adults, could be found in Southeastern Queensland, Australia. The SGS was sampled from over 20 species of plants (Loch, 2000). It was also feared that populations of *T. basalis* would be low during summer, when SGS egg mass numbers were low. Clarke & Walter (1993) hypothesized that the poor survival rate of *T. basalis* over summer, coupled with the scarcity of SGS hosts during this period in southeastern Queensland, may be limiting populations of *T. basalis* and thus reducing its impact as a biological control agent.

However, later studies seemed to confirm the presence of *T. basalis* as the main egg parasitoid of *N. viridula* in Australia. Loch & Walter (1999) stated that their survey data demonstrate that *T. basalis* is the major parasitoid of the SGS in south-eastern Queensland and that the species regularly parasitizes several other host species at high rates. Their results show that almost 50% of the 204 *N. viridula* egg packages collected were parasitized.

Furthermore, survey results emphasized that *T. basalis* is the only parasitoid species in Australia that could have a significant impact in SGS biological control. Two other parasitoid species recovered from SGS eggs, *Xenoencyrtus* sp. (Hymenoptera: Encyrtidae) and *Anastatus* sp. (Hymenoptera: Eupelmidae), only emerged at very low rates. (Loch & Walter, 1999). Furthermore, according to Loch (2000), *T. basalis* has been recorded parasitizing at least 25 species of bugs from the families Pentatomidae,

Scutelleridae, Alydidae, and Coreidae. This wide host range of *T. basalis* coupled with the seasonal phenology of these different host species indicates that *T. basalis* is unlikely to be host limited during summer in southeastern Queensland.

Loch & Walter (1999) stated that the degree to which SGS populations are suppressed by *T. basalis* in Australia is difficult to assess. They also noted that biological control in Australia could not be classed as completely successful on the Darling Downs in south-eastern Queensland, mainly because of the fact that *N. viridula* remains a significant agricultural pest in that area, despite high rates of egg parasitism by *T. basalis.* An accurate assessment of the positive returns from biological control achieved through the introduction of *T. basalis* would be exceedingly difficult to measure, even though the species is so widely established and firmly embedded in the ecology of the area (Loch & Walter 1999).

Thus, it would appear that, to a degree, *T. basalis* is indeed successful in controlling *N. viridula* in Australia. Earlier reports on the success of *T. basalis* as a bio-control agent were in all probability exaggerated, prompting the critical reviews by various later authors. However, recent studies delivering strong supportive data, seem to confirm that *T. basalis* is indeed well established in Australia, and is the major egg-parasitoid of *N. viridula.*

1.2.2. Hawaii

N. viridula was first collected in Hawaii on the island of Oahu in 1961 and by 1963 had spread to all seven the major islands (Davis, 1964) Upon its discovery in Hawaii, the Hawaii Department of Agriculture (HDOA) initiated a biological control program aimed at reducing *N. viridula* to sub-economic status (Davis, 1964). It was widely regarded as a success, but small scale releases of *T. basalis* were performed on the island of Oahu in 1976, 1977 and 1983, using colonies initiated from field collections (Jones, 1995). During initial studies conducted by Davis (1964) and Davis (1967) very promising results concerning the biological control of *N. viridula* were obtained. Davis

(1967) found that field collected *N. viridula* egg-packages collected on the island of Oaha over a period of 6 months in 1963 had an average of 94.9% of the eggs parasitized. He also states that from 1964 to 1966, only an occasional egg package which was 90 to 97% parasitized, was found. On the other hand, parasitism rates found on two other islands, were considerably lower, with only 58% of the egg packages showing parasitism on the island of Maui and only 34.4% showing parasitism on the island of Hawaii.

Recent studies, however, indicate that bio-control success employing *T. basalis* on *N. viridula* is not as high as the earlier studies indicated. Jones (1995) declared that predators were considerably more efficient at finding *N. viridula* egg packages during both the years that his study was conducted. Jones's study was conducted in response to growers of macadamia on the island of Hawaii complaining of very high numbers of *N. viridula* in the orchards which caused significant nut damage. He also reported that the ant species *Monomorium floricola* (Jerdon) and *Pheidole megacephala* (F.) were regularly seen preying on stinkbug eggs. Consequently, strong doubt is cast on the prominent role attributed to *T. basalis* in the biological control of *N. viridula* in Hawaii. In the macadamia agroecosystem, results clearly indicated that predation is much more important than *T. basalis* in limiting stink bug survival through the egg stage (Jones, 1995).

Jones and Westcott (2002) also stated that it is clear that biological control of *N. viridula* is not such a "landmark" example of success in either Hawaii or Australia as suggested by some previous authors. They suggested that the high mortality of *T. basalis* females at summer temperatures may suggest that they won't be able to regulate populations of *N. viridula* that are at low levels and thus either spatially or temporally patchy. Also, suitable egg packages in macadamia nut orchards are rare and may act as a limiting factor concerning the biological control of *N. viridula* (Jones & Westcot, 2002).

Very low parasitism rates were also found during another study conducted by Jones, Westcott, Finson & Nishimoto (2001) where only 2.8% parasitism and 0.1% parasitism were found at two of the study sites. However, they could also find no conclusive results demonstrating that the two dominant ant species have a significant effect on *N. viridula* populations in terms of adult and egg predation.

In conclusion, therefore, it would appear that *T. basalis* is currently not regarded as a satisfactory bio-control agent on *N. viridula* in Hawaii. By reviewing the literature, it would appear that the main reason for this is the relatively low densities of *N. viridula* in the orchards (macadamia) and the low survival rate during the summer months. (Jones 1995, Jones & Westcott, 2002). This has also been suggested as a problem concerning *T. basalis* success rates in Australia (Clarke, 1990; Clarke & Walter, 1993). However, in a recent study conducted by Johnson, Follett, Taylor & Jones (2005), it has been shown that *T. basalis* will accept eggs of *N. viridula* and the Hawaiian endemic *Coleotichus blackburniae* (koa bug) on an equal basis and used cues for host acceptance similarly in both hosts. According to Louda, Pemberton, Johnson & Follett (2003) this shows the importance and validity of pre-release testing of native species to predict potential non-target effects. On the other hand, this may provide an alternative host for *T. basalis*, should *N. viridula* populations become low, thereby maintaining population numbers of the egg parasitoid.

1.2.3. California

According to Hoffmann, Davidson, Wilson, Ehler, Jones & Zalom (1991), *N. viridula* was first discovered in California, USA in 1986 in a processing tomato field. Tomatoes are reported to be a major crop of California and *N. viridula* is known to damage this crop. Stinkbug feeding damage to fresh market tomatoes can reduce crop quality and result in economic losses to producers. The SGS has also been associated with fungal and bacterial diseases of crop plants (Hoffmann *et al.,* 1991). Hoffmann *et al.* (1991) also state that soon after the discovery of the SGS in California, it fast became apparent that this

new pest could significantly threaten a variety of crops. Subsequently, by reviewing the records of *T. basalis* as bio-control agent in Australia and Hawaii, it was deemed necessary to import the parasitoid as control measure against *N. viridula* in California.

In co-operation with several government institutions the USDA-ARS European Parasite Laboratory, (Behoust, France) collected *T. basalis* from the Mediterranean areas of France, Italy and Spain as part of a pest management program aimed at managing pentatomid pests of tomatoes in California (Awan, Wilson & Hoffmann, 1990). The regions were specifically selected because of the similarity of climatic conditions to that of California (Hoffmann *et al.,* 1991).

In a study conducted by Awan *et al.* (1990), the three different populations from France, Italy and Spain were examined to determine the differences, if any, concerning immature developmental time, fecundity, adult longevity, sex ratio of progeny, length of oviposition period, percentage of host parasitism, mating, egg selection, oviposition and egg marking times. High parasitism rates were found during the laboratory experiments conducted, with an average of 75% showing after three days of adult parasitoid emergence. All in all the difference observed in the three geographic populations appeared to be minor. This reflected the possibility that all the populations collected represented the same biotype (Awan *et al.,* 1990).

Subsequently *T. basalis* was released at selected sites in Contra Costa, Fresno, Kern, San Joaquin, Stanislaus, Sacramento, San Diego, Solano and Yolo, all counties situated in California. In this regard several hundred *T. basalis* were released in 1987, whilst 180 000 individuals were released in 1988 and 1989 (Hoffmann *et al.,* 1991).

Several post-release studies were conducted by Hoffmann *et al.* (1991), during which several promising findings were made. For instance, It was determined that the SGS has a general lack of effective indigenous parasites, providing convincing support for the decision to import and release *T. basalis*

in California. Also, it was demonstrated that *T. basalis* also parasitizes eggs of two other stink bug species common to the area, *i.e.* the consperse stink bug (*Euschistus conspersus* Uhler) and the red-shouldered stink bug (*Thyanta pallidovirens* Stål). Hoffmann *et al.* (1991) mention that a positive offspin of the availability of these alternate hosts suggest that *T. basalis* could survive, and by implication maintain critical population sizes even in the absence of the main host, *N. viridula.* This would also increase the likelihood of *T. basalis* becoming an effective biological control agent in California, while controlling indigenous stink bug pests as well. High rates of parasitism was found during the study with almost 80% of the eggs being parasitized under field conditions. Hoffmann *et al.* (1991) also state that, at the time of the study their results indicate that *T. basalis* appeared to be established in Northern California.

No literature concerning more recent post-release studies could be found, and it is assumed that *T. basalis* has enjoyed considerable success in controlling *N. viridula* in California from earlier studies.

1.2.4. South Africa

It was only fairly recently that *T. basalis* was released in Southern Africa. According to Bruwer (1987), the southern green stinkbug, *N. viridula* has become a huge problem on macadamia nuts produced in South Africa, especially after the application of pesticides in an attempt to control the pest chemically. Consequently, it was decided that biological control would be attempted as an alternative control measure for *N. viridula.* In South Africa, *N. viridula* apparently favours nuts in its host range since Haddad & Louw (2006) report that some individuals belonging to the genus *Nezara,* possibly *N. viridula*, have also been sampled in pistachio orchards near Prieska, albeit in low numbers.

Two races of the wasp parasitoid *T. basalis* was imported from Hawaii and the USA state of Florida. Mass releases in South Africa began in September-October of the same year and establishment has apparently been successful. (Bruwer, 1987; Van den Berg, 1995). However, no recent literature on the current status of *T. basalis* as a biological control agent could be traced.

2. Material and Methods

These studies were conducted together with the studies concerning *A. raptoria*, described in the previous chapter. Thus, the study site, and experimental layout of each trial will not be discussed in detail. The details concerning the methods used during the trials, can be found in Chapter 2.

During the *A. raptoria* bio-ecology studies, the presence of *T. basalis* was confirmed in *A. raptoria* egg-packages. Further to determining parasitism success on *A. raptoria* egg packages and investigating this egg parasitoid as a possible bio-control agent, the effect of temperature on parasitoid emergence rates was given specific attention, since this issue relates to degree day models (see Chapter 7). Consequently, the following studies were conducted.

2.1. Statistical significance of parasitoid loads versus stinkbug hatching success

The number of A. raptoria eggs parasitized by T. basalis could hold great implications for future research. If *T*. basalis loads are heavy, this wasp may in future be implemented in the biological control of *A. raptoria.* To determine if the difference between parasitized eggs and normal, hatching eggs, is indeed significant, a paired t-test was done. This was done using GraphPad InStat ©, version 3.00. The program was used to calculate median values of parasitoids and nymphs that successfully emerged. From these values, a paired t-test was done, determining the one-tailed P-value.

2.2. Effect of temperature on parasitoid emergence rates

As was the case during the study done on *A. raptoria* egg-packages, higher temperatures should increase the rate of development. By noting the day of parasitoid emergence at varying temperatures, the acceleration in emergence rate could be determined. These data sets were compared to determine if development indeed occurred at a faster rate at high temperatures.

2.3. Temperature thresholds for *T. basalis*

There is a developmental threshold temperature for each different species of insect. First off, one finds the lower developmental threshold. No development occurs when temperatures are below this level. Insects also have an optimum temperature range in which they will develop most rapidly. Then, there is maximum temperature (termed upper cutoff) above which development is terminated (Johnson, Bessin & Townsend, 1998). By investigating the success rates of parasitism at different temperatures, it can be determined what the optimum temperature, as well as the upper and lower temperature thresholds for *T. basalis* is. These thresholds are determined experimentally and are different for each species (Higley & Wintersteen, 1997).

3. Results and Discussion

3.1. Statistical significance of parasitoid loads versus stinkbug hatching success

By comparing all successfully emerged parasitoids and all successfully hatching A. raptoria nymphs, medians could be obtained. The parasitoids showed a median value of 88.29 and the nymphs a median of 17.86. The mean difference was equal to -70.429. By doing the paired t-test the following statistics were obtained. The one-tailed P value is 0.0088, which is considered very significant with the P-value < 0.05. Also, the degrees of freedom was t = 3.245 with 6 degrees of freedom.

The P value of 0.0088 shows that the parasitoid loads were indeed, significantly more than successful egg eclosions. This shows that *T. basalis* does indeed hold great promise as biological control agent. Further studies as to the exact success rate of parasitism can be conducted in the future. A One-way Analysis of Variance (ANOVA) could not be done, as only two values were compared.

3.2. Effect of temperature on parasitoid emergence rates

Since insects are cold-blooded animals, temperature plays a major role in their growth and development (Johnson *et al.,* 1998). Temperature controls the developmental rate of many organisms and plants and invertebrate animals, including insects and nematodes, require a certain amount of heat to develop from one point in their life cycles to another. This measure of heat is known as physiological time (Anon, 2003)

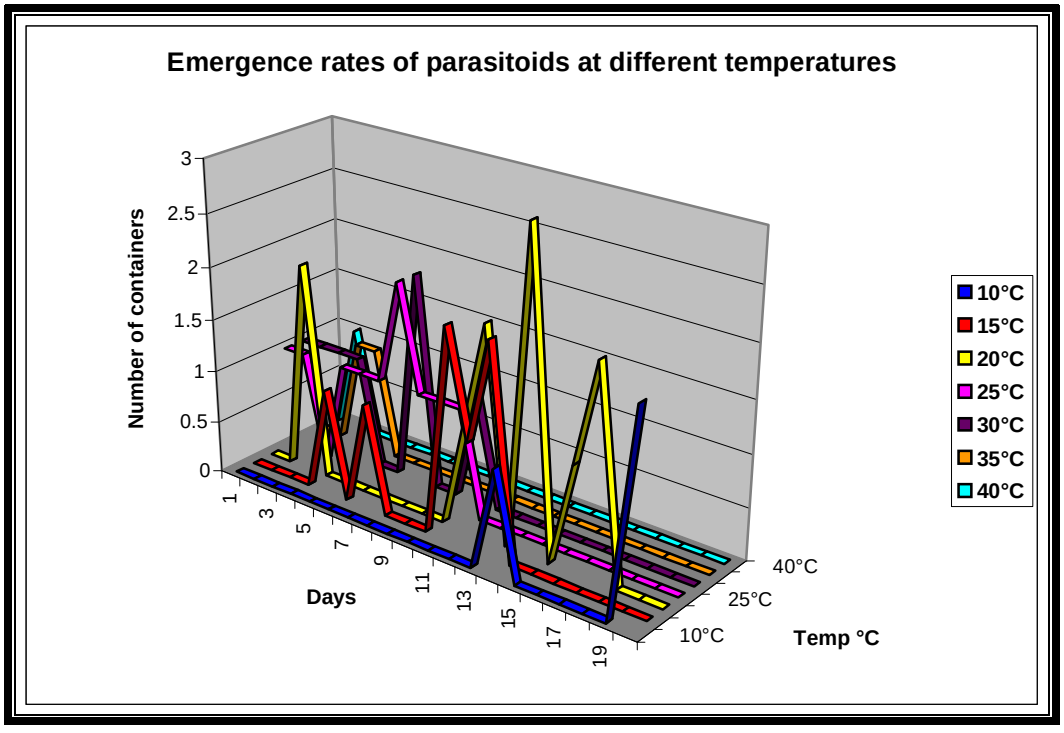


Figure 1: Developmental succession of parasitoids (*T. basalis*) at temperatures ranging from 10°C to 40°C.

Figure 1 shows that development did indeed occur faster at higher temperatures. Parasitoids maintained at 40°C developed in the first few days (day 2), closely followed by those at 35°C (days 3 & 4). Results further show a definite trend for development to be delayed at lower temperatures. Parasitoids at 10°C first emerged on day 14 of the study.

3.3. Temperature thresholds of *Trissolcus basalis.*

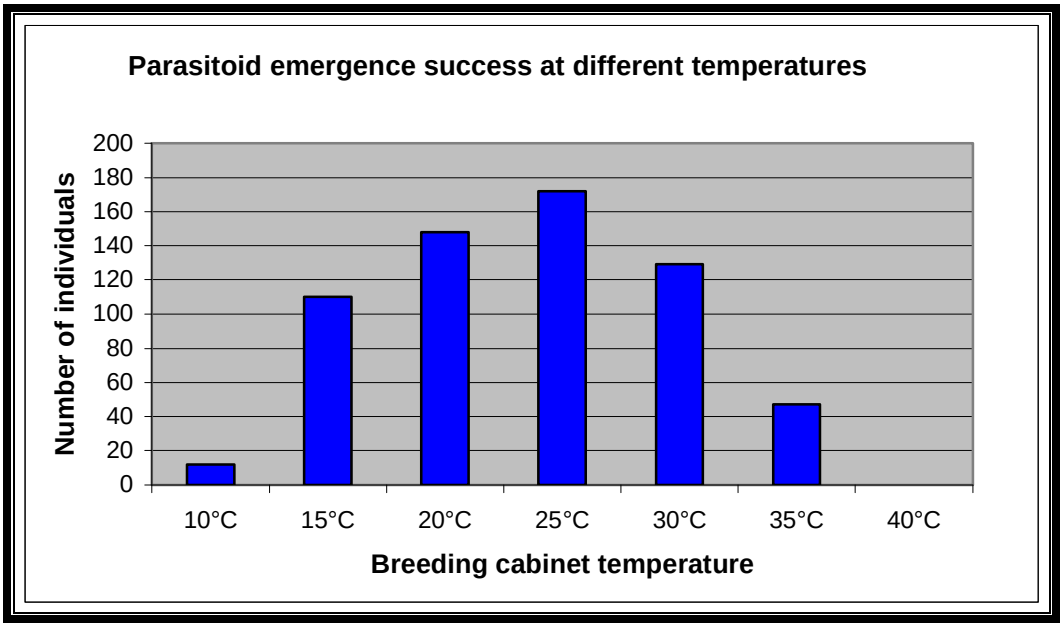


Figure 2: Parasitoid emergence success from *A. raptoria* eggs at various temperatures.

Figure 2 clearly shows that successful development of the parasitoids increased towards mid-range temperatures and then again decreased, as temperatures got higher. From this data, approximate lower and upper temperature thresholds for the parasitoids could be determined.

Based on this temperature thresholds could be determined for the successful emergence of *T. basalis* parasitoids (Table 1). Optimal developmental temperature appears to be between 20°C and 30°C. This is the temperature range at which most of the parasitoids successfully emerge from the egg packages.

Table 1: Upper and lower temperature thresholds for the parasitoid *Trissolcus basalis.*

	Lower threshold	**Upper threshold**
***Trissolcus* basalis**	**< 10°C**	**35°C < 40°C**

4. Conclusion

When one considers the history of this parasitoid, it appears as if great success has been achieved in deploying it for the control of *N. viridula.* By considering the parasitoid loads found on *A. raptoria* eggs, it was determined that 66.9% of eggs maintained between 20°C and 30°C were parasitized, although not all emerged successfully. When compared with the 79.9 % of *N.viridula* eggs parasitized in California, it can be regarded as reasonably successful. If one considers that all *A. raptoria* eggs were parasitized by naturally occurring populations of *T. basalis*, and that *A. raptoria* is actually not the documented preferred host of this parasitoid, great potential as biological control agent can be expected.

Should natural parasitoid populations be augmented by means of mass release, much higher parasite loads could be expected. Nectar sources can also be planted around orchards or as part of the existing cover crops between tree rows to attract and sustain *T. basalis* populations. Certain kairomones, like that secreted by *N. viridula*, can also attract this parasitoid. Mattiacci, Vinson, Williams, Aldrich, & Bin (1993) has reported on the identification of a volatile in the defensive secretion of *N. viridula* that attracts *T. basalis.* If kairomone sources were to be placed in orchards, much greater numbers of *T. basalis* could be attracted, with the result that greater percentages of A. raptoria egg packages will be parasitized. In Brazil there is currently a programme that supplies soybean growers with egg packages of *N. viridula* parasitized by *T. basalis* (Panizzi *et al.,* 2000). If such programs could be implemented in South Africa, much greater success can be expected.

The temperature thresholds found in the case of *T. basalis* also shows that this wasp will be able to survive nearly anywhere in South Africa. With a lower developmental threshold of below 10°C, it should be tolerant to colder nights, such as those found in the more arid regions within which Prieska and Green Valley Nuts fall. The upper developmental threshold was found to be between 35°C and 40°C. This also indicates that *T. basalis* will experience little difficulty surviving in hot, arid regions. Moreover, it would appear that the optimal temperature preference falls between 20°C and 30°C, a fairly wide range.

In terms of chemical control, care has to be taken when applying chemicals. Chemicals should be chosen which does not target the parasitoid and rather specifically targets pest species. Weedon *et al.* (2004) mention that *T. basalis* was little affected by applications of permethrin, but was susceptible to methyl parathion, especially within the first six hours. The methyl parathion also killed some wasps as they exited the host eggs, probably from spray residue occurring on the egg chorion becoming ingested as they chewed through.

5. References

Anonymous. 2003. *About degree-days.* Statewide IPM Program, Agriculture and Natural Resources, University of California. Retrieved from Http://www.ipm.ucdavis.edu/WEATHER

Awan, M. S., Wilson, L. T. & Hoffmann, M. P. 1990. Comparative biology of three geographic populations of *Trissolcus basalis* (Hymenoptera: Scelionidae). *Environmental Entomology* **19:** 387-392.

Bruwer, I. J. 1987. Parasiete ingevoer om neutplaag biologies te beheer. *Citrus and Subtropical Fruit Research Institute, Information Bulletin* **175**: 1 -2.

Clarke, A. R. 1990. The control of *Nezara viridula* L. with introduced egg parasitoids in Australia. A review of a 'landmark' example of classical biological control. *Australian Journal of Agriculture* **41:** 1127 – 1146.

Clarke, A. R. & Walter, G. H. 1993. Biological control of green vegetable bug (*Nezara viridula* (L.)) in eastern Australia: Current status and perspectives, pp. 223-225. In: *Pest control and Sustainable Agriculture.* S. A. Corey, D. J. Dall & W. M. Milne (Editors). CSIRO Division of Entomology, Canberra. (Original article not seen).

Davis, C. J. 1964. The introduction, propagation, liberation, and establishment of parasites to control *Nezara viridula* variety *smaragdula* (Fabricius) in Hawaii (Heteroptera: Pentatomidae). *Proceedings of the Hawaiian Entomological Society* **18(3):** 369-375. (Original article not seen).

Davis, C. J. 1967. Progress in the biological control of the southern green stink bug, *Nezara viridula* variety *smaragdula* (Fabricus) in Hawaii (Heteroptera: Pentatomidae). *Mushi*. **39:** 9-16. (Original article not seen).

Haddad, C. R. & Louw, S vd M. 2006. Phenology and potential biological control of the stink-bug *Atelocera raptoria* Germar (Hemiptera: Pentatomidae) in pistachio orchards. *African Plant Protection* **12:** 24 – 27.

Higley, L. & Wintersteen, W. 1997. *Using Degree Days in an Integrated Pest Management Program.* Iowa State University, University Extension. Retrieved from Http://www.extension.iastate.edu/Publications

Hoffmann, M. P., Davidson, N. A., Wilson, L. T., Ehler, L. E., Jones, W. A. & Zalom, F. G. 1991. Imported wasp helps control green stink bug. *California Agriculture* **43(3):** 20 – 22.

Johnson, D., Bessin, R. & Townsend, L. 1998. *Predicting insect development using degree days.* University of Kentucky Entomology. Retrieved from Http://www.uky.edu/Agriculture/entomology

Johnson, M. T., Follett, P. A., Taylor, A. D. & Jones, V. P. 2005. Impacts of biological control and invasive species on a non-target native Hawaiian insect. *Oecologia* ***1*42:** 529 – 540.

Jones, V. P. 1995. Reassessment of the role of predators *Trissolcus basalis* in biological control of the southern green stink bug (Hemiptera: Pentatomidae) in Hawaii. *Biological Control* **5:** 566−572.

Jones, V. P., Westcott, D. M., Finson, N. N. & Nishimoto, R. K. 2001. Relationship between community structure and southern green stink bug (Hemiptera: Pentatomidae) damage in macadamia nuts. *Environmental Entomology* **30:** 1028–1035.

Jones, V. P. & Westcott, D. 2002. The effect of seasonal changes on *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) in Hawaii. *Biological Control* **23:** 115–120.

Loch, A. D. 2000. Abundance, distribution, and availability of *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) hosts in a soybean agricultural system in southeastern Queensland. *Biological Control* **18:** 120−135.

Loch, A. D. & Walter, G. H. 1999. Multiple host use by the egg parasitoid *Trissolcus basalis* (Wollaston) in a soybean agricultural system: biological control and environmental implications. *Agricultural and Forest Entomology* **1(4):** 271-280

Louda, S. M., Pemberton, R. W., Johnson, M. T. & Follett, P. A. 2003. Nontarget effects – The Achilles' heel of Biological Control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology* **48:** 365 – 396.

Mattiacci, L., Vinson, S. B., Williams, H. J., Aldrich, J. R. & Bin, F. 1993. A long-range attractant kairomone for egg parasitoid *Trissolcus basalis,* isolated from defensive secretion of its host, *Nezara viridula. Journal of Chemical Ecology* **19:** 1167–1181**.** (Original article not seen).

Meats, A. & Castillo Pando, M. S. 2002. Ratio-dependant parasitism with *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) on egg rafts of *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae): effect of experimental variables and compatibility of 'ratio' and 'Holling' models. *Australian Journal of Entomology* **41:** 243-252.

Panizzi, A.R., McPherson, J.E., James, D.G., Javahery, M. & McPherson, R.M. 2000. Stink bugs (Pentatomidae), pp. 421-474. In: Schaefer, C. W. & Panizzi, A. R. (Editors), 2000. *Heteroptera of Economic Importance.* CRC press, London.

Todd, J. W. 1989. Ecology and behavior of *Nezara viridula*. *Annual Review of Entomology* **34:** 273–292.

Van den Bosch, R. & Messenger, P.S. 1973. *Biological Control.* Intext Educational Publishers, New York. 180 pp.

Van den Berg, M. A. 1995. *Pests attacking macadamia in South Africa*. The 6th Conference of the Australasian Council on Tree and Nut Crops, Lismore, Australia.

Weedon, C.R., Shelton, A.M., Li, Y. & Hoffmann, M.P. 2004. *Trissolcus basalis* (Hymenoptera: Scelionidae). Biological Control, a guide to natural enemies in North America, Cornell University. Retrieved from Http://www.nysaes.cornell.edu/ent/biocontrol

Appendix 1

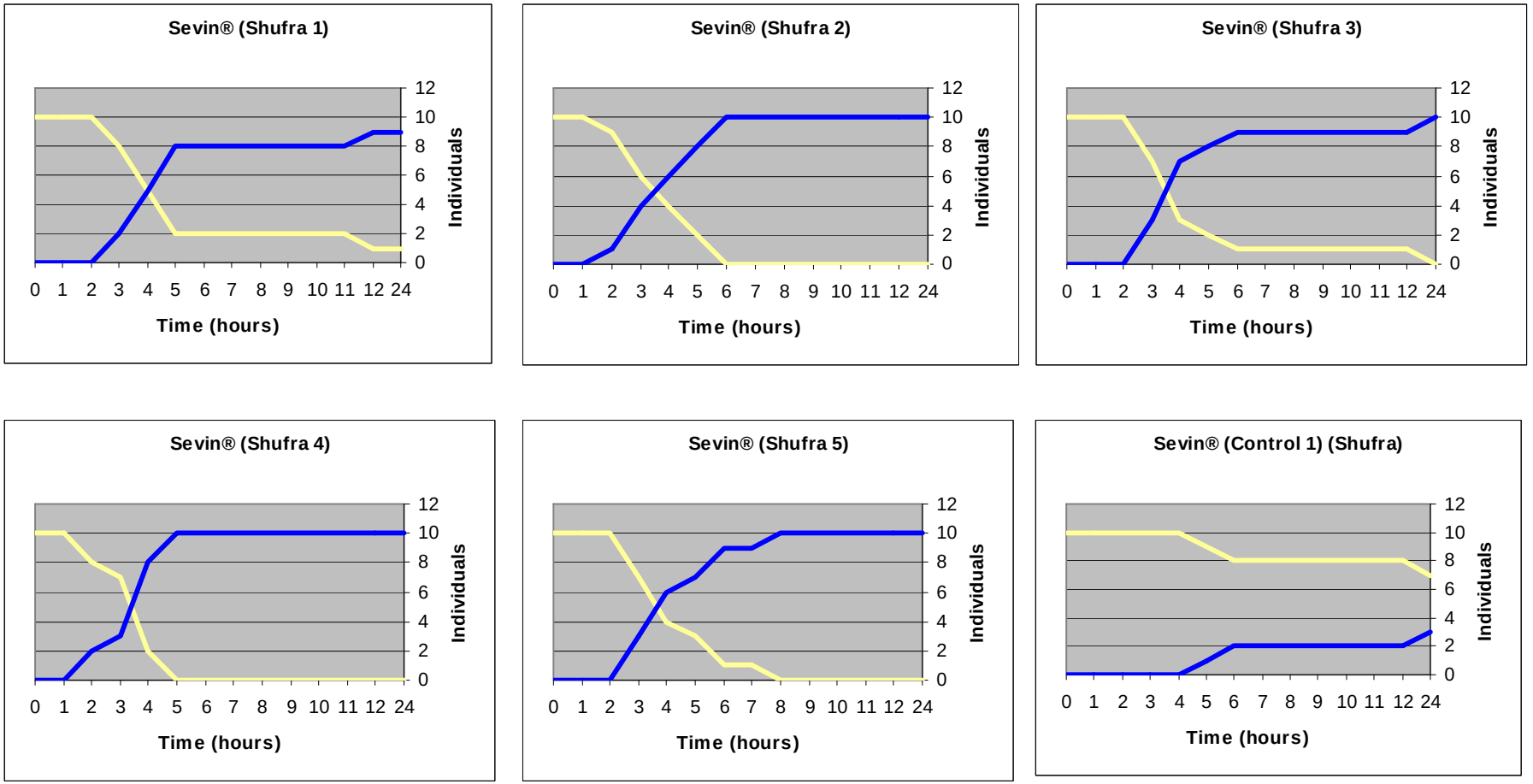


Figure 1: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Shufra trees using Sevin®.

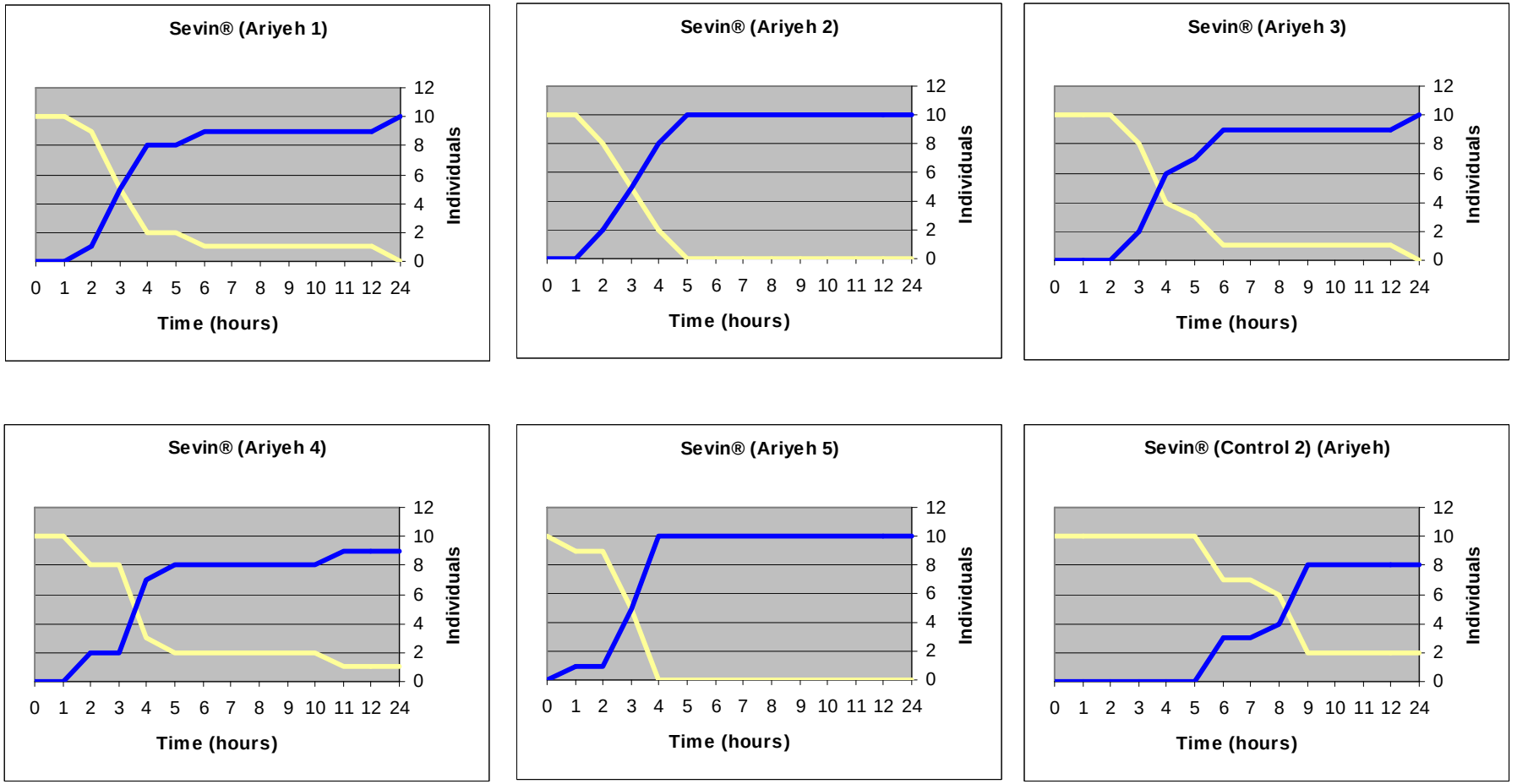


Figure 2: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Ariyeh trees using Sevin®.

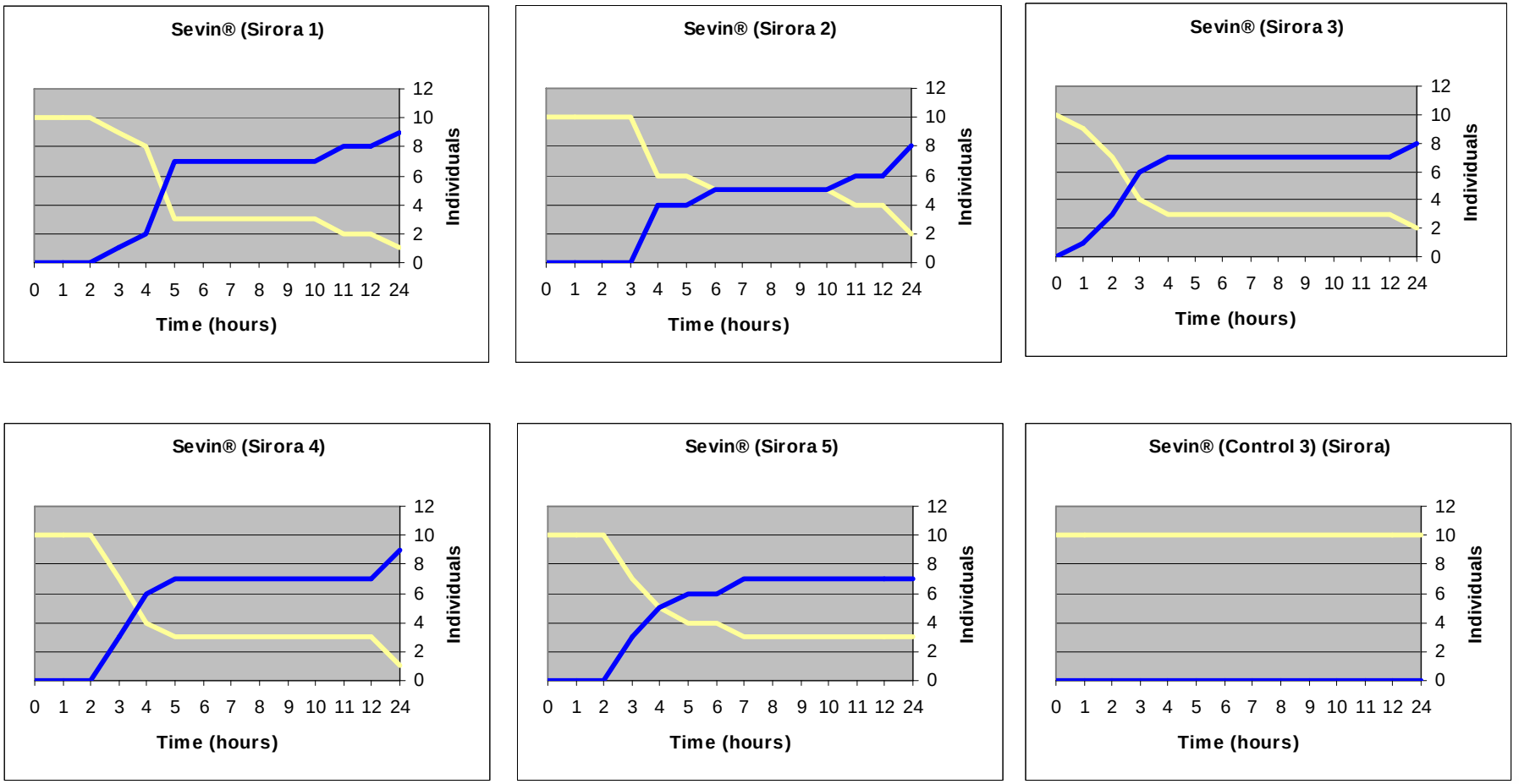
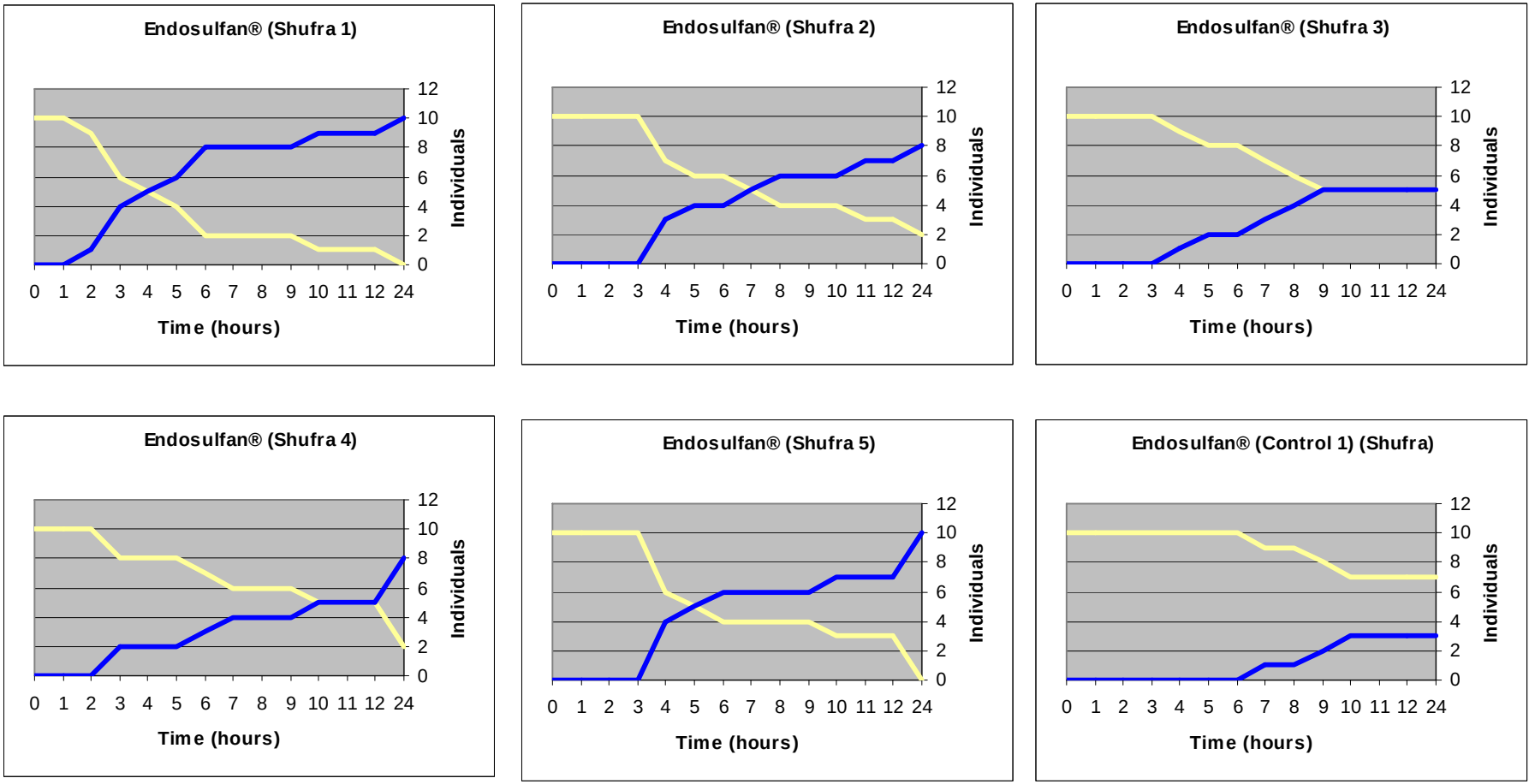


Figure 3: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Sirora trees using Sevin®.



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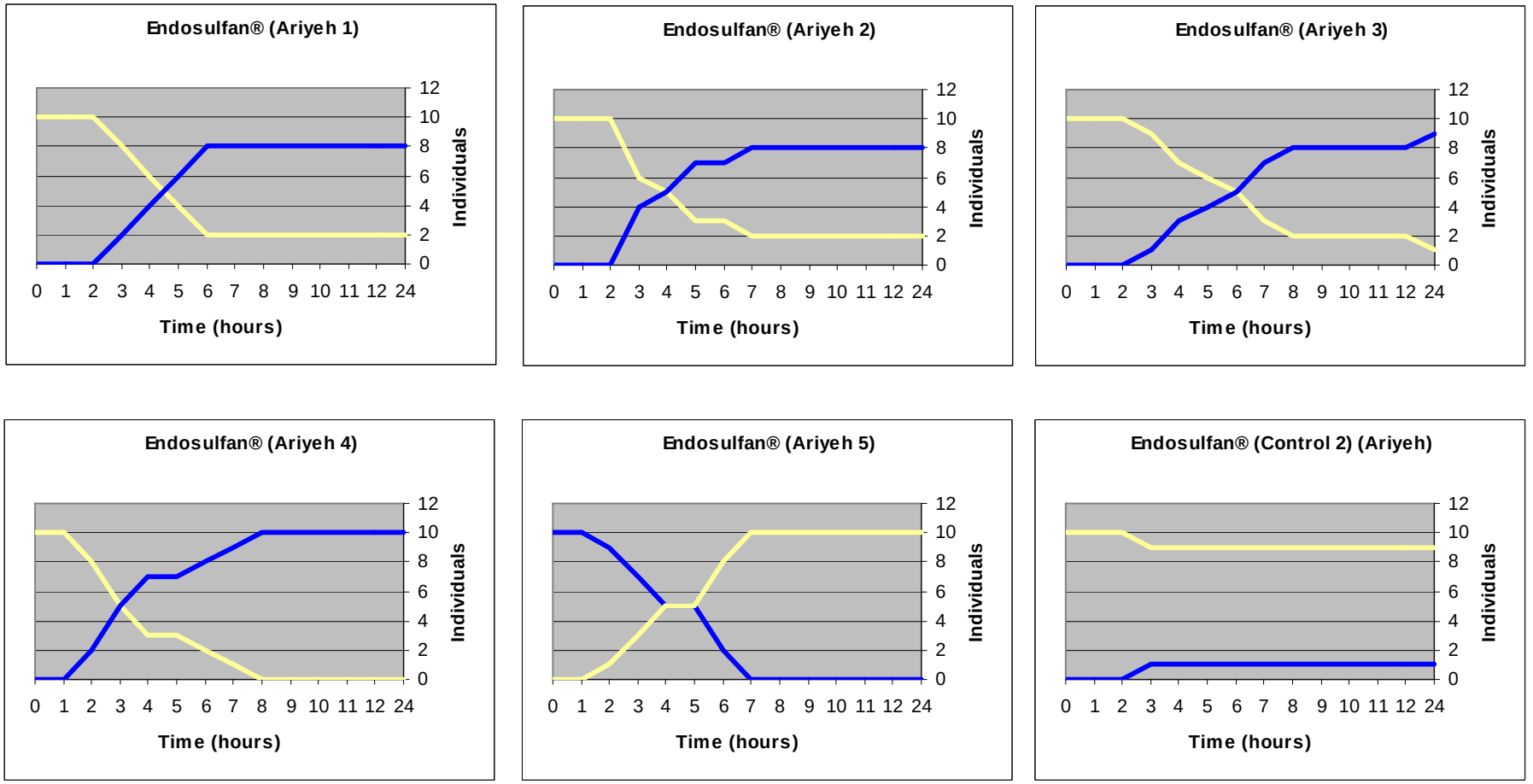

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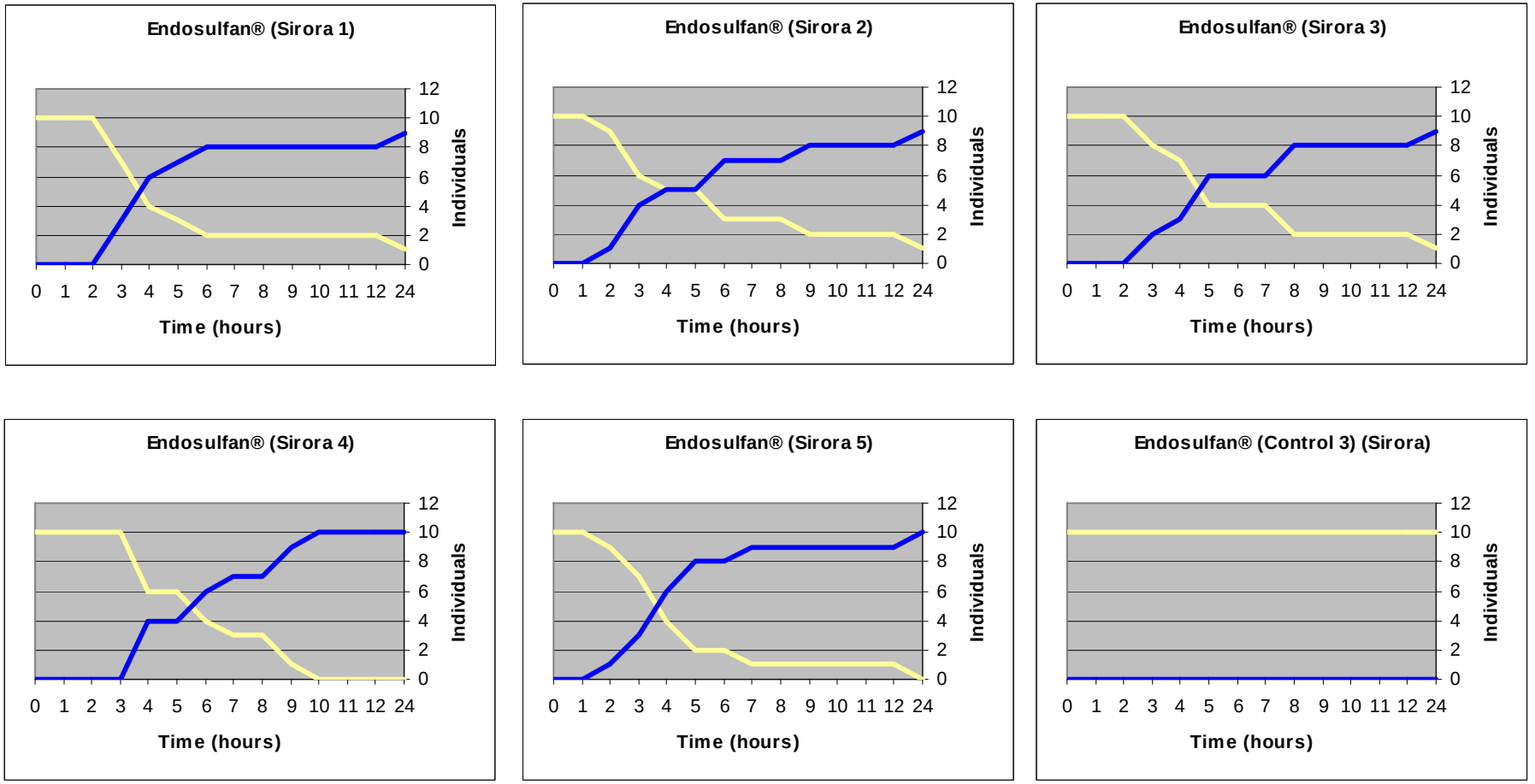


Figure 6: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Sirora trees using Endosulfan®.

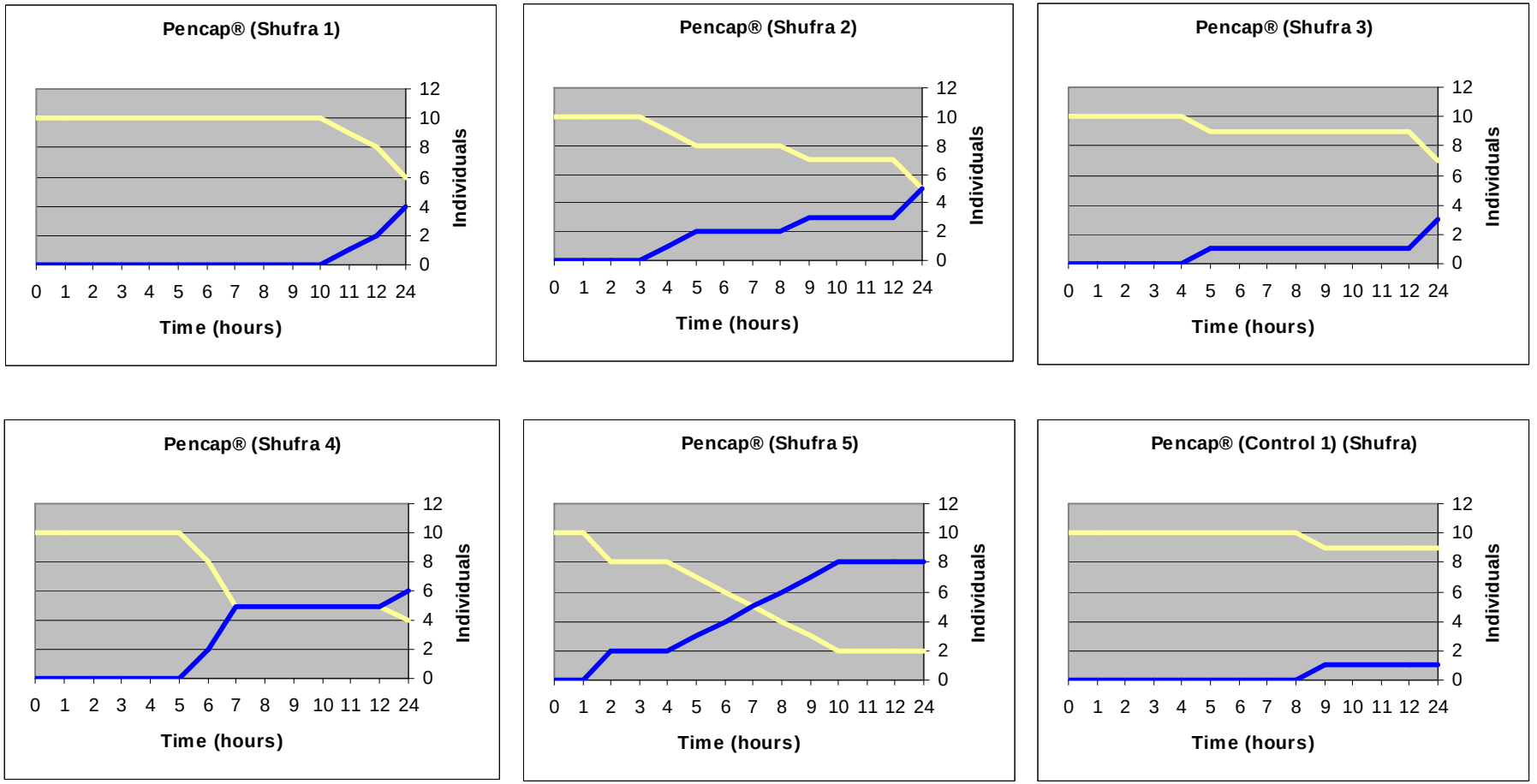


Figure 7: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Shufra trees using Pencap®.

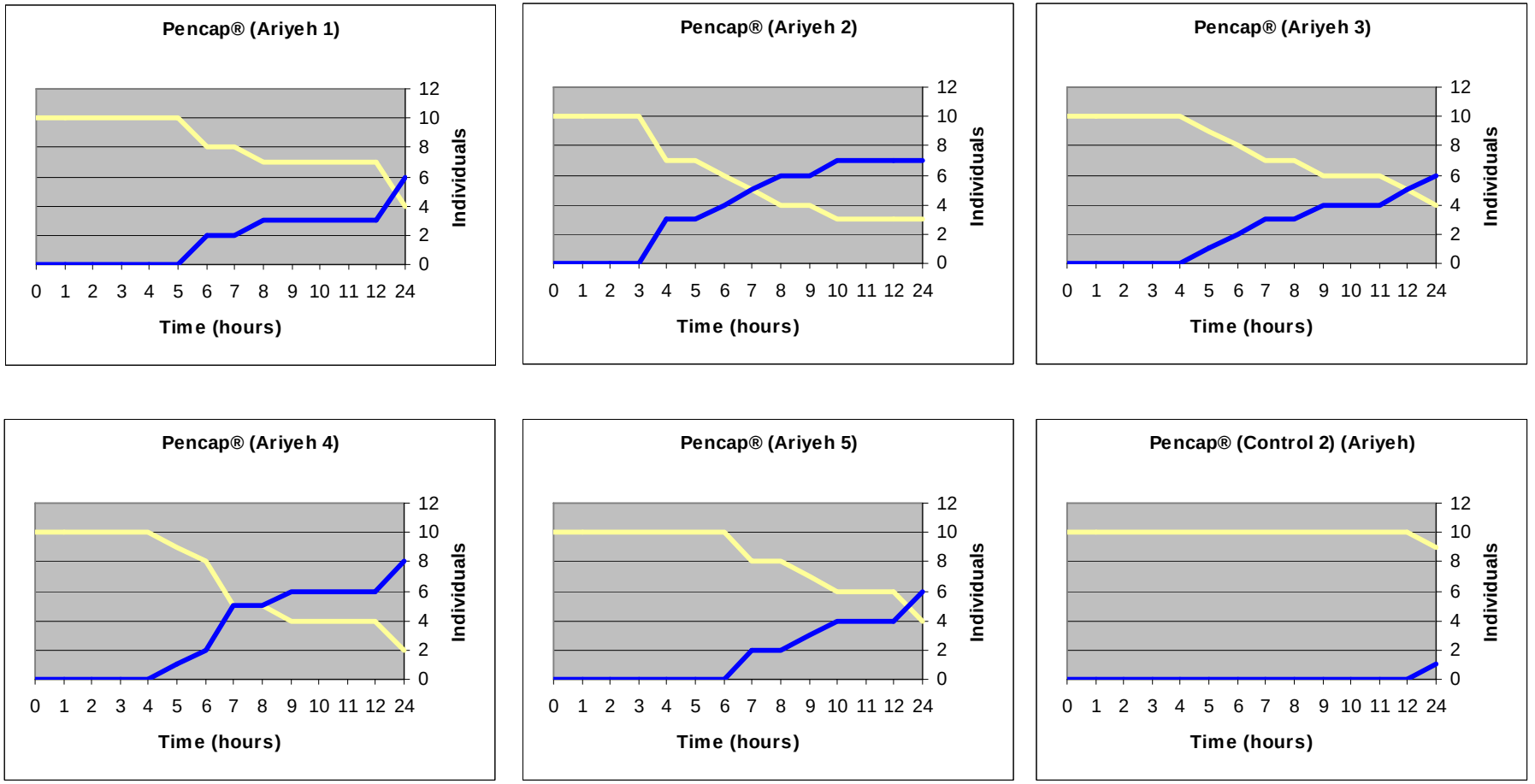


Figure 8: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Ariyeh trees using Pencap®.

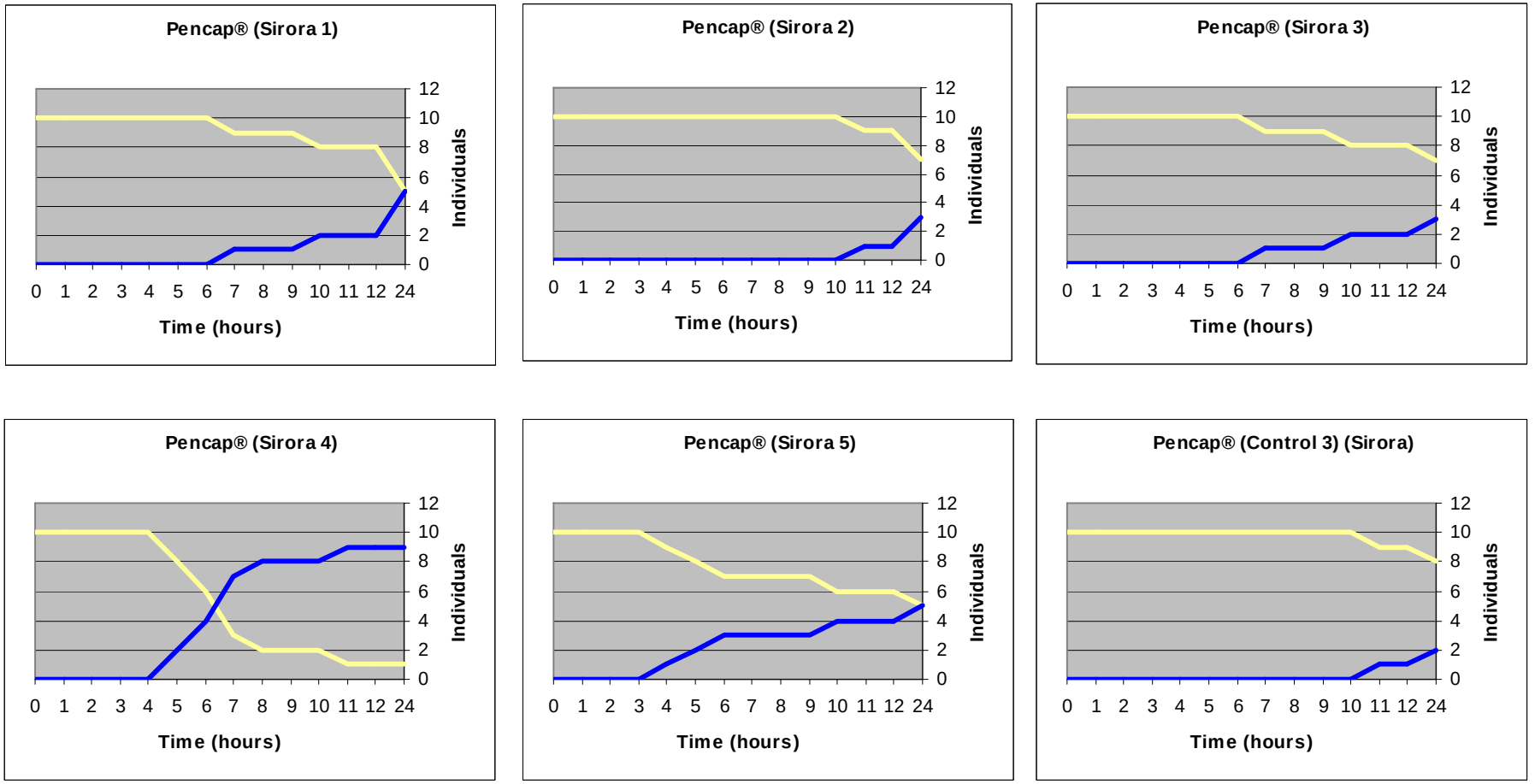


Figure 9: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Sirora trees using Pencap®.

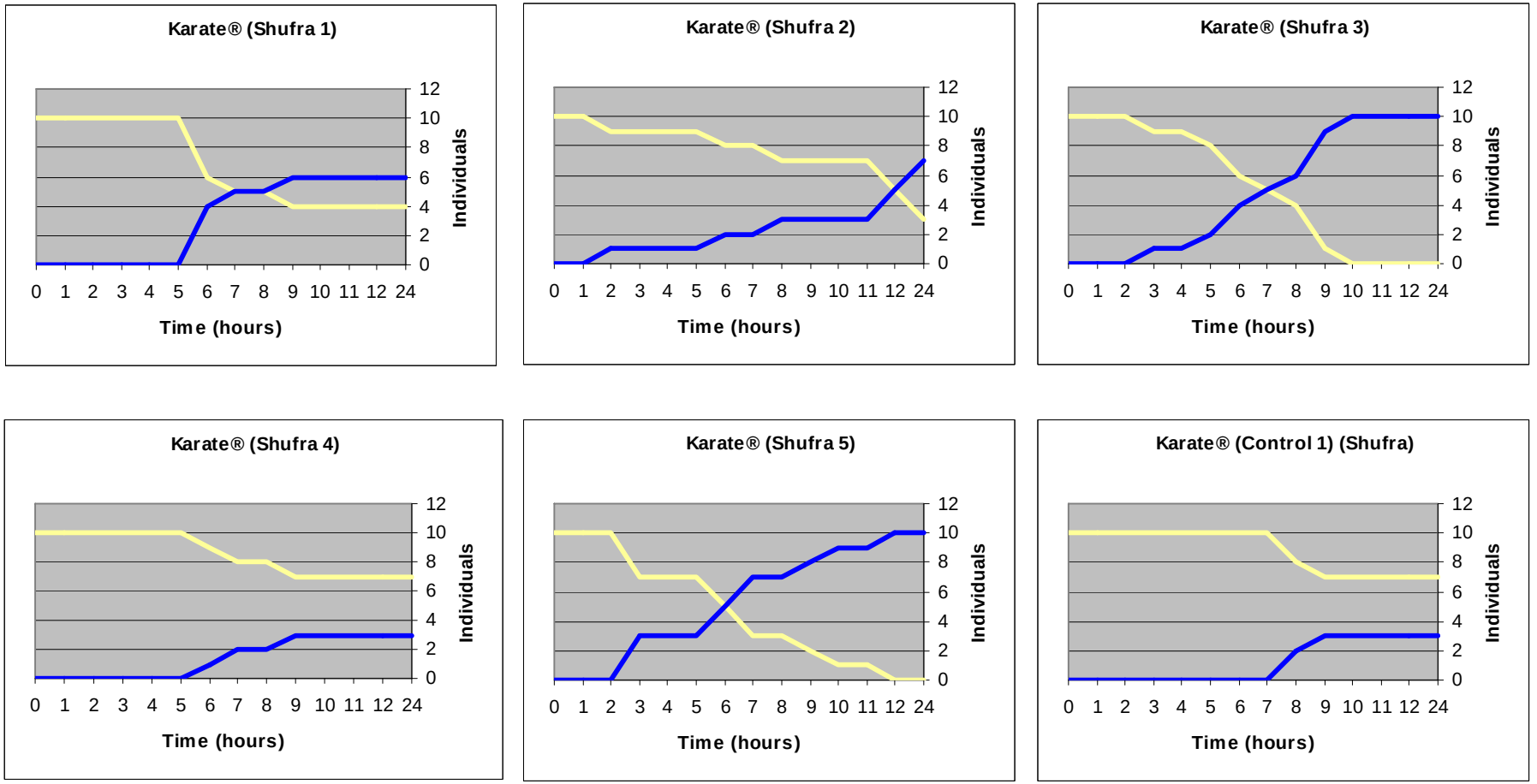
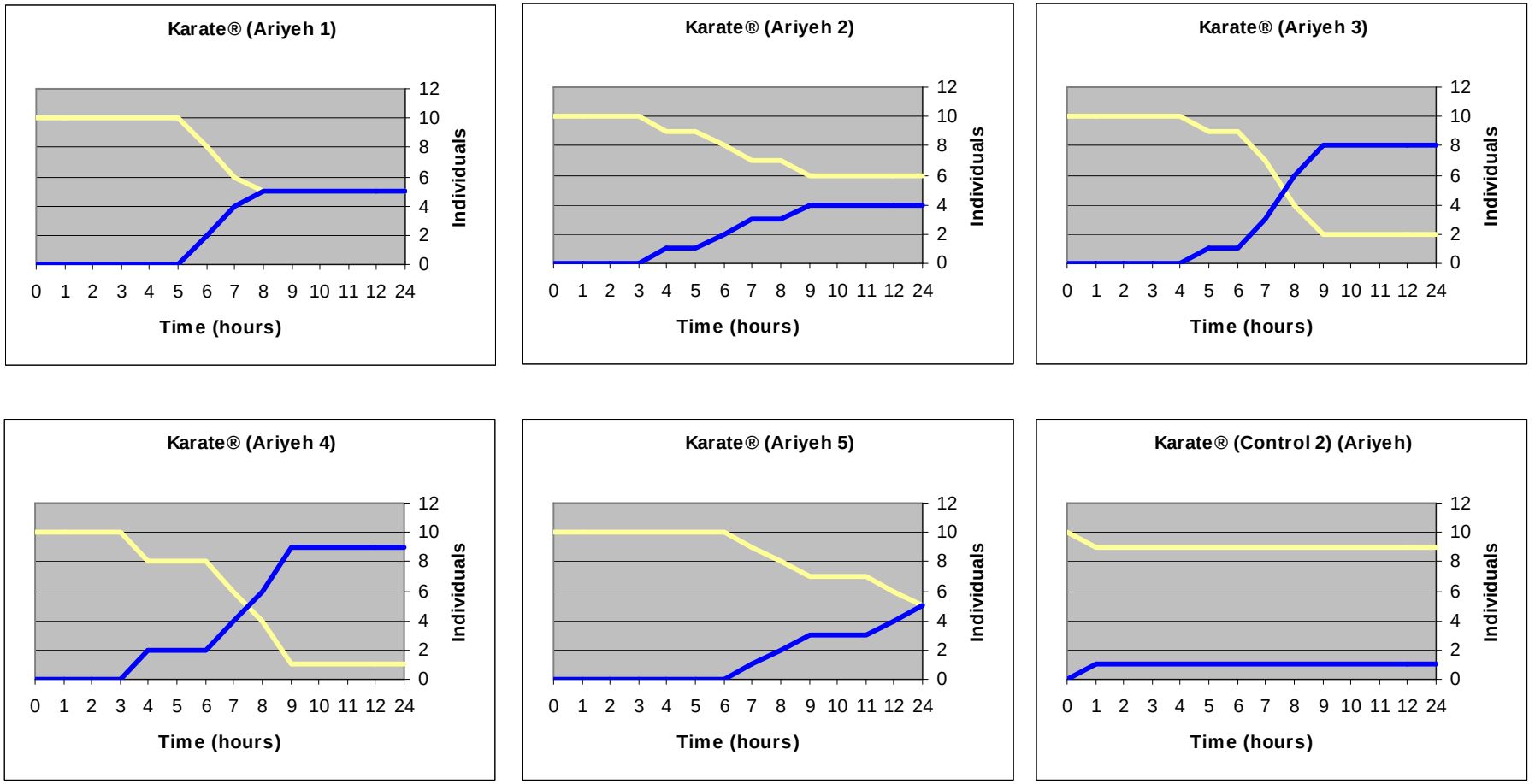


Figure 10: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Shufra trees using Karate®.



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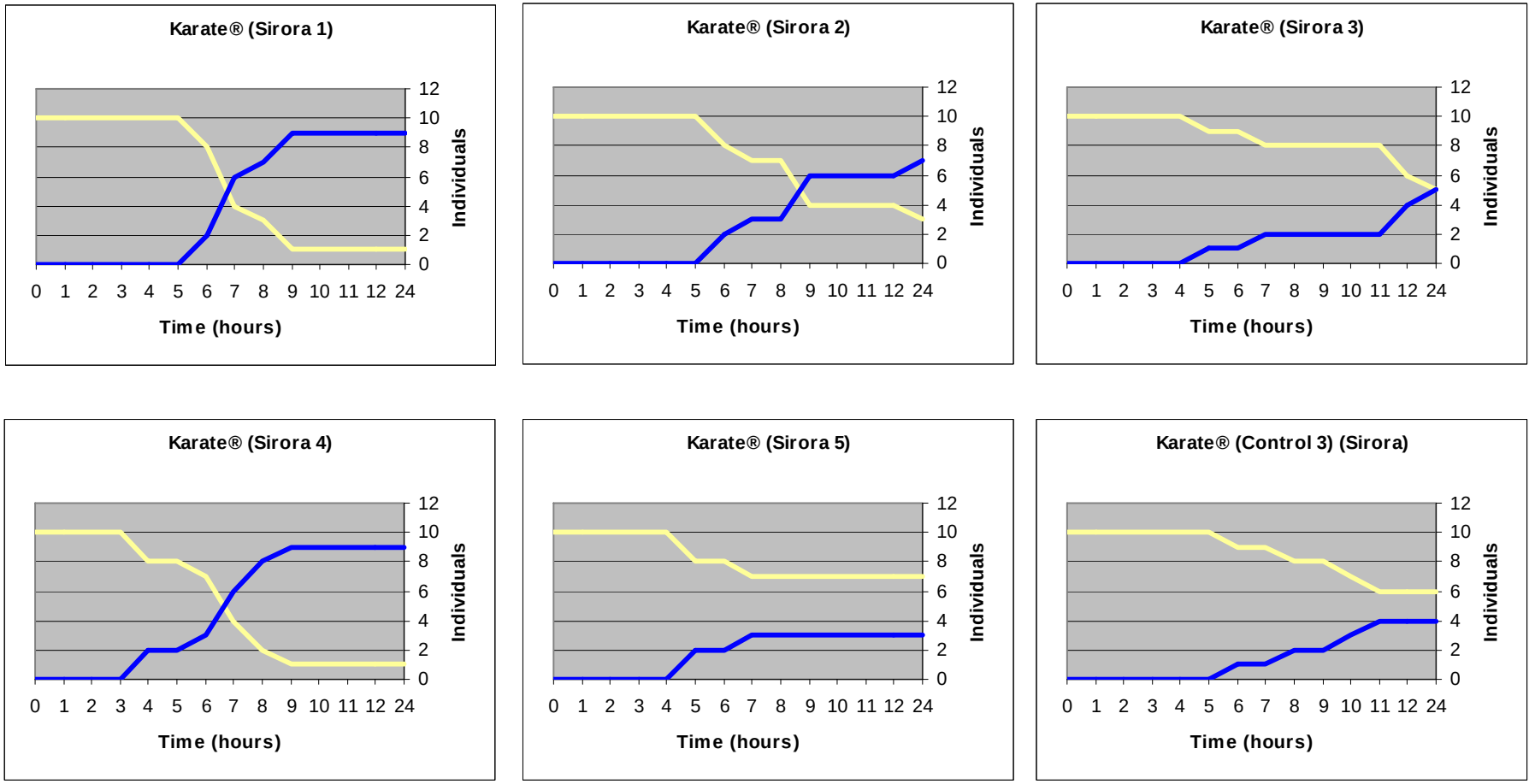

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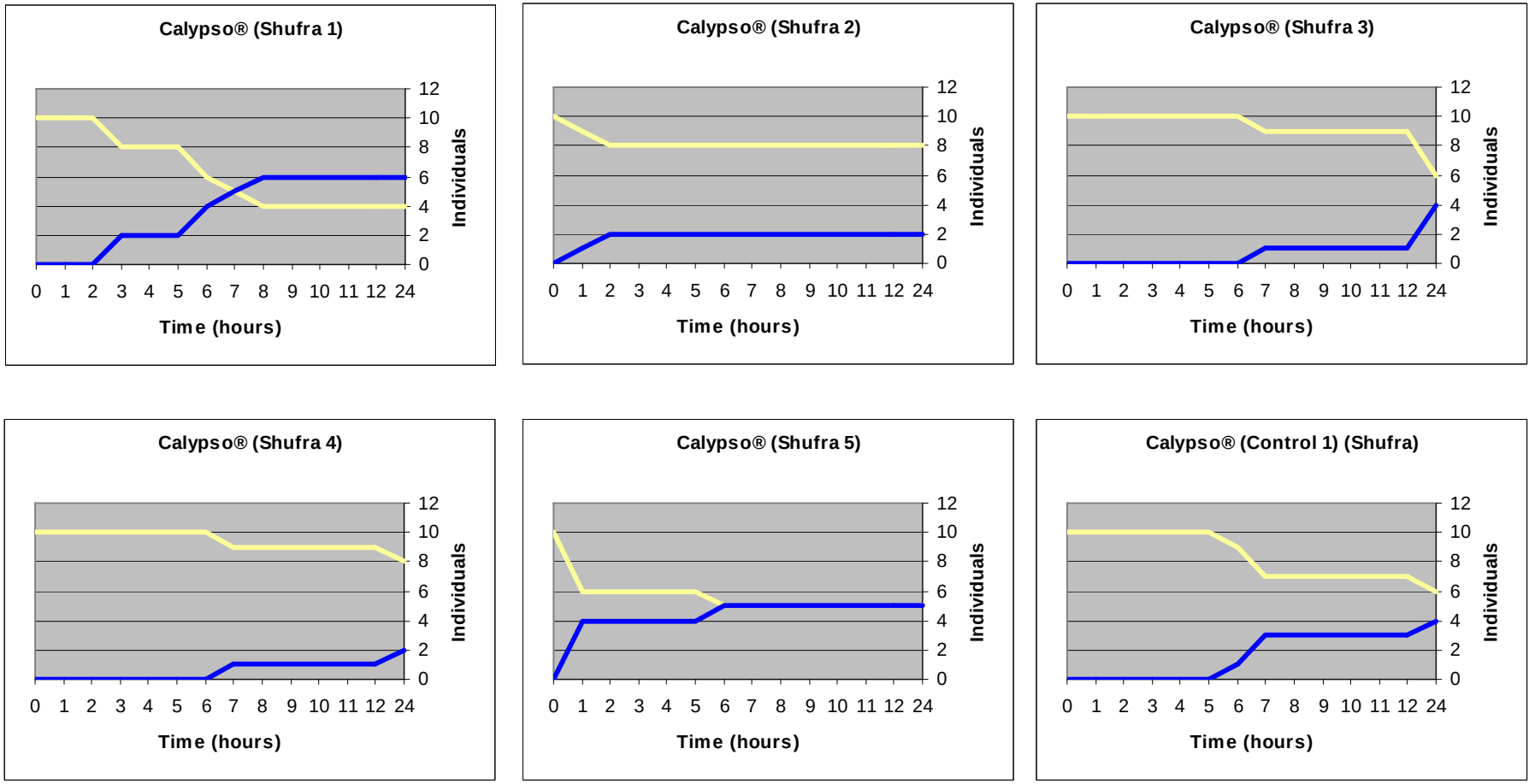


Figure 13: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Shufra trees using Calypso®.

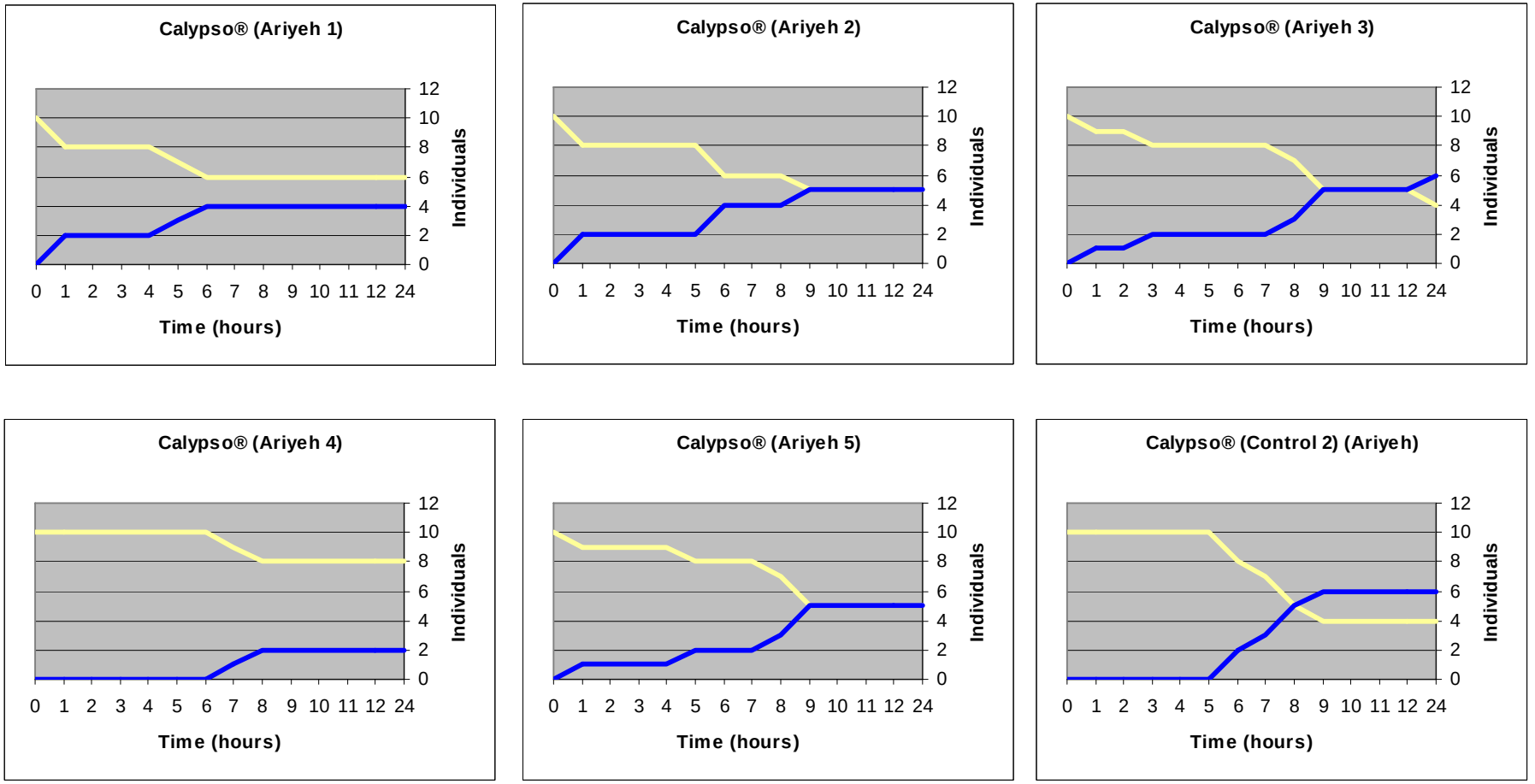


Figure 14: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Ariyeh trees using Calypso®.

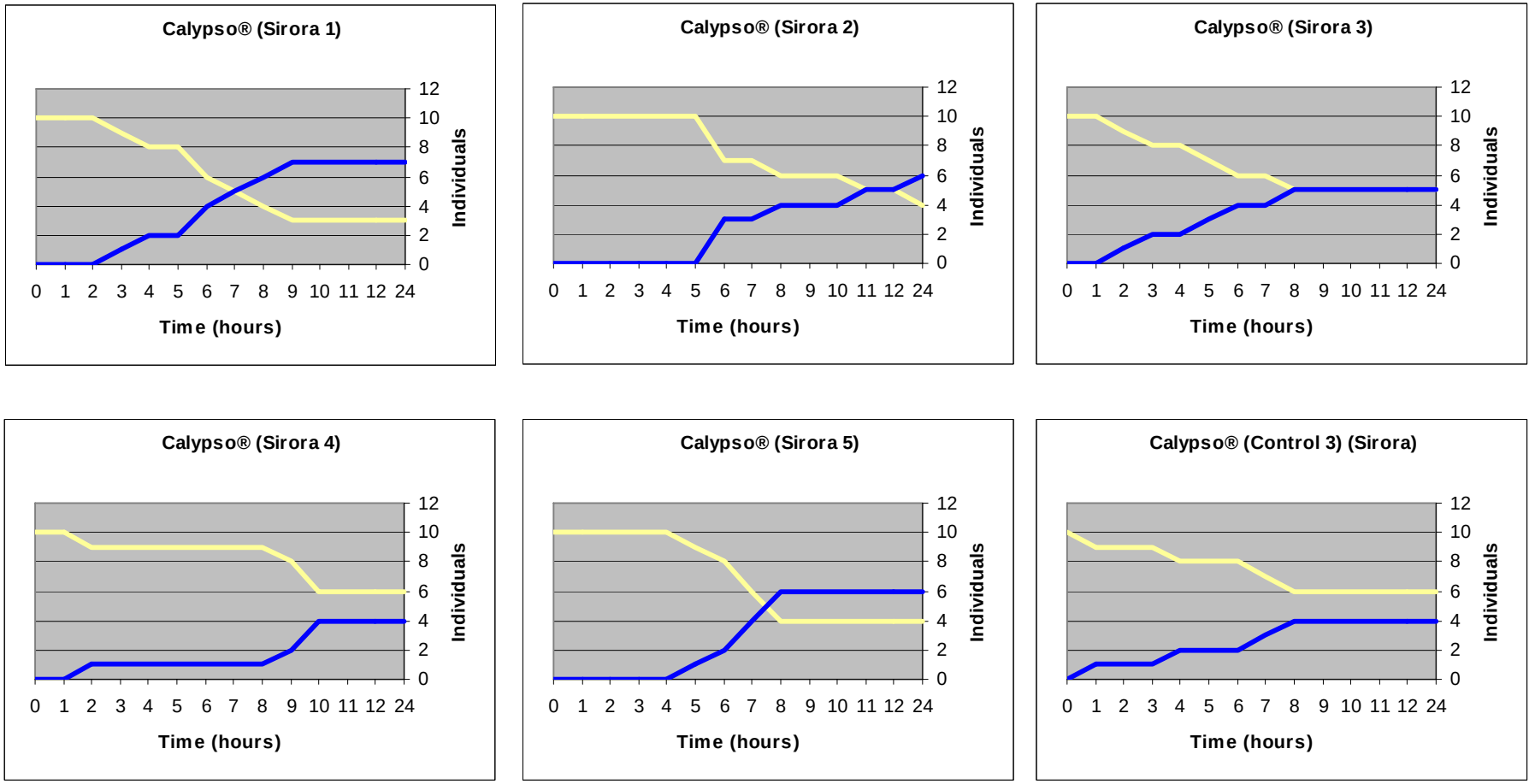


Figure 15: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Sirora trees using Calypso®.

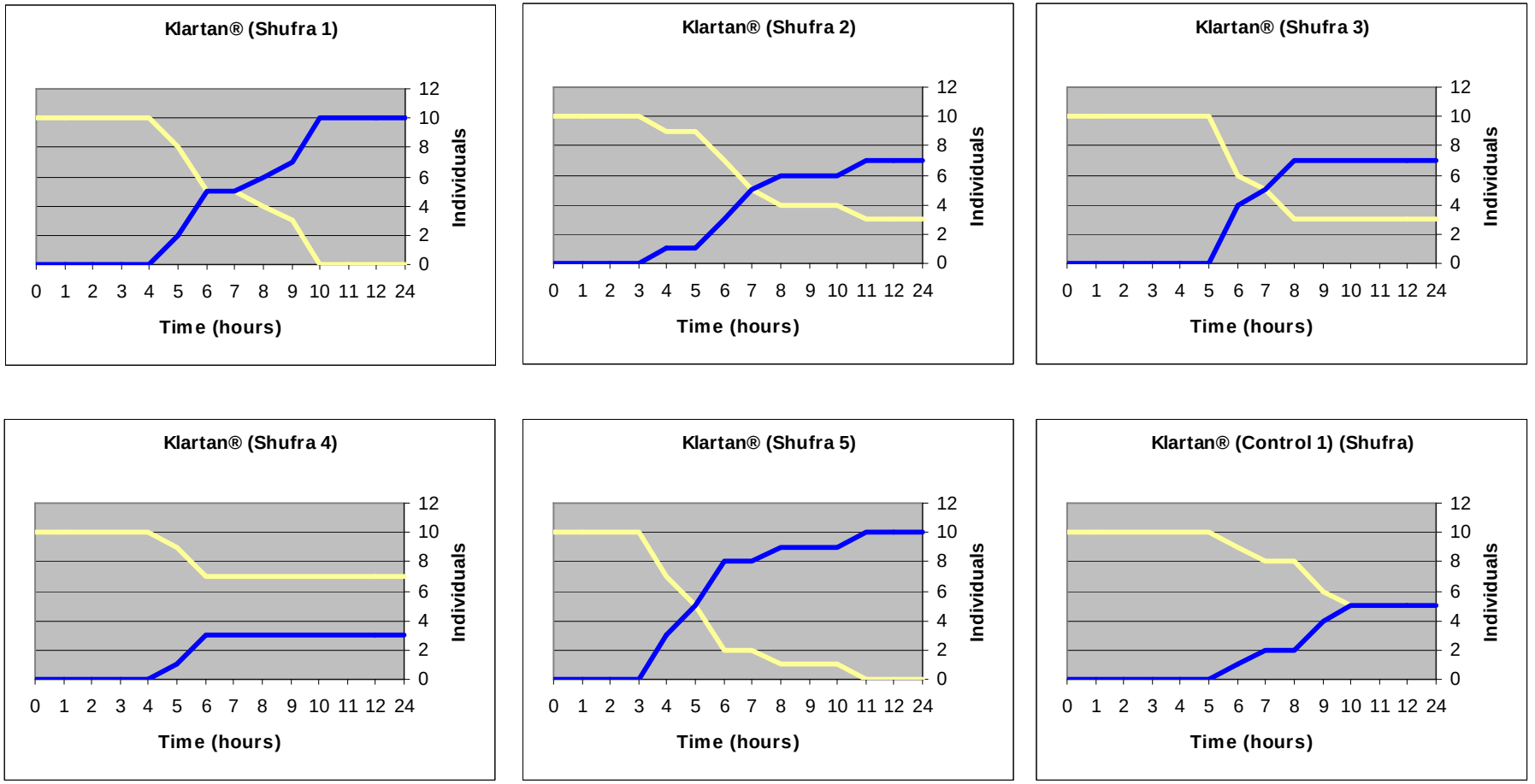


Figure 16: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Shufra trees using Klartan®.

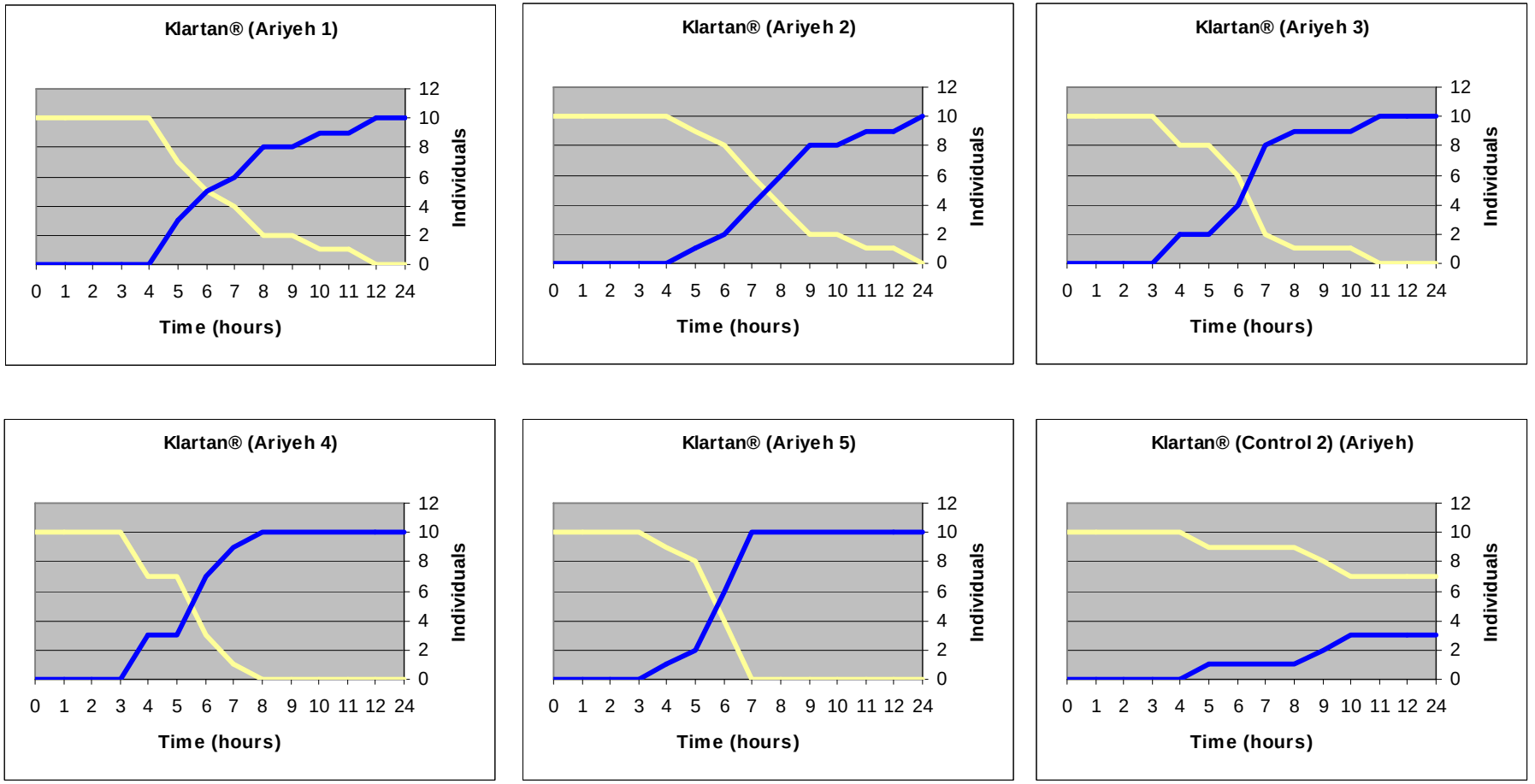


Figure 17: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Ariyeh trees using Klartan®.

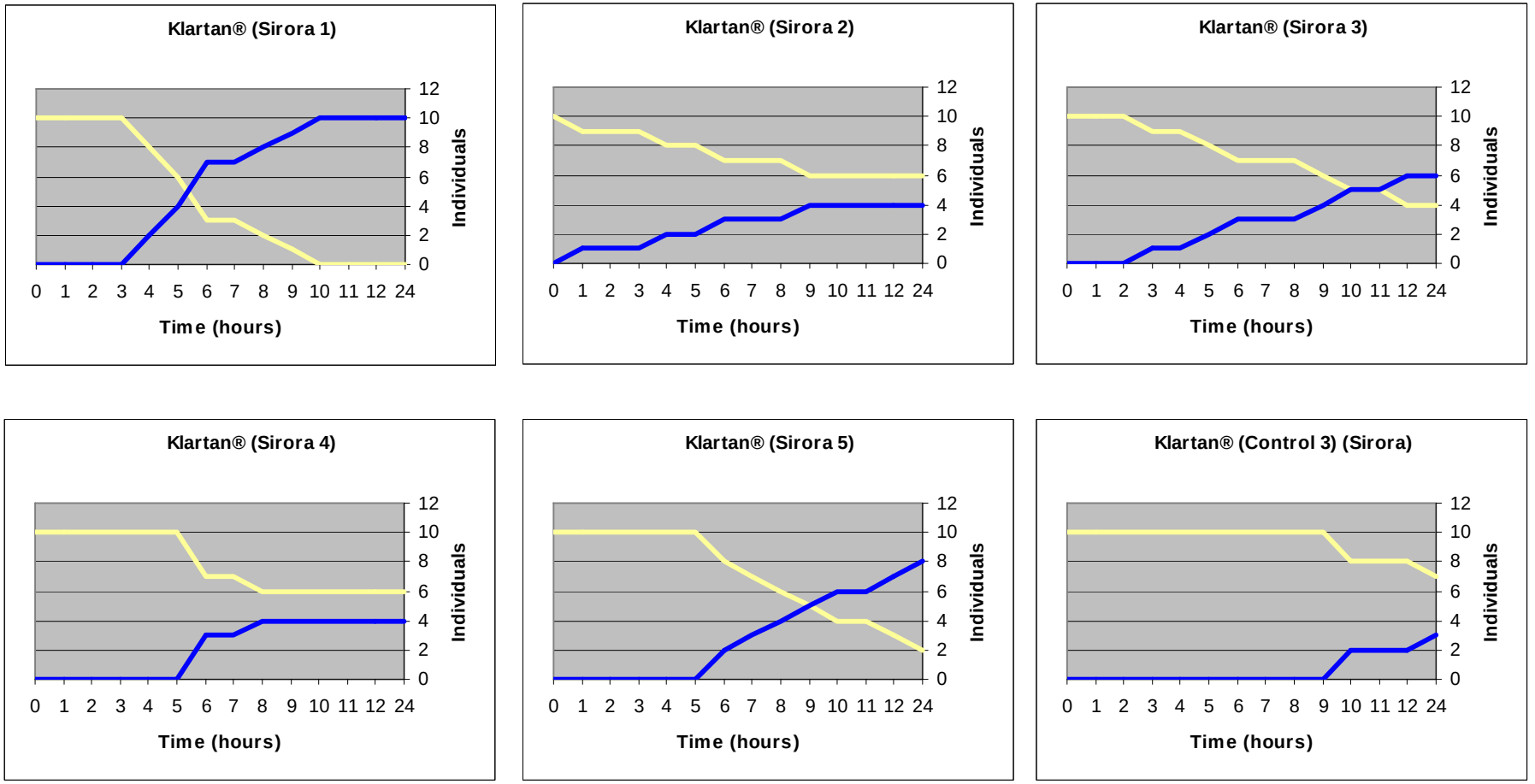


Figure 18: A pesticide bioassay conducted on Atelocera raptoria on pistachio cultivar Sirora trees using Klartan®.

CHAPTER 4

Diurnal pesticide bioassay on *Atelocera raptoria* (Hemiptera, Pentatomidae)

1. Introduction

1.1. Aim

The purpose of the study was to conduct a bioassay of the pesticides currently being deployed by Green Valley Nuts (GVN) for the control of the powdery stinkbug, *Atelocera raptoria.* This stinkbug is a primary pest on pistachio nuts, causing tissue necrosis and premature nut drop.

The study was conducted at the GVN site to test the effectiveness of the pesticides while applied to the pistachio trees. The stinkbugs received no direct contact to the pesticides which were applied to young pistachio trees. This was done under controlled conditions after which the insects were placed onto the trees. This simulated the situation at GVN where the pesticides are sprayed in the orchards. Adult *A. raptoria* are strong flyers and they leave the area when there is disturbance during the spraying process. Individuals would therefore seldom come into direct contact with the pesticide. Non alate nymphs would, however, be sprayed directly. It is thus of utmost importance that the pesticides have a lasting action so that they can either be ingested by sap feeding insects on the trees during the feeding process or make contact with the insect at a later stage.

Due to the fact that several bioassays were conducted (see later chapters), this study will be referred to as Bioassay 1.

1.2. Bioassays

A pesticide bioassay can take on many forms and overall there are no set ways in which these bioassays are to be performed. In most cases bioassays are specifically set up according to the pesticide being tested and the type of insect the pesticides are being tested upon.

There are, however, set guidelines that should be followed during every bioassay and there is a difference between studies conducted in controlled laboratories and studies conducted by simulating natural conditions.

Unterstenhöfer (1976) states that laboratory experiments are conducted in set conditions. Controlled temperature and light and the exclusion of rain and wind create artificial environmental conditions which can also be varied as required. On the other hand, during field studies, environmental conditions prevail, which cannot be determined by the person running the trial. This means that the methodical procedure for field trials are completely different from that of laboratory trials.

1.4. Selectivity

As shown in Chapter 3, *Trissolcus basalis* is an important biological control agent for *Atelocera raptoria* occurring in the orchards at Green Valley Nuts. It is therefore important to select pesticides that would have as little as possible detrimental effect on *T. basalis*. According to Unterstenhöfer (1970), a discussion of the technical and economic possibilities for developing active materials to fit into an integrated pest control program has to begin by examining the requirements that have to be satisfied by the compounds used for control. These requirements mainly concern the spectrum of activity. He further states that the active ingredients are required to eradicate the pests, and at the same time they must not only spare the natural enemies of the pests as indifferent species but, also encourage their population build-up. In other words, they must have a selective action on the target species only.

Unterstenhöfer (1970), also states that selectivity can be achieved in two ways. Firstly, by using selective compounds such as pesticides that possess active ingredients with a spectrum of activity limited to specific species. This is known as physiological selectivity. Secondly, by applying compounds that has an otherwise broad spectrum of activity in such manner that they are able to produce their insect toxicity only against the pest species requiring control. Both these approaches ensure contact of the toxic dose only with the target species and avoids or minimizes contact with any beneficial insects.

Diercks (1967) stated that there are several possibilities towards reducing the probability of contact between a lethal dose and a beneficial insect. Firstly, by using the lowest possible concentration on the most susceptible developmental stage of a pest, which is also the weakest point in the pest population cycle. This approach is known as optimal exploitation and basically exploits the point at which the population offers the least resistance to the pesticide. Secondly, Diercks (1967) states that in direct association with above-mentioned strategy, is the approach to apply of the pesticide at the most favorable time. Although these strategies were proposed 40 years ago, the principles still apply today.

In conclusion Unterstenhöfer (1970) advocates the adoption of all possible ways and means for achieving results and that ecological selectivity, last but not least, deserves special attention. These can be listed as a) choice of lowest dosage for suppression of pest population, b) application of the product at the most suitable time, c) use of attractants for the purpose of increasing the probability of contact between active ingredient and pest with reduced probability of contact between active ingredient and beneficial insects and d) production of suitable formulations.

1.5. Field Trials

As mentioned earlier, there are no fixed methods in which to conduct field trials. In almost all cases, these trials are driven by the specific requirements, pest insects, type op pesticide and environmental conditions prevalent at the study site. Several examples show how field bioassays were conducted in a variety of manners, with each catering for the specific needs of the situation. Examples include studies on various pests, including cabbage root fly and turnip root fly, on turnips and swedes (Kelly & Miller, 1978), trials conducted on the effect of nikkomycins on various insects and mites (Zoebelein & Kniehase, 1985), the control of *Elaeidobius kamerunicus* on oil palm (*Elaeis* guineensis) (Ng, Schonlau & Weber, 1986), insecticide efficacy studies on *Frankliniella occidentalis* (Thysanoptera: Thripidae) on a variety of horticultural crops (Herron, Rophail, Gullick, 1996), the comparative efficacy of *Beauveria*

bassiana and *Bacillus thuriengiensis* for the control of the Colorado potato beetle (Lacey, Horton, Chauvin & Stocker, 1999), pesticide evaluations conducted on avocado thrips (Philips & Faber, 1999), the influence of several pesticides on the mortality and fecundity of the aphidophagous coccinellid (*Adalia bipunctata*) (Olszak, 1999), the efficacy of Chinaberry extracts and certain pesticides against the pea leafminer (Abou-Fakhr Hammad, Nemer & Kawar, 2000), the efficacy of *Ocimum basilicum* and *Ocimum gratissimum* essential oil extracts for the control of *Callosobruchus macalatus* (Keita, Vincent, Schmit, Arnason & Belanger, 2001), and lastly the insecticide efficacy of several pesticides for the control of *F. occidentalis* (Broughton & Herron, 2007)

During a study conducted by Villanueva-Jimenez & Hoy (1998), several bioassays were conducted with the objective of implementation into an IPM program. This study was aimed at finding pesticides that reduce harmful influence on the encyrtid wasp, *Ageniaspis citricola* Logvinovskaya, which was introduced as part of a classical biocontrol project for the citrus leafminer, whilst simultaneously examining the efficacy of the pesticides on the pest species, the citrus leafminer. This study correlates very closely with the study conducted at GVN. However, even though both studies were conducted towards the same goals, the methods used differ completely because of the difference in pest species, biological control agents and pesticides.

Furthermore, when examining different bioassays conducted on other Hemipteran species, the same trend can be seen. These include a study conducted by Tillman (2002) and Tillman, Mulrooney & Snodgrass (2003) on *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) and *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae). Another example, conducted by Cortes, Sanchez, Riis, Bellotti & Calatayud (2003), on HCN toxicity to the burrowing bug, *Cyrtomenus bergi* (Hemiptera: Cydnidae), shows the same.

All above-mentioned examples re-emphasize the statement that every bioassay should be conducted according to the existing requirements and variables.

2. Material and Methods

1) At GVN three pistachio cultivars, i.e. Shufra, Sirora and Ariyeh are planted in the orchards. Bagged young trees of all three cultivars were used during this *A. raptoria* bioassay study that was conducted during February 2005. Six pesticides, i.e. Sevin®, Endosulfan®, Pencap®, Karate®, Calypso® and Klartan® were used during the bioassays and each pesticide was tested on its own.

2) Same brood, equally fit, male and female adult *Atelocera raptoria* individuals were used for these trials, since it is assumed that both sexes occur on the trees and both cause damage to the nuts. Under usual spraying programs, pesticides will be applied indiscriminately on both sexes, as well as immature *A. raptoria* nymphs. Only adults were used for this trial because of the difficulty in obtaining enough nymphs of the same instar and the assumption that the nymphs would be more susceptible to the pesticides. It is thus logical to assume that the efficacy of the pesticide on adult specimens should also occur on immature stages. Sampling of the specimens used in the trials was conducted in the orchards of GVN.

3) In the experimental setup six trees of each cultivar were used in each trial. Thus, in total, 18 trees were used for an entire trial. Of these 18 trees, five trees from each cultivar were used as trial trees and one tree from each cultivar acted as control. The entire study consisted of six trials and 108 trees were therefore used. Every trial occurred over a period of 24 hours. During each period each trial was further subdivided into two phases. Phase 1 consisted of the first 12 hours and phase 2 of the last 12 hours of the trial. Monitoring took place at the beginning of each hour during the first phase and then again at the end of the second phase. The study was therefore conducted over a period of six days. In between every trial, a day was used for sampling *A. raptoria* specimens in the GVN orchards and preparing for the next trial. Every tree was covered with a gauze sleeve (see below) and stocked with 10 adult insects. In

total, for the 18 trees needed in every trial, 180 insects were used. Due to the fact that 6 trials were conducted, 1080 insects were used during the entire study. Trees were obtained from the GVN nursery and arranged inside an empty greenhouse that was used for all the trials (Fig. 1). This was done to cover the trees from rain during the trial period. Rain would wash away or dilute pesticides on trees after application and cause irregularities in the results. The trees were assembled in four rows, in sequence from north to south, as Shufra, Ariyeh, Sirora and Control (Fig. 2).

Figure 1: Greenhouse used in diurnal bioassay study using pesticides on *Atelocera raptoria*.

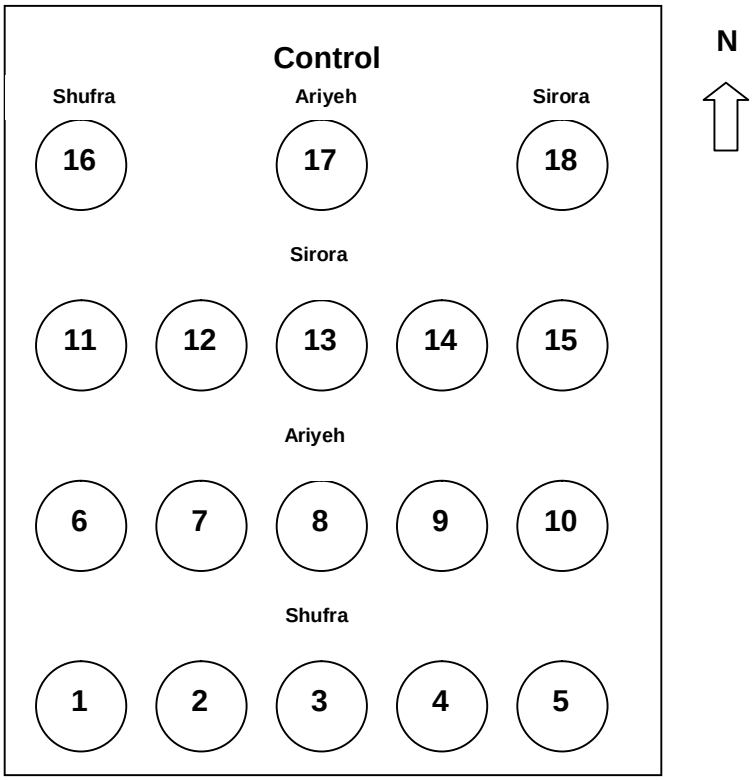


Figure 2: Trial setup in diurnal bioassay study using pesticides on *Atelocera raptoria*.

After the trees were arranged in the correct order, they were left overnight to acclimatize to the conditions inside the greenhouse. A data logger measuring relative humidity and temperature was also placed inside the greenhouse. The data logger would take readings every half-hour. This was done over the entire study period. The data logger was placed inside a protective cover in the center of the greenhouse at 18:00 on the day prior to the commencement of trials and readings were taken until the morning after the study was terminated.

4) *A. raptoria* specimens were sampled on trees occurring in the GVN orchards. Sampling was conducted by hand whilst scouting the different orchard blocks. Sampled material was placed in paper bags provided with fresh pistachio leaves. General monitoring conducted every day at GVN was also used to supplement the trial insect collection. This monitoring was done using sheets and beating sticks and all sampled insects were placed inside glass terraria for containment. In total, three terraria were used which consisted of rectangular glass tanks covered with a gauze lid. The base of the terrariums were filled with soil and covered with laboratory paper towels. The

terrariums were all exposed to the same conditions in terms of temperature and humidity. Freshly cut pistachio leaves were provided as sustenance for the captured specimens.

5) The methodology used with the application of the pesticides was conducted in the same manner for all six trials. Pesticides were prepared according to the label specifications of each individual pesticide. To spray the trees, a Stihl SR 420 versatile backpack mist blower was used which has a displacement capacity of 56.5 cm^3 and is fitted with a 15 liter tank. All pesticide formulations were mixed in accordance with a 15 liter volume (Table 1). Measurements were done using a 5ml syringe and a 10ml measuring cylinder. 10ml buffering liquid (Aquarite®) was also added to each mixture of 15 liters. Pesticide concentrate was measured and added to the 15 liter tank, filled with tap water, after which the buffering agent was added. The tank was shaken to ensure a even pesticide mixture. Facemasks and rubber gloves were worn throughout this mixing and application procedure for protection.

Table 1: Specified dosages of six pesticides for three different mixtures used in diurnal bioassay study on *Atelocera raptoria*.

Product	Dose (ml) / 100 L	Dose (ml) / 15 L	Dose (ml) / 5 L
Sevin®	225	33.75	11.25
Endosulfan®	100	15	5
Pencap®	70	10.5	3.5
Karate®	20	3	1
Calypso®	15	2.25	0.75
Klartan®	30	4.5	1.5

Each trial commenced at 08:00 during the trial day. This was seen as time 0 at the start of the first phase. Spraying of the trees occurred at approximately 06H45 every morning and the entire tree was thoroughly wetted each time (Fig. 3). During this procedure the three control trees were removed from the greenhouse to avoid pesticide contamination

Figure 3: Application of pesticides to trial trees using a mist blower.

6) Directly after the application of the pesticide, net sleeves were placed on each of the trees. Once the sleeve has been fitted, the top and bottom of the nets are tied down - the bottom end around the trunk to prevent specimens escaping and the top end pulled shut and tied to a suspension spanning the width of the greenhouse. In so doing the suspension provided stability for the net sleeves, providing a sturdy structure (See Fig. 4). After the nets were in place, 10 randomly selected insects per tree were placed inside the nets.

7) Data recording commenced after all the netted trees were supplied with insects. Phase 1 readings were noted as time 0 and hour 0 immediately after placement of the specimens when all insects were still alive. Following on this monitoring was conducted every hour, on the hour, during the 12 hour period. Monitoring was conducted by visual inspection through the net sleeves during which specimen survival and mortality was noted. Recordings were conducted

in the same sequence and inspection on every tree continued until all 10 insects were spotted and accounted for (Fig. 4).

This procedure covered a period from 08:00 to 20:00, after which phase 1 was complete. The trees were then left overnight for the continuation of phase 2, which encompassed another 12 hours. The next one-off monitoring was done the following morning at 08:00, which completed the 24 hour cycle. All specimens still alive after the completion of the trial were removed and killed in 70% ethanol. Nets were removed from the trees after the completion of each trial and washed. The trees used were taken back to the nursery at GVN and replaced with new trees in preparation for the next trial. The same procedures were followed for all six trials.

Figure 4: Visual inspection of survival and mortality rates of *Atelocera raptoria* on netted trees during diurnal pesticide bioassay.

3. Results and Discussion

3.1. Temperature and relative humidity (RH)

After the completion of the study, data was extracted from the data logger and analyzed to construct a graph to illustrate temperature and relative humidity fluctuations during the study period (Fig. 5). Rainfall caused temperatures to drop, hence the two coldest days. For a period following on this RH was also very high. As can be expected, relative humidity would become lower as the temperature rises. Consequently, the highest values for RH were noted during nighttime, when temperatures were at their lowest At the lowest, temperature would drop to approximately 16°C, causing the RH to rise to as high as approximately 97%.

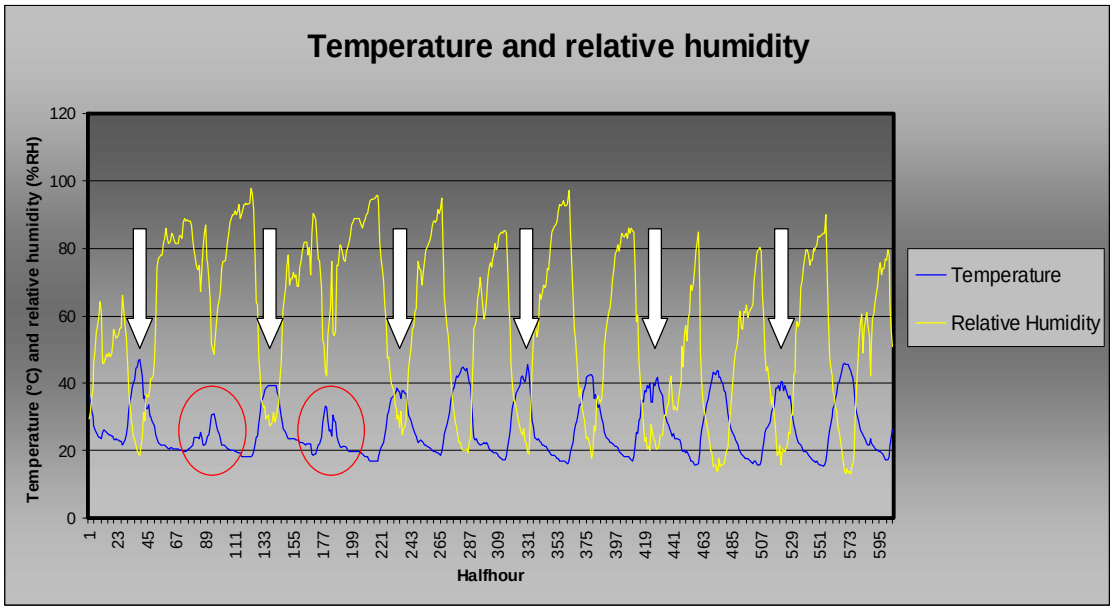


Figure 5: Temperature and relative humidity readings taken over 13 days in February 2005 during the diurnal pesticide bioassay on *Atelocera raptoria* . Arrows indicate the commencement of the six different trials and circles the coldest days on which rain occurred.

The greenhouse where the trials were conducted was sheltered from winds and natural air flow, causing abnormal conditions in some cases. The perspex roofing of the greenhouse would also amplify any sunlight. Temperatures would therefore rise to very high levels during the day (up to 47°C), averaging 45°C during the hottest time of the day (12:00 – 14:00), during trial days.

Relative humidity would drop to around 20% during this time. All trials were conducted during sunny days, causing conditions to be very similar during all trial days.

Thus, it was deemed unlikely that fluctuating temperatures and RH would cause serious irregularities in the results. It was borne in minds that specimens could die from desiccation and that premature evaporation of the pesticides could occur during and following application. This would, however, be a uniform effect and apply throughout the study.

3.2. Pesticide bioassay

A point of concern during this study was that the insects would not feed on the trees and consequently not come into contact with the pesticides by means of feeding. However, this was underscored by the high temperatures that triggered the insects to feed readily, as was witnessed through visual inspection.

By compiling all data accumulated over the 24 hours of every trial, a complete data set could be compiled. This included the total specimen mortality as measured every hour. This was done for all 108 trees from all six trials during the entire study. Due to the fact that only 10 specimens were placed in every tree, the number of surviving specimens per trial tree could easily be calculated. From these, data graphs could be constructed that are easy to interpret and which point out several important events during the pesticide activity period. These graphs are provided in Appendix 1, Figs. 1- 18.

Figure 6 demonstrates the process of pesticide activity as found during the bioassays conducted. The blue line represents the live insects (survival) on the trial tree with the yellow line indicating the total mortalities observed on the trial tree. This method of interpretation will be used to discuss, in detail, pesticide activity occurring over time during the trials conducted. Using this method, pesticide activity can be well monitored, providing valuable indications as to the efficacy of the pesticide being examined.

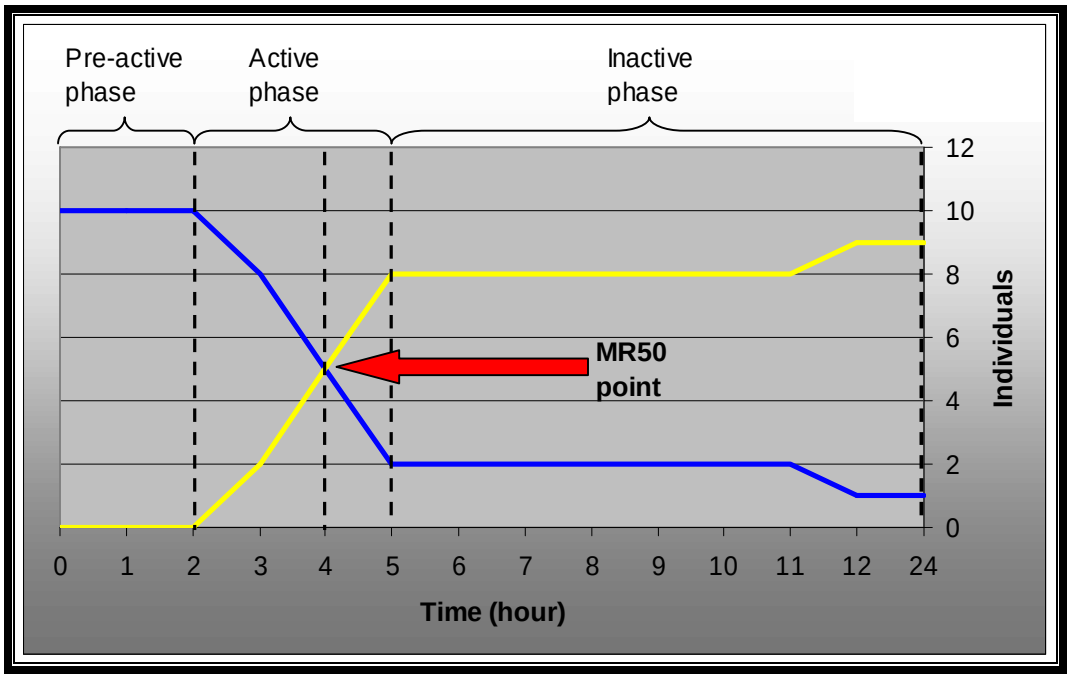


Figure 6: Graphical representation of pesticide activity that was determined for bioassays on *Atelocera raptoria*.

The first phase is referred to as the pre-active phase. This phase starts as pesticide application commences. It represents the time preceding the point where pesticide activity can be identified. Some pesticides would show a much shorter pre-active phase. This time is probably dependant on the active ingredient, mode of transfer and the concentration of the pesticide.

The second phase in Figure 6 is referred to as the active phase. This is the phase in which the effect of the pesticide could clearly be seen. This is also the time in which most mortalities would take place. It can be assumed that nearly all mortalities occurring in this phase are due to exposure to the pesticide. In most cases, the mortality rate 50% (MR50) point also falls within this phase. This represents the point where 50% of the trial insects have died and 50% have survived. This is not the same value as the well-known LD50 value. LD is for "Lethal Dose" and LD50 is the concentration of a product, applied all at once, which causes the death of 50% (one half) of a group of test animals. The LD50 is one way to measure the short-term poisoning potential (acute toxicity) of a product. Thus, the LD50 value measures the amount of active ingredient needed to kill 50% of the trial subjects (Anon, 2005) and the value is therefore applied to express the toxicity of a substance.

During these trials, the MR50 point only represents the point where 50% mortality occurs, regardless of dosage. As all pesticides were mixed according to recommended dosages, the LD50 value does not apply.

The third and final stage in Figure 6 is referred to as the inactive phase. Mortalities due to pesticide exposure during this phase is regarded as an exception, *e.g.* insects not feeding for 5 hours into the trials and then suddenly starting to feed. Therefore all deaths occurring during this phase are lumped and ascribed to desiccation from the high temperatures in the greenhouse or other diverse influences. This phase is of special significance in the control trials.

Statistical analyses were conducted on the datasets generated from all the trees of all six trials. Using Graphpad Instat® mean mortalities were determined and these are presented in Figs. 7 – 24.

The maximum percentage mortalities over all five trial trees from each cultivar of every trial were also examined. These figures were compared to the maximum percentage mortalities of the control trees of the same cultivar in each trial. These graphs clearly demonstrate the difference between the mortality rates found on trial trees versus control trees (Figs. 25 – 30).

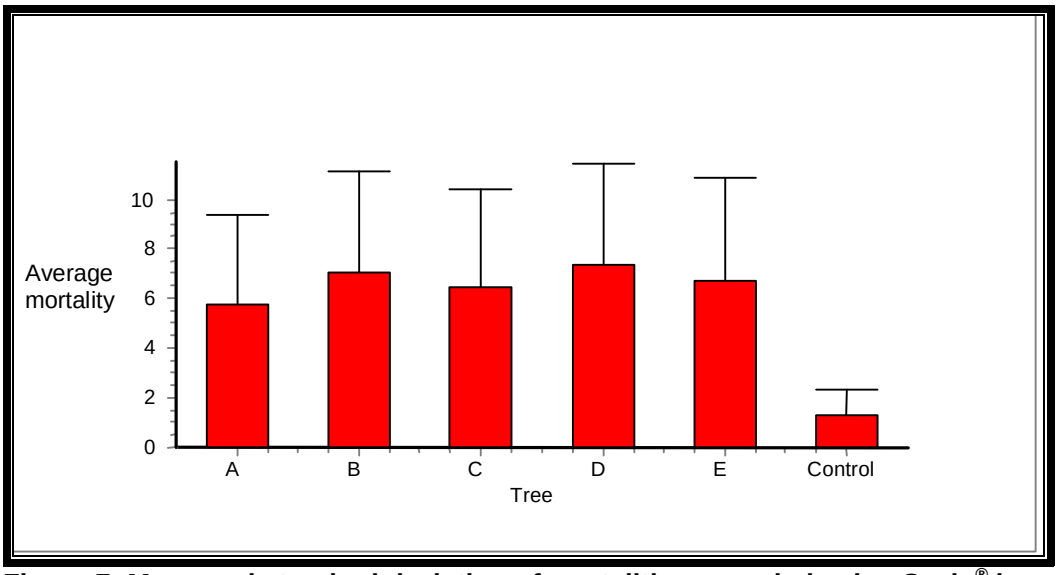


Figure 7: Mean and standard deviation of mortalities recorded using Sevin® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

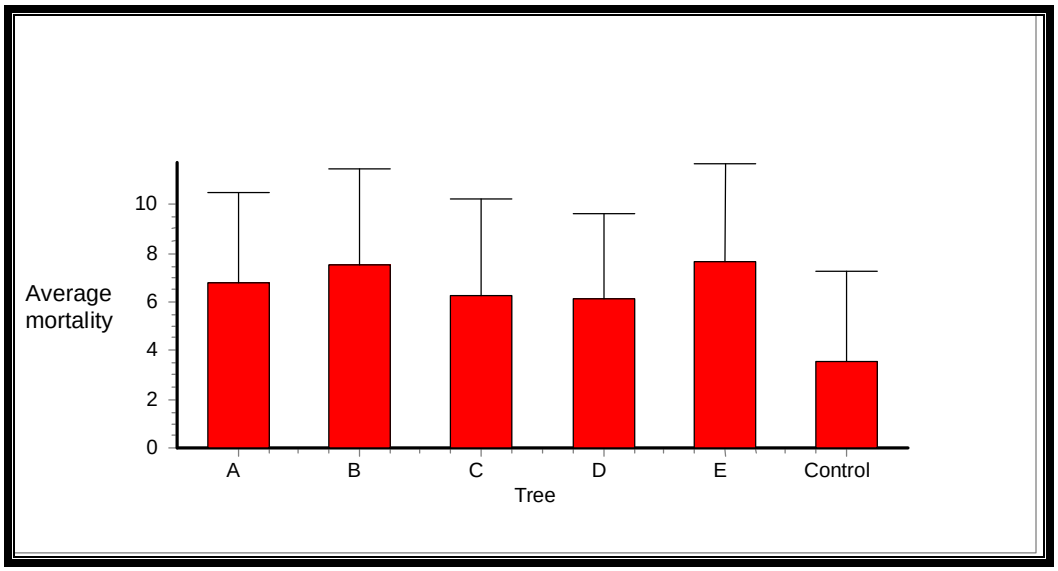


Figure 8: Mean and standard deviation of mortalities recorded using Sevin® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

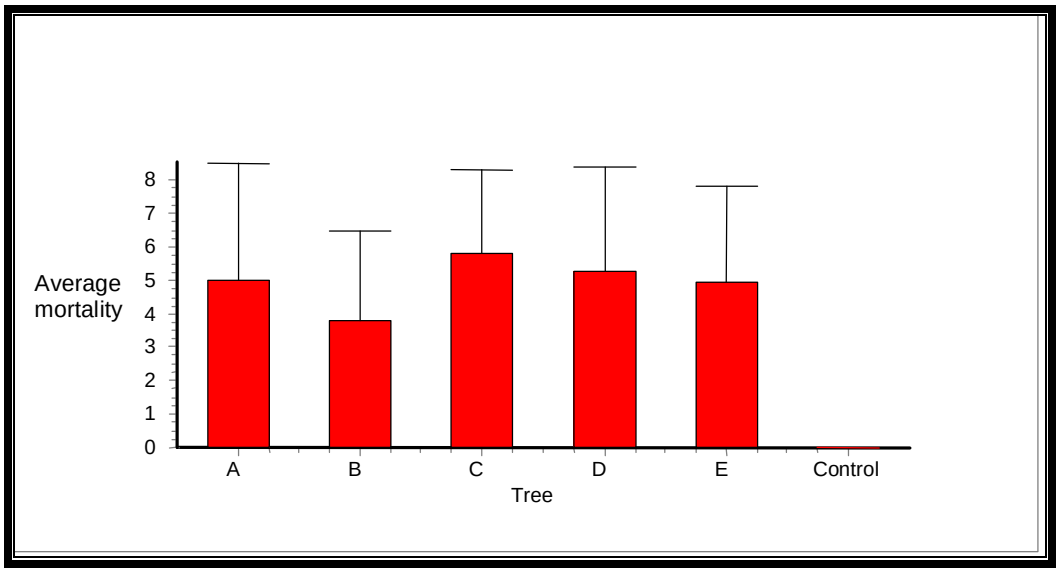


Figure 9: Mean and standard deviation of mortalities recorded using Sevin® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

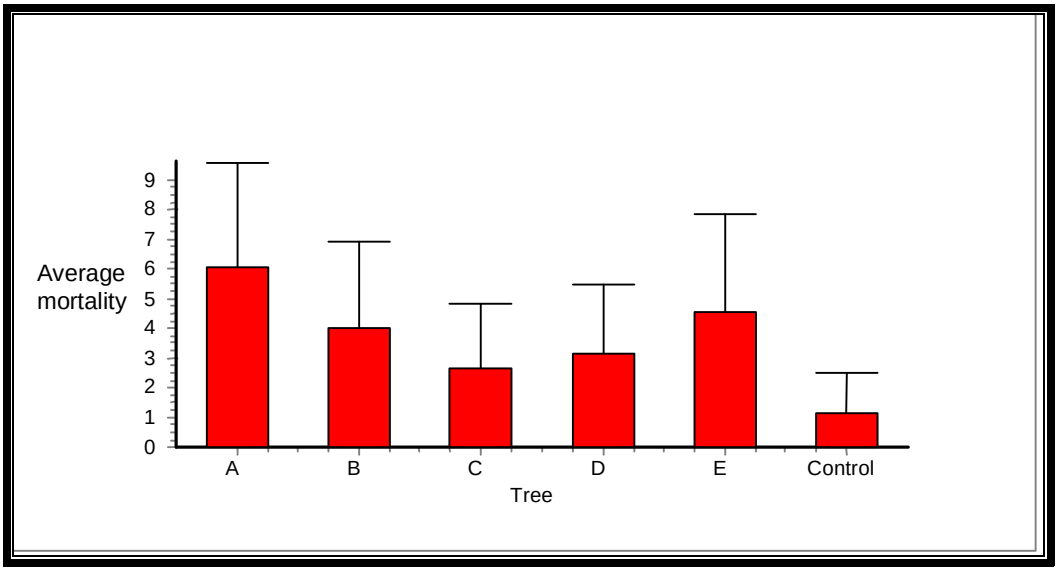


Figure 10: Mean and standard deviation of mortalities recorded using Endosulfan® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

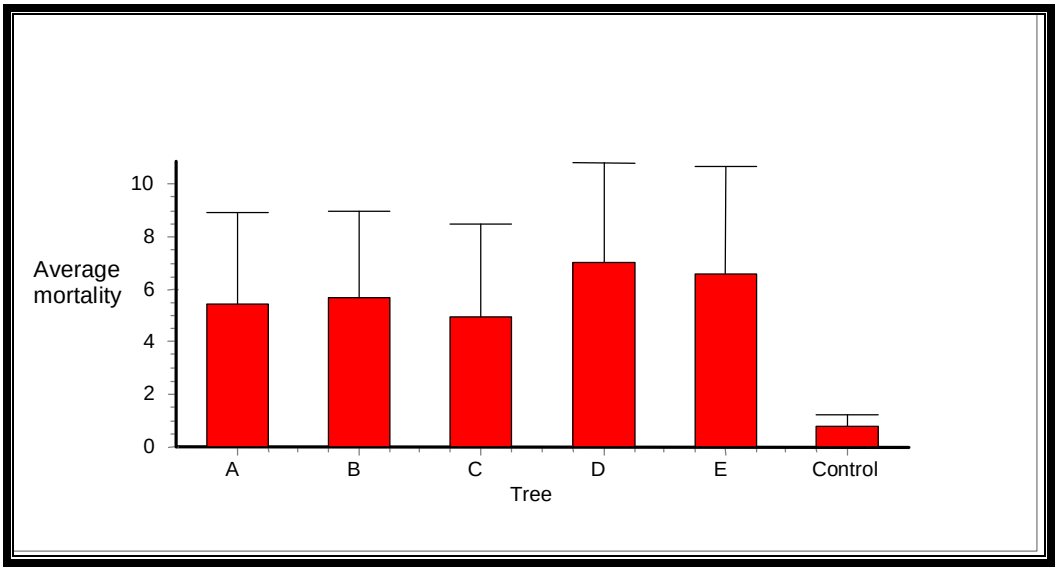


Figure 11: Mean and standard deviation of mortalities recorded using Endosulfan® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

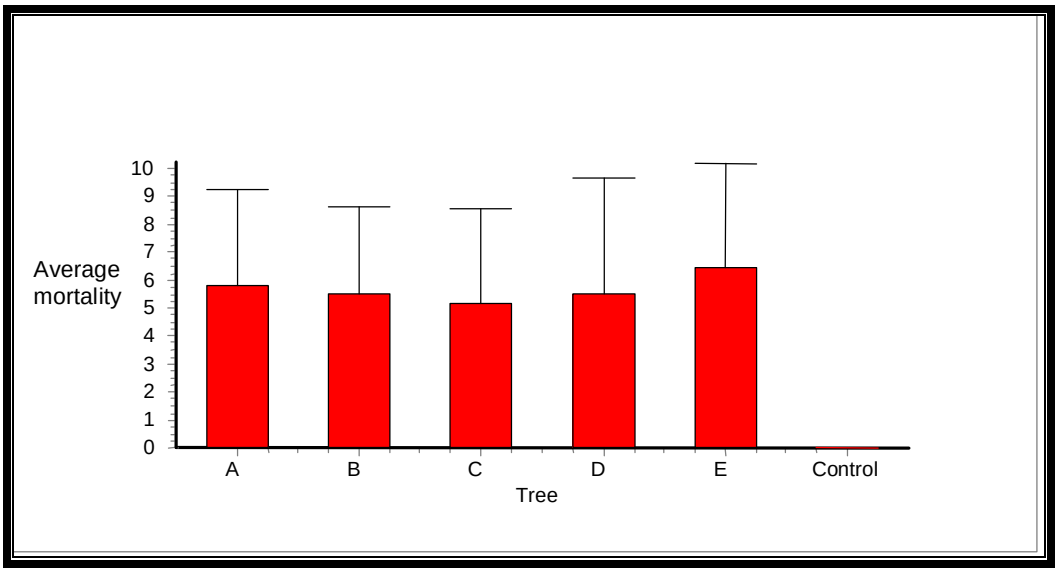


Figure 12: Mean and standard deviation of mortalities recorded using Endosulfan® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

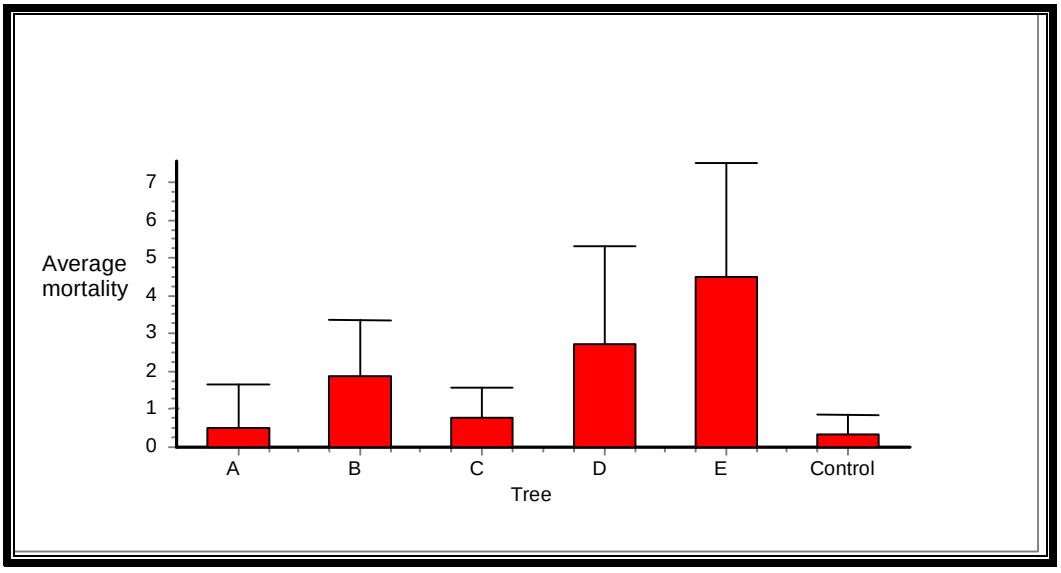


Figure 13: Mean and standard deviation of mortalities recorded using Pencap® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

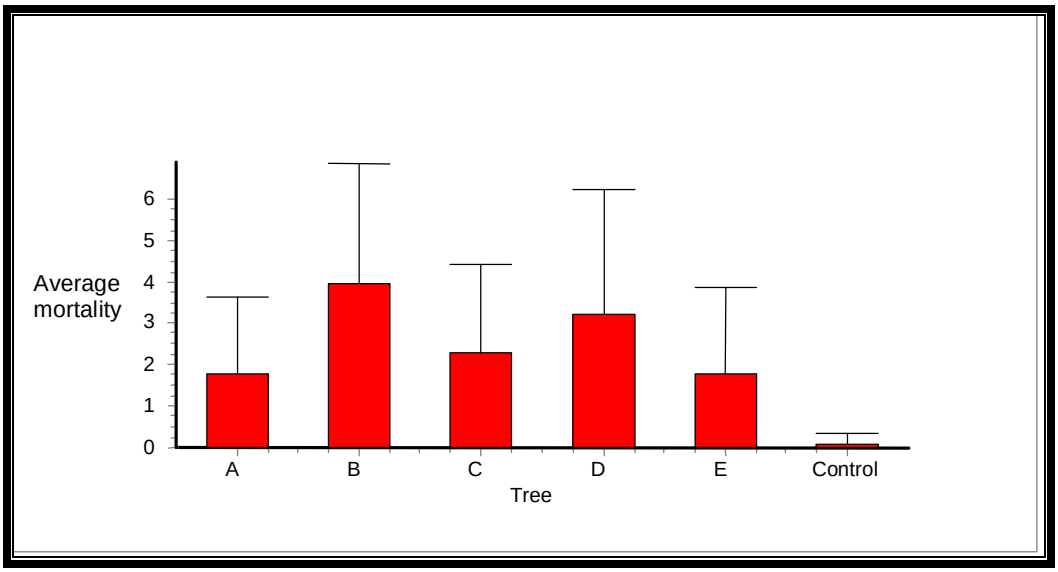


Figure 14: Mean and standard deviation of mortalities recorded using Pencap® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

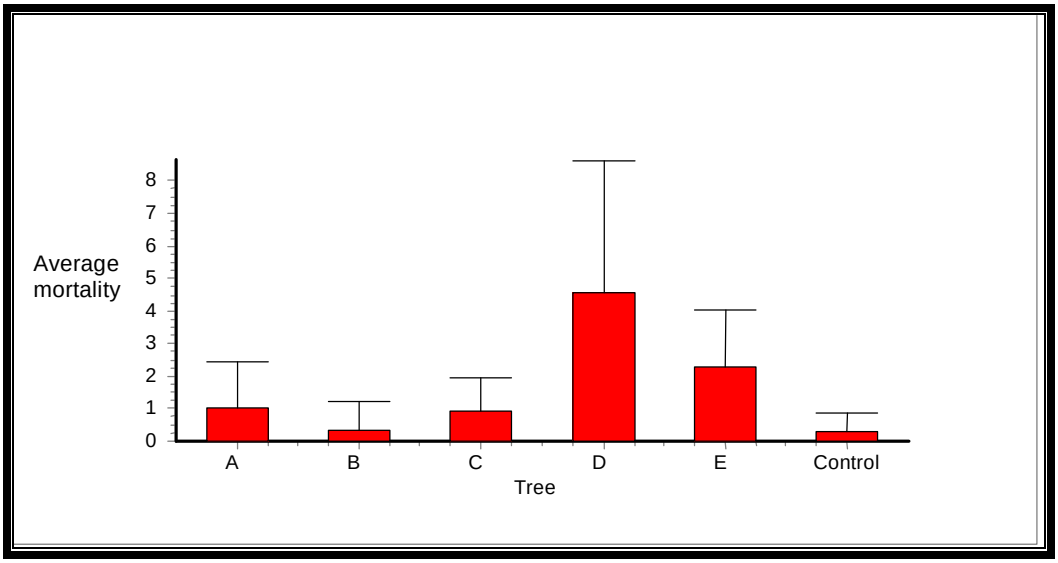


Figure 15: Mean and standard deviation of mortalities recorded using Pencap® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

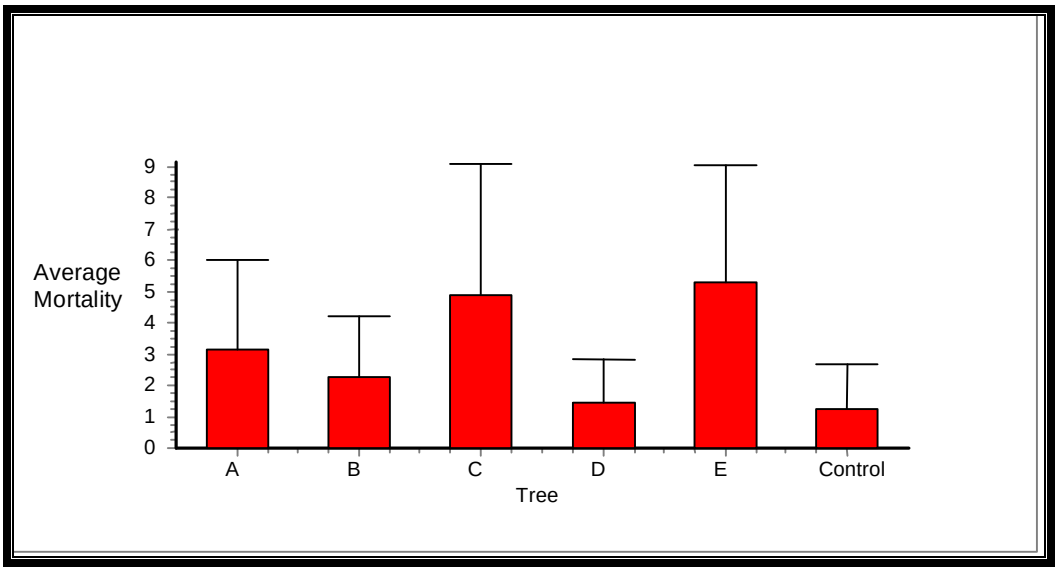


Figure 16: Mean and standard deviation of mortalities recorded using Karate® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

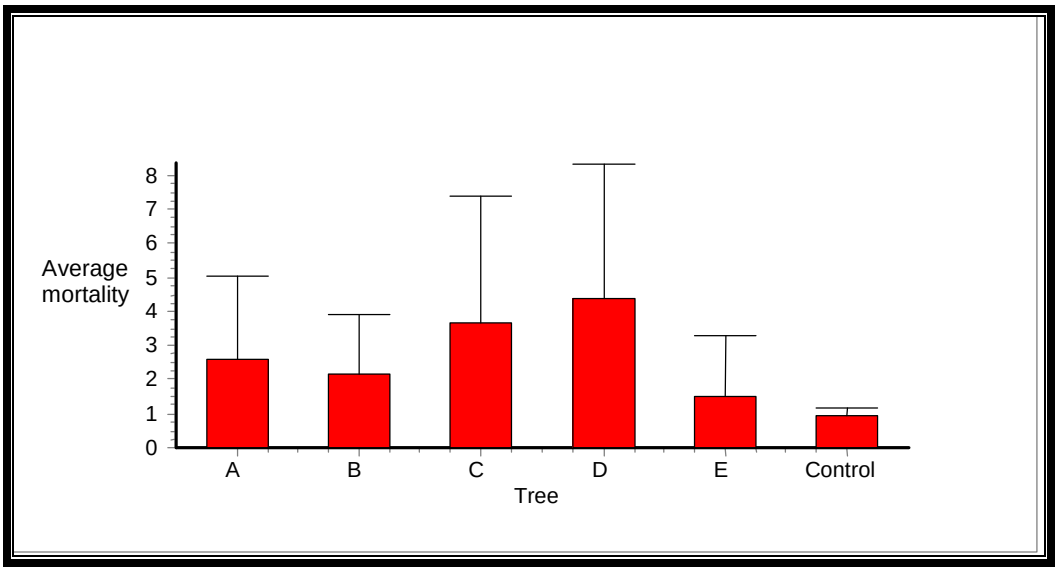


Figure 17: Mean and standard deviation of mortalities recorded using Karate® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

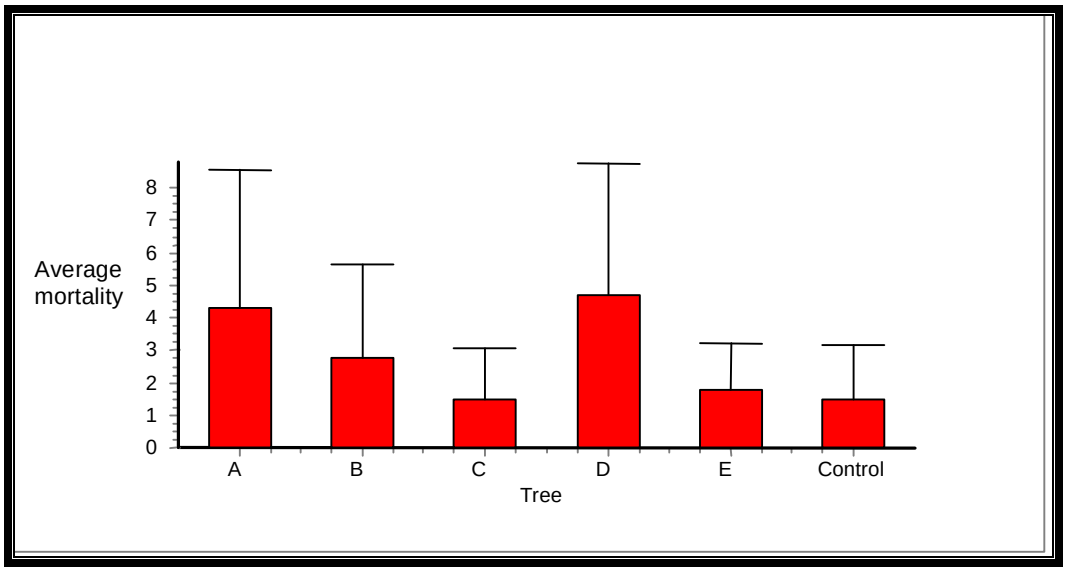


Figure 18: Mean and standard deviation of mortalities recorded using Karate® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

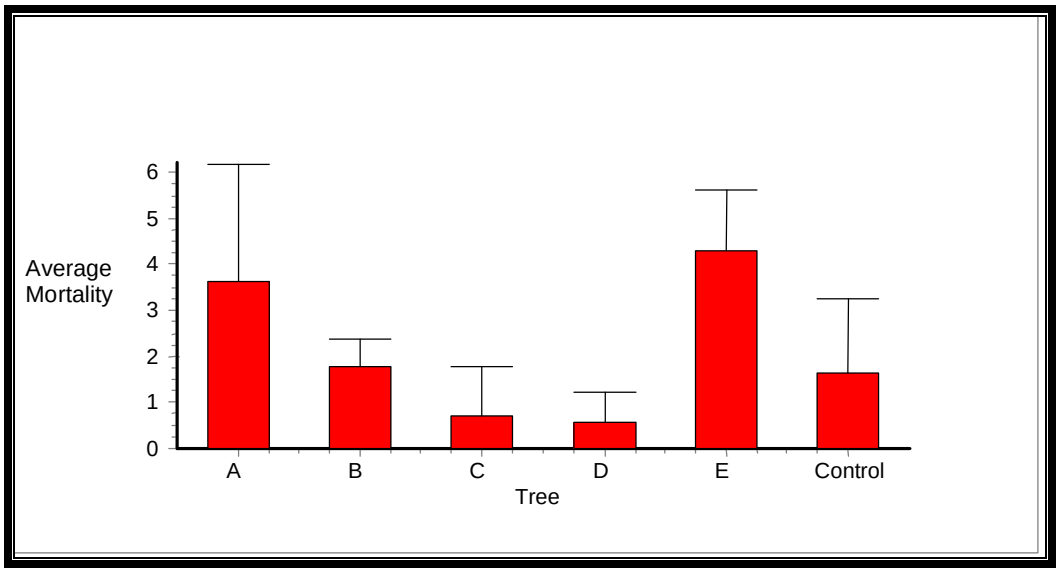


Figure 19: Mean and standard deviation of mortalities recorded using Calypso® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

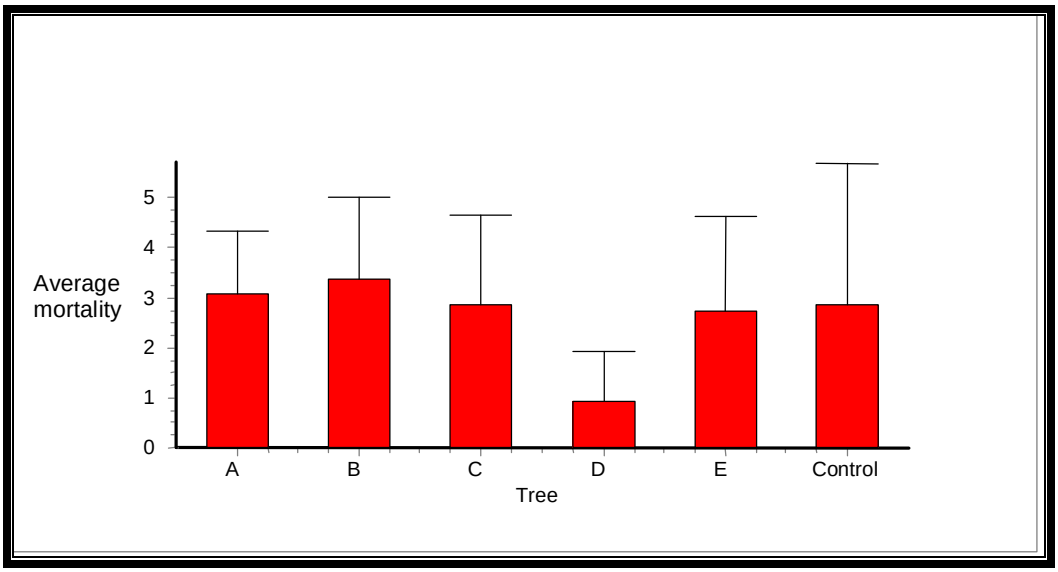


Figure 20: Mean and standard deviation of mortalities recorded using Calypso® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

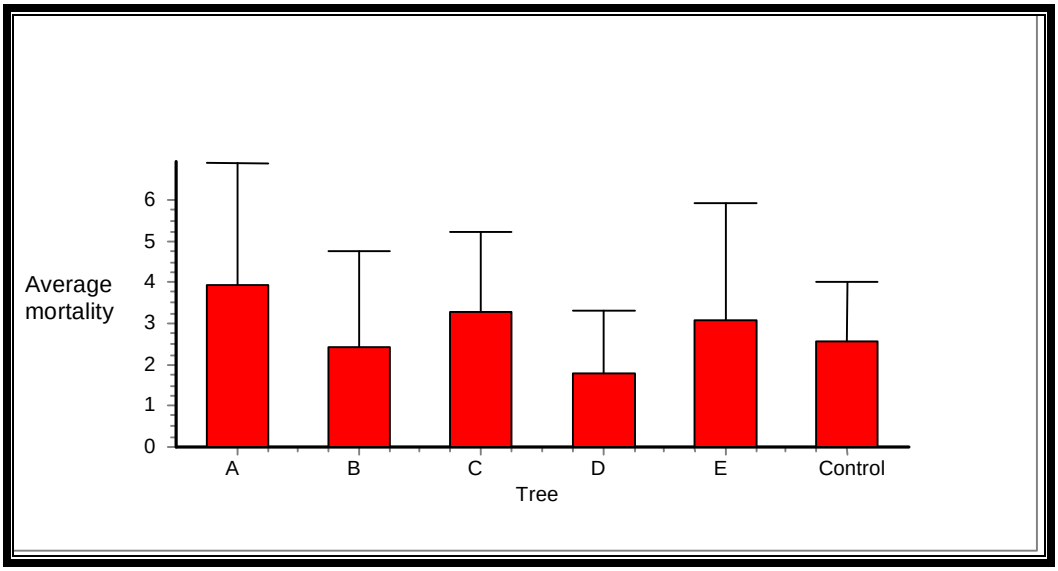


Figure 21: Mean and standard deviation of mortalities recorded using Calypso® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

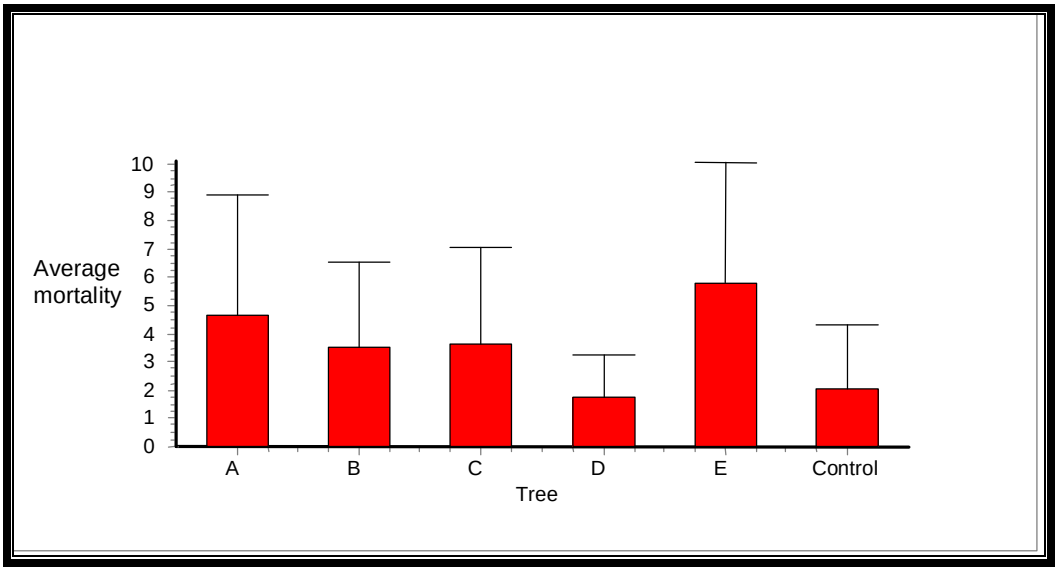


Figure 22: Mean and standard deviation of mortalities recorded using Klartan® in *Atelocera raptoria* bioassays occurring on Shufra cultivar.

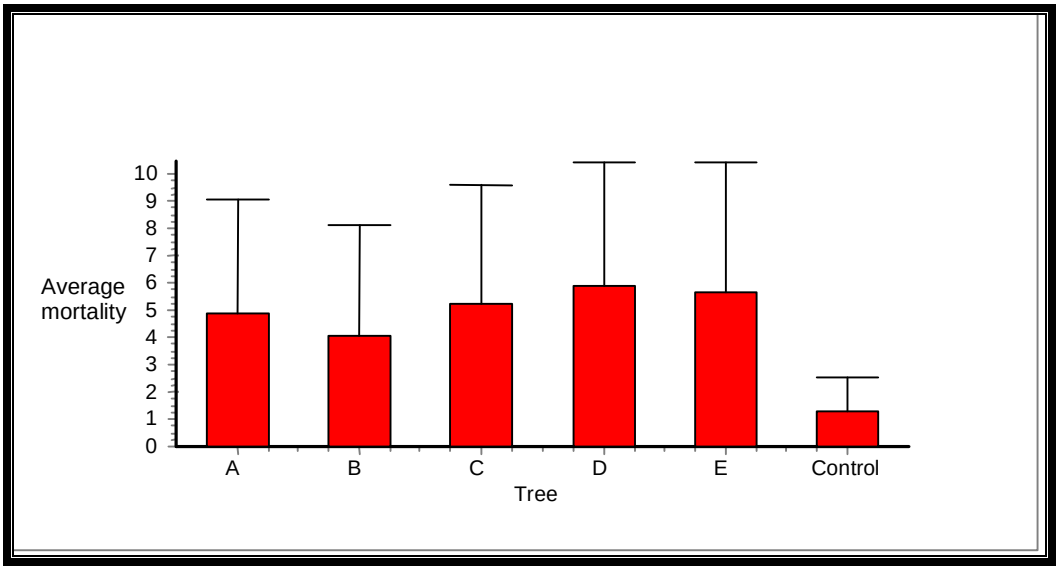


Figure 23: Mean and standard deviation of mortalities recorded using Klartan® in *Atelocera raptoria* bioassays occurring on Ariyeh cultivar.

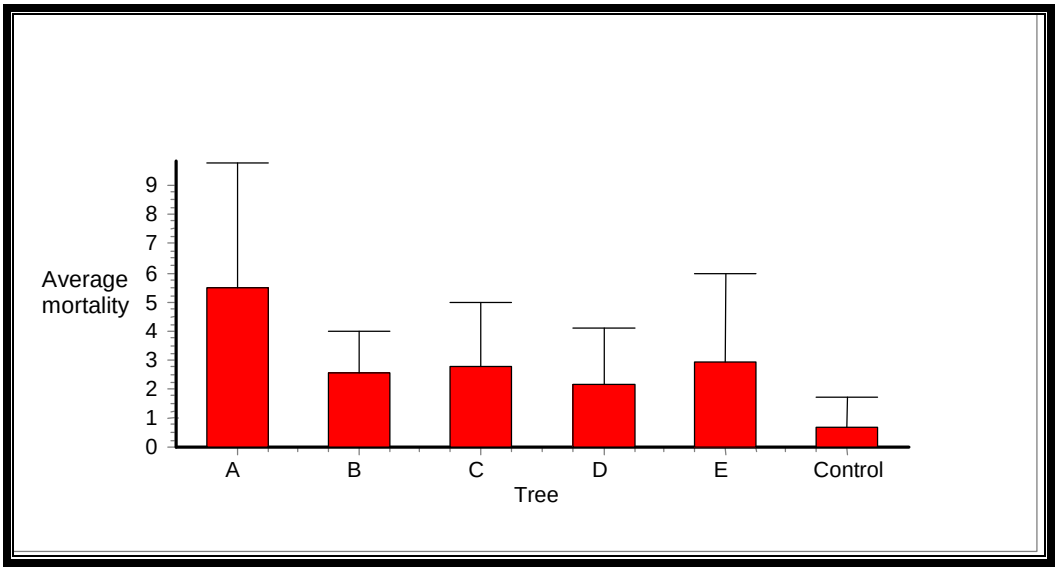


Figure 24: Mean and standard deviation of mortalities recorded using Klartan® in *Atelocera raptoria* bioassays occurring on Sirora cultivar.

3.2.1. Sevin® (Trial 1)

(A) Shufra

On the first cultivar, Shufra, very promising results were obtained. In all five trial trees, a very short pre-active phase was noted, with the longest being two hours after pesticide application. During the active phase, all trees showed a MR50 point. The active phase on trees lasted between 3 and 5 hours on each trial tree. Mean values showed the average mortality rates to be around six, which is among the highest recorded in all trials. The Shufra control tree also suffered some mortalities. The first two trial insects died after four hours and another died after 12 hours. Mortalities did, however, occur much later than those noted in the trial trees and it can thus be assumed that heat or other unknown circumstances were responsible for these mortalities.

(B) Ariyeh

On this cultivar, again, a very short pre-active phase was noted, with mortalities occurring after only one hour on most of the trial trees. Again, all trial trees showed a MR50 point. The active phase lasted between 4 – 5 hours in all trial trees. The mean mortality rates were again approximately six. The

Ariyeh control, however, showed very abnormal results with eight of the ten trial insects dying during the 24 hours. This is the highest control mortality rate found in the entire study. However, mortalities only began occurring after five hours, after the active phase of all the trial trees were almost complete. It is thus logical to assume that other diverse circumstances were responsible for the mortalities.

(C) Sirora

The Sirora cultivar showed the weakest results. In all trial trees, the pre-active phase lasted only 1 – 3 hours, similar to the other cultivars. The active phase also lasted mostly the same time as those found on the other two cultivars. This was found to be between 3 and 5 hours. However, the main difference was that mortalities occurred at a much lower rate than those of the other two cultivars. The mean mortality figures show it to be below six, considerably lower than the other cultivars. Although all trial trees had a MR50 point, mortalities were found to be lower. The control tree showed perfect results, with no trial insect mortalities during the 24 hour cycle.

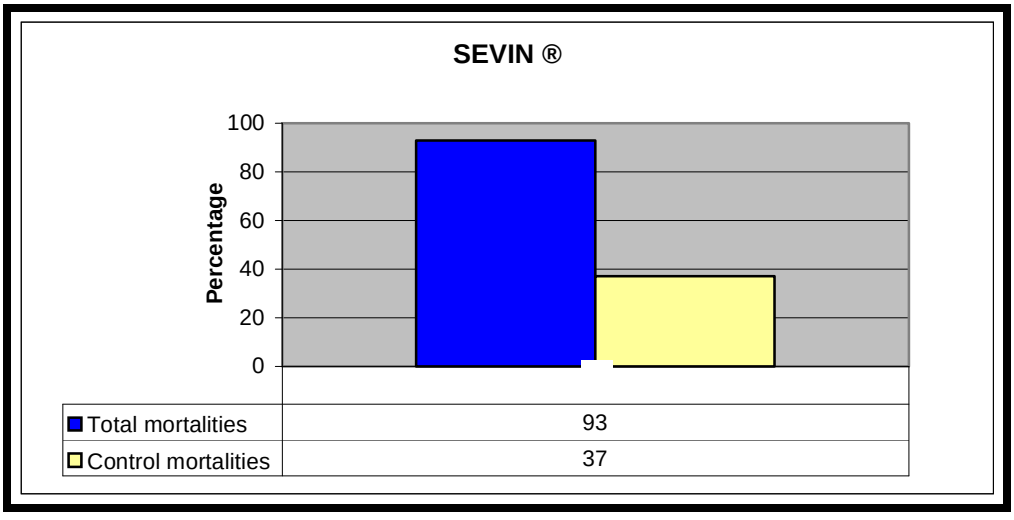


Figure 25: Total trial tree mortalities versus control tree mortalities using Sevin® in a *Atelocera raptoria* bioassay.

The total mortality percentage of the fifteen trial trees was 93%, against the 37% mortalities recorded for all the control trees (Fig. 25). However, the latter figure is heavily influenced by the mortality rates found on the Ariyeh control. It

would appear, however, that the Ariyeh control tree was a drastic outlier and may have given rise to inaccurate results.

3.2.2. Endosulfan ® (Trial 2)

(A) Shufra

On Shufra the weakest results in Trial 2 were obtained. With most of the trial trees, the pre-active phase lasted approximately three hours. On average, this is a bit longer than those noted during Trial 1. The main difference noted, however, is the considerably longer active phases noted in all the trial trees, which apparently lasted from 7 - 24 hours. All trial trees showed a MR50 point. In most of the trees, no inactive phase could be distinguished showing that this pesticide has a longer post application effect. Lower mean values were also noted, with the average mortality rates well below six in most trial trees. The control tree also showed notable mortality rates, with three of the trial insects dying in the 24 hour trial.

(B) Ariyeh

On Ariyeh trial trees different results than those found on Shufra were recorded. Short pre-active phases on all trial trees could be noted, this period lasting between 1 to 2 hours. The active phase was also shorter, ranging from 4 - 7 hours. All trial trees reached the MR50 point fairly early in the trial. All trial trees also clearly showed an extensive inactive phase. Statistically, very high mean mortality values were found with an average of approximately seven in some trees. The control showed good results, with only one insect dying fairly early in the trial.

(C) Sirora

On Sirora trees much the same results as those found on the Ariyeh trees were obtained. Again, short pre-active phases were noted, lasting between 1 - 3 hours in the trial trees. The active phases were recorded to be a bit longer, lasting between 4 – 8 hours. Clear inactive phases could nonetheless be distinguished in all trial trees. All trial trees also showed a MR50 point. Again,

very high mean mortalities could be seen, averaging around six. No mortalities took place on the control tree.

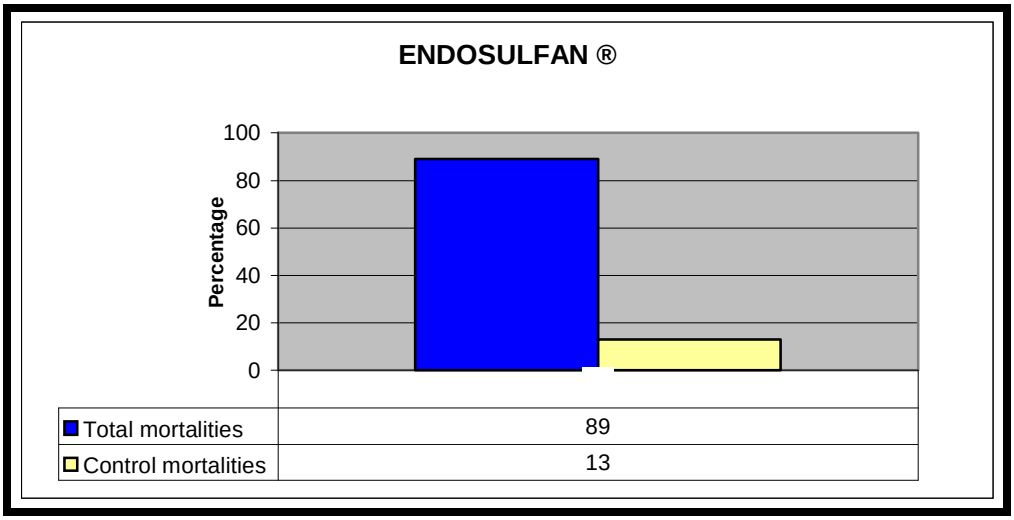


Figure 26: Total trial tree mortalities versus control tree mortalities using Endosulfan® in a *Atelocera raptoria* bioassay.

Very high trial tree mortalities were recorded, showing an average of 89% for all three cultivars. This is almost as high as rates found during Trial 1. However, fairly low control mortalities, only 13%, were recorded on the control trees (Fig. 26). It would appear that the Shufra trees showed irregular results, differing noticeably from those found on the other two cultivars.

3.2.3. Pencap® (Trial 3)

(A) Shufra

Considerably lower mortality rates could be seen in this trial. The pre-active phase differed greatly between the trial trees, ranging from 1 – 4 hours in nearly all trial trees. Only one tree showed an effect after 10 hours. Only two of the trial trees reached the MR50 point, with the other trees having mortality rates below 50%. Statistically, almost all the trees had a mean mortality value below three, with only one tree higher at around five. This is a great deal lower than those found in the previous two trials. The control showed only one mortality over the 24 hour period.

(B) Ariyeh

Better results were obtained on the Ariyeh cultivar. The pre-active phase lasted between 3 and 6 hours, with the active phase lasting around 6 hours in most of the trial trees. No clear inactive phase could be distinguished on the trial trees. Nonetheless, all trial trees reached the MR50 point, showing higher mortality rates than in the case of the Shufra trees. Statistically, higher mean mortality rates were also found, averaging between 2 and 4. Only one mortality was noted in the control tree, only occurring in phase two of the trial.

(C) Sirora

Sirora showed almost the same results as those found on Shufra, showing a combination of long pre-active phases and low mortality rates. Yet, again, only one tree had a clear MR50 point with the rest of the trees below the 50% mortality mark. Statistically, very low mean mortality rates could be seen, averaging at 1 – 2 with only one tree determined as 4. On the control tree only two mortalities were recorded.

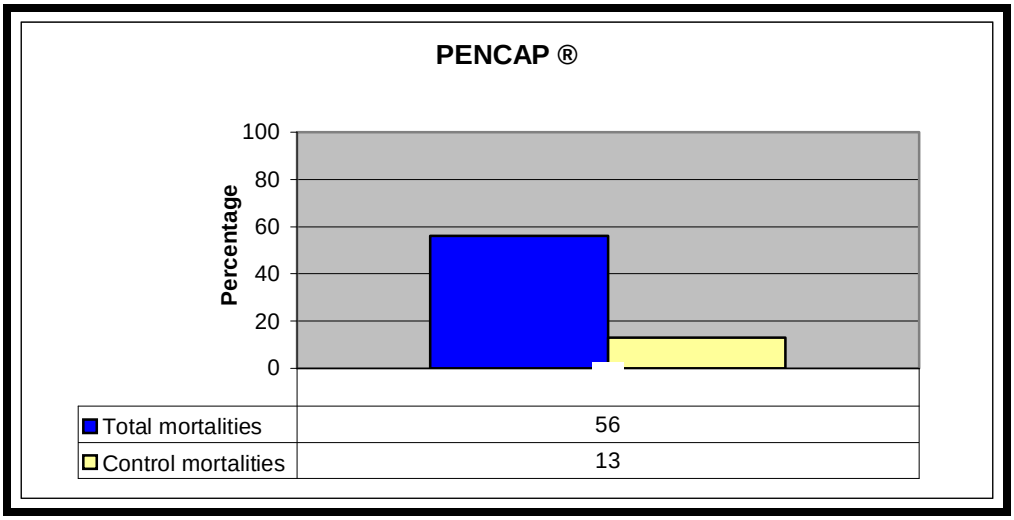


Figure 27: Total trial tree mortalities versus control tree mortalities using Pencap® in a *Atelocera raptoria* bioassay.

As can be seen, noticeably lower average percentage mortalities (i.e. 56%) were recorded. Once again, mortality percentage on the control was fairly low at 13% (Fig. 27).

3.2.4. Karate® (Trial 4)

(A) Shufra

The results for this trial showed substantial fluctuations. The pre-active phase on this cultivar showed highly irregular fluctuation between 1 - 5 hours. The same could be said for the active phase, differing between 3 - 10 hours. In some cases, clear inactive phases could be distinguished, while in other trees, none were perceptible. Conversely, four of the five trial trees showed a MR50 point, with only one tree showing below 50% mortality. Statistical results were also found to be very irregular, averaging from just above one to almost six. The control tree showed three mortalities, but these only occurred after 7 hours.

(B) Ariyeh

Slightly better results were obtained on the Ariyeh cultivar. The pre-active phase appeared to be much the same, falling between 3 - 6 hours, whilst the active phase also lasted between 3 - 6 hours. Somewhat higher mortality rates were, however, observed, with three of the trees reaching the 50% mortality mark. In this case, clearer inactive phases could also be noted. Statistically, slightly higher mean mortality rates were also noted, ranging from 2 – 4 in this cultivar. Only one mortality occurred on the control tree.

(C) Sirora

Fairly good results were obtained on the Sirora cultivar. The pre-active phase was much the same for all five trial trees, differing from 3 – 5 hours. Moderately short active phases could also be noted, ranging from 3 – 6 hours. Of the five trial trees, four showed a MR50 point, with only one tree not reaching 50% mortality. Inactive phases could also clearly be seen in most of the trees. Statistically, the mean mortality figures were low, ranging from as low as 1 up to around 4. The control tree suffered four mortalities, which are the highest found on any of the control trees during this trial.

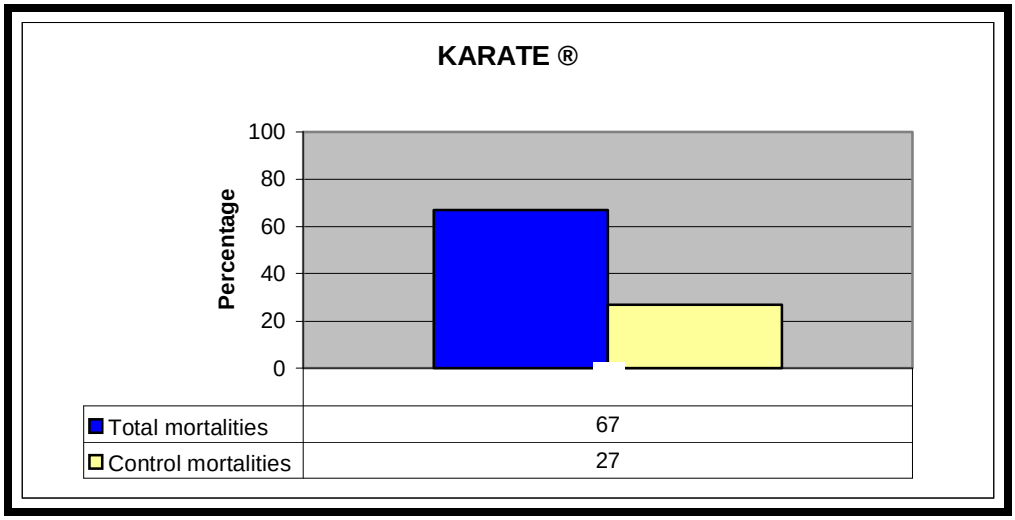


Figure 28: Total trial tree mortalities versus control tree mortalities using Karate® in a *Atelocera raptoria* bioassay.

As was the case in the previous trial, fairly low mortality rates were found, with a total mortality percentage of only 67%. The three control trees showed a 27% mortality overall (Fig. 27). This is a bit higher than those found in the previous two trials, but still considerably lower than the trial trees figure.

3.2.5. Calypso® (Trail 5)

(A) Shufra

Overall, this trial showed the worst results. On this cultivar almost no pre-active phase could be seen in the trial trees. The same can be said for the active phase, with only one tree showing the trend found in the earlier trials. Only two trees reached the MR50 point with the other three showing very low mortalities. In two of the trees, Shufra 2 and Shufra 4, only two mortalities occurred over the whole 24 hour period. Statistically, very low mean mortality values were obtained, with only two of the trees above two. In this case, the control tree suffered more mortalities than some of the trial trees, with four insects dying in the 24 period.

(B) Ariyeh

Results were slightly better on this cultivar and with the majority of the trees, the active phase began almost immediately and steadily declined towards a maximum of 9 hours. Of the trial trees, three reached the MR50 point, but stayed on that level for the entire first phase of the trial. The two other trees had very low mortality rates. Statistically, mean mortality rates were a bit higher, averaging around four for the majority of the trees. The control tree showed high mortality rates, crossing the MR50 mark and suffering 60% mortalities of trial insects.

(C) Sirora

On the Sirora trees the best results of the three cultivars used in the trial were obtained. Pre-active phases ranged from 1 – 5 hours, with active phases averaging at about 6 hours. Of the five trial trees, four crossed the MR50 point, more than in any of the other two cultivars. However, the control tree again showed high mortalities, with four of the insects dying in the first eight hours of the trial. Mean mortality values were between 2 - 4, with the control tree averaging approximately three.

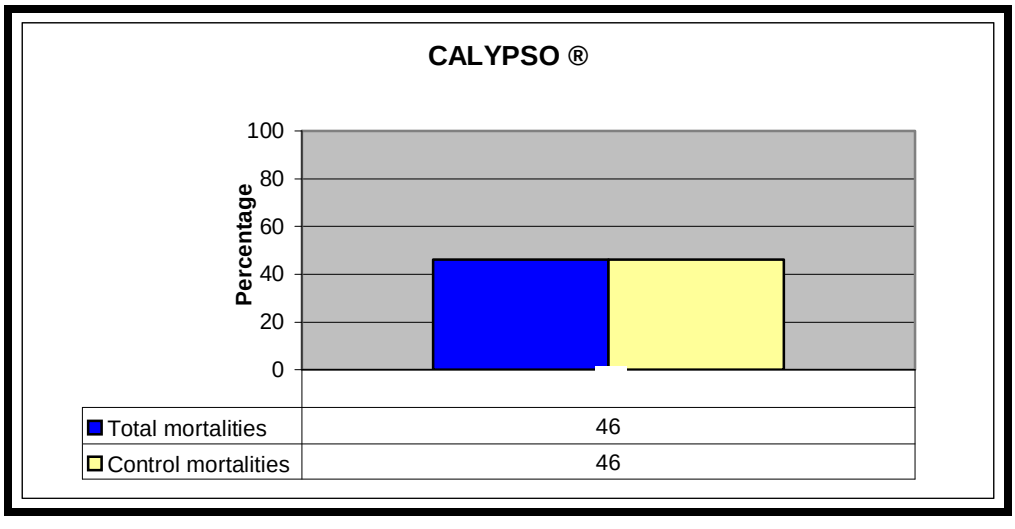


Figure 29: Total trial tree mortalities versus control tree mortalities using Calypso® in a *Atelocera raptoria* bioassay.

The maximum percentage values obtained during the Calypso® trial revealed poor pesticide activity. Very low mortality rates were found, with only 46% of the trial insects over a period of 24 hours. However, the exact same number of insects also died on the three trial trees (Fig. 29). This is the highest mortality rates found on the trial trees in the whole study. In all probability, most of the insects died due to heat and subsequent desiccation. Thus, it can be assumed that this pesticide delivered even lower results than shown in the graph. Clearly, this was the weakest pesticide tested during the trials.

3.2.6. Klartan® (Trial 6)

(A) Shufra

In the final trial run during this study, much better results were noted than those found in the previous trial. In this trial, extended pre-active phases of between 3 – 5 hours were recorded. This is longer than that of most of the previous trials. Even so, widely ranging active phases were found, ranging from only 3 hours up to 9 hours in some of the trees. Of the five trial trees, four reached the MR50 point, with only one failing to do so. The average mortality rates were found to be between 4 - 5 in most of the trees. Conversely, high mortality rates could be seen in the control tree, with half of the insects dying in the trial.

(B) Ariyeh

As found in some of the earlier trials, much better results were recorded on this cultivar. Constant pre-active phases could clearly be seen, ranging from 3 – 4 hours. A fairly constant active phase could also be defined, falling between 4 - 8 hours. All trial trees showed a MR50 crossing point and 100% mortality occurred in all of the trial trees after the conclusion of phase 2 (24 hours). Very high mean mortality rates were found, ranging from 5 – 6 in all the trial trees. The control tree suffered three deaths during the trial.

(C) Sirora

Very diverse results were found on the Sirora cultivar. Very short (1 hour) to very high (10 hours) active phases could be noted. Also, only two of the trees showed a clear inactive phase with three trees crossing the MR50 point. Drastically lower mean mortality rates were also noted during the statistical analysis, with only one tree being above five. The four other trees averaged around three during the trial. The control tree showed three mortalities again during the trial. Mortalities, however, only began occurring after 9 hours.

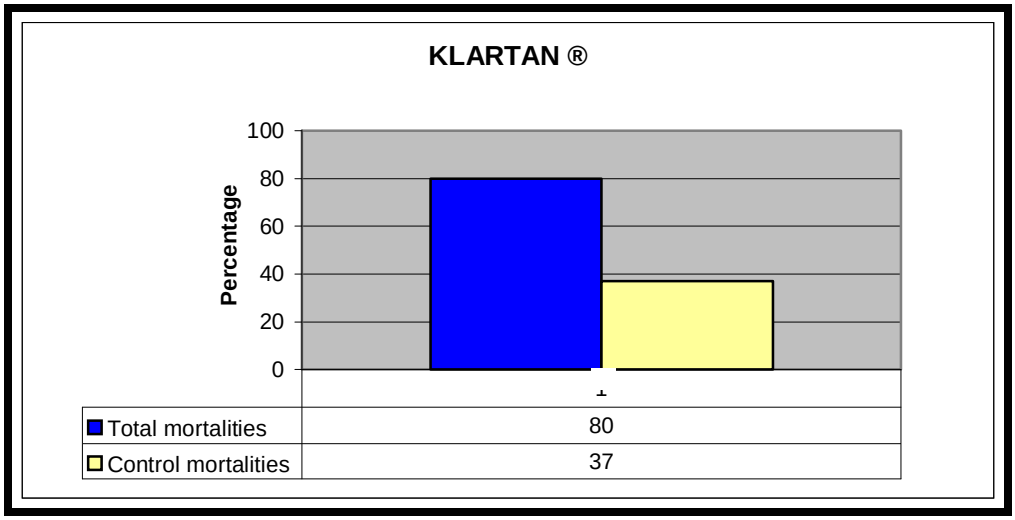


Figure 30: Total trial tree mortalities versus control tree mortalities using Klartan® in a *Atelocera raptoria* bioassay.

When comparing the average mortality percentages of the trial trees and the control trees, it could be seen that high trial tree mortalities occurred during this trial. These were found to be 80%, which is the third highest found in all the trials conducted during this study. Control tree mortality was seen to be 37%, which is also fairly high compared to the other trials (Fig. 30).

4. Conclusion

All in all, good results were obtained during this study. It is clear that some of the pesticides have much greater efficacy than others. By comparing the

percentage mortalities found, the pesticides can be categorized from high to low efficacy (Table 2).

In general, however, it is apparent that the high temperatures in the study greenhouse did have an effect on the survival of the insects used in the trials. It can thus be assumed that mortality rates were lower than those shown by the results. In the three most effective pesticides, control mortality rates were nevertheless much lower than the trial tree mortality rates. The fact that the insects were never directly sprayed with the pesticide, and only underwent secondary contact, reflects on the potency of these three pesticides. This is of great importance when one considers the method of pesticide application as it is conducted at GVN. The adult *A. raptoria* stinkbugs are strong flyers, which mean that most of the target adults would simply fly away during spraying activities. Only the non-alate nymphs would be exposed to direct contact to the pesticide. It is therefore of great importance that the pesticides used in a management program at GVN show good secondary contact capabilities.

As far as cultivars are concerned it would appear as if the results on the cultivar Ariyeh were the most meaningful in nearly all the trials. Overall, it would appear that cultivar Shufra delivered the weakest results.

Table 2: Percentage mortalities recorded in six trials conducted in a pesticide bioassay *Atelocera raptoria* bioassay.

Pesticide	Trial tree mortalities (%)	Control tree mortalities (%)
1) Sevin®	93	37
2) Endosulfan®	89	13
3) Klartan®	80	37
4) Karate®	67	27
5) Pencap®	56	13
6) Calypso®	46	46

5. References

Abou-Fakhr Hammad, E.M., Nemer, N.M. & Kawar, N.S. 2000. Efficacy of Chinaberry tree (Meliaceae) and certain insecticides against the pea leafminer. (Diptera: Agromyzidae). *Journal of Agricultural Science* **134:** 413 – 420.

Anonymous, 2005. *What is an LD50 and a LC50?.* Canadian Centre for Occupational Health & Safety. Retrieved from Http://www.CCOHS.ca

Broughton, S. & Herron, G. A. 2007. *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) chemical control : Insecticide efficacy associated with three consecutive spray strategy. *Australian Journal of Entomology* **46:** 140 -145.

Cortez, M.L., Sanchez, T., Riis, L., Bellotti, A.C. & Calatayud, P.-A. 2003. A bioassay to test HCN toxicity to the burrowing bug, *Cyrtomenus bergi. Entomologica Experimentalis et Applicata* **109:** 235 - 239.

Diercks, R. 1967. Integrierter Pflanzenschutz im Blickfeld der Praxis. *Gesunde Pflanzen* **19:** 8-13. (Original article not seen)

Herron, G. A., Rophail, J. Gullick, G.C. 1996. Laboratory-based insecticide efficacy studies on field-collected *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implication for its management in Australia. *Australian Journal of Entomology* **35:** 161 - 164.

Keita, S. M., Vincent, C., Schmit, J., Arnason, J.T. & Belanger, A. 2001. Efficacy of essential oil of *Ocicum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus macalatus* (Fab.) [Coleoptera: Bruchidae]. *Journal of Stored Products Research.* **37:** 337 - 349.

Kelly, J.R. & Miller, M.A. 1978. Effectiveness of ®Curaterr against pests of swedes and turnips in the British Isles. *Pflanzenschutz-Nachrichten Bayer* **31:** 258 - 282.

Lacey, L.A., Horton, D.R., Chauvin, R.L. & Stocker, J.M. 1999. Comparitive efficacy of *Beauveria bassiana, Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. *Entomologica Experimentalis et Applicata* **93:** 189 - 200.

Ng, K.Y., Schonlau, H.J. & Weber, P. 1986. Integrated pest control in oil palm (*Elaeis guineensis)* with ®Alsystin. *Pflanzenschutz-Nachrichten Bayer* **39:** 30 - 46.

Olszak, R.W. 1999. Influence of some pesticides on mortality and fecundity of the aphidophagous coccinellid *Adalia bipunctata* L. (Col., Coccinellidae). *Journal of Applied Entomology* **123:** 41 - 45.

Philips, P. & Faber, B. 1999. Avocado thrips subproject 2: Pesticide evaluations and phenology in the field. *California Avocado Research Symposium*. Pp 27 - 35.

Tillman, P.G. 2002. Toxicity of Indoxacarb™ to the tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae), and the big-eyed bug, *Geocoris punctipes* (Hemiptera: Lygaiedae). *1st International Symposium on Biological Control of Arthropods.* Honolulu, Hawaii, USA, January 14-18, 2002

Tillman, P.G., Mulrooney, J.E. & Snodgrass, G.L. 2003. Comparison of susceptibility of *Geocoris punctipes* and *Lygus lineolaris* to insecticides for control of the tarnished plant bug. *Southwestern Entomologist* **28 (1):** 1 - 8.

Unterstenhöfer, G. 1970. Integrated pest control from the aspect of industrial research on crop protection chemicals. *Pflanzenschutz-Nachrichten Bayer* **23:** 264 - 272.

Unterstenhöfer, G. 1976. The basic principles of crop protection field trials. *Pflanzenschutz-Nachrichten Bayer* **29:** 83 - 180.

Villanueva-Jimenez J.A. & Hoy, M.A. 1998. Toxicity of pesticides to the citrus leafminer and its parasitoid *Ageniaspis citricola* evaluated to assess their suitability for an IPM program in citrus nurseries. *Biocontrol* **43:** 357 - 388.

Zoebelein, G. & Kniehase, U. 1985. Laboratory, greenhouse and field trials on the effect of nikkomycins on insects and mites. *Pflanzenschutz-Nachrichten Bayer* **38:** 203 - 304.

CHAPTER 5

Nocturnal pesticide bioassay and residual pesticide efficacy study on *Atelocera raptoria* (Hemiptera, Pentatomidae)

1. Introduction

This study was conducted on site at Green Valley Nuts (GVN) in the Northern Cape Province during February 2006. The purpose of this study was to conduct a nocturnal pesticide bioassay using three different pesticides for the control of *Atelocera raptoria*, a key-pest on pistachio. In January 2005, similar trials were conducted at GVN using six different pesticides which were, at the time, deployed by GVN for the management of *Atelocera raptoria*. The pesticides that were tested then were Sevin®, Endosulfan®, Calypso®, Pencap®, Karate® and Klartan®. Of these, Sevin® and Endosulfan® were found to be the most effective and GVN reverted to using only these two in subsequent spraying strategies.

As a follow up to the trials conducted in 2005 these two pesticides, together with Decis®, were used in this study. It is common practice at GVN to mix foliar nutrients with the pesticides and to apply the cocktail during a single spraying event, since it saves time and expenses. During this bioassay study the implementation of such an approach at GVN was investigated and therefore one of the primary aims was to determine whether foliar nutrients influence the efficacy of pesticides in any way.

As was the case during the 2005 trials, the stinkbugs received no direct contact to the pesticides, allowing the secondary contact action of the pesticides to be investigated. All in all conditions during a spraying event at GVN were simulated and therefore pesticides were applied to young pistachio trees during nocturnal hours under controlled conditions, after which the insects were placed onto the trees. At GVN pesticide application occurs during the night in order to minimize evaporation and spray drift. Nocturnal studies also allowed feeding activity of the insects during the night and whether lower temperatures may influence results to be investigated.

It is well known that insects are affected by temperature in many ways. According to Mellanby (1939), insects are cold blooded, and their metabolism and activity is greatly influenced by the temperature of their bodies which, in turn, is almost entirely dependant on the surrounding environment. Low temperature will inhibit activity, whereas higher temperature will usually stimulate the insect. All arthropods are poikilothermic, meaning they cannot regulate their own body temperature and their body temperature rises and falls with the environmental temperature (Borror, Triplehorn & Johnson, 1992). Insects are thus dependable on environmental temperature to regulate metabolism, physiological processes and body temperature.

Albeit that lower temperatures during night time should cause adult *A. raptoria* to be less active, lowering their flight capabilities, these insects are excellent flyers and would still fly away during spaying activities in the orchards. The secondary contact action of the pesticides are thus of great importance, since insects migrating in and out of the orchards on account of disturbances would still be exposed to the pesticide during feeding. In such a scenario non-alate nymphs would receive direct contact spray as well.

Another study was included as follow-up to the diurnal and nocturnal bioassays, whereby the residual efficacy of the pesticides was also examined. The residual efficacy of a pesticide entails the effectiveness against the target insect over an extended period of time. This period will usually differ depending on the pesticide used and the target species. Pesticide residue levels on crops are of great concern in modern agriculture. According to Blasco, Font & Pico (2006), pesticides have been linked to a wide spectrum of human health hazards, ranging from short-term impacts to chronic effects due to pesticide exposure. Hence the presence of pesticides in food receives worldwide attention because of their wide use in agriculture to control organisms that spoil the crops. Due to these health risk factors, the detection of pesticide residue levels in food has been implemented in many countries. Analytical methods are needed to screen, quantify, and confirm pesticide residues in fruit and vegetables for both research and regulatory purposes (Torres, Pico & Manes, 1996). Consequently a variety of analytical procedures and methods towards the testing of pesticide residue levels in food arose

(Blazquez, 1973; Chiang, Dean & McDaniel, 1985; Chen & Wang, 1996; Torres et al., 1996; Blasco et al., 2006). Due to the strict residue regulations applied in practice, modern pesticides usually have a shorter residue lifespan, which may affect residual efficacy.

According to Rojas de Arias, Lehane, Schofield & Fournet (2003), the limiting factors for activity of applied pesticides usually include the initial insecticide dosage, the nature of the sprayed surface, as well as the age of the insecticide deposits. Environmental conditions such as humidity and temperature will also influence insecticide efficacy over extended periods of time. Furthermore, on porous surfaces, such as mud, the insecticide deposit seems to lose activity much faster than on non-porous surfaces.

As was the case when conducting normal efficacy bioassays on pesticides, the protocol and methods used, seems to depend entirely on the specific pesticide used, it's mode of action and the insect target species. This can be demonstrated by examining different residue efficacy trials in the literature. Radical differences occur in each study. During laboratory trials Zoebelein & Kniehase (1985) found that pesticide efficacy lasted only 5 days post application. During an integrated pest control study conducted on oil palm (Elaeis guineensis), pesticide efficacy was found to be 100% after approximately six days (Ng, Schonlau & Weber, 1986). Zacharda & Hluchy (1991) examined the long-term efficacy of 16 different pesticides. Again, vastly different results were obtained, depending on the pesticide used. During a study conducted by Bostanian & Racette (1997) the residual toxicity of lambda-cyhalothrin on leaves from a treated apple orchard declined to one-third of its original level within 3 weeks. These examples all indicate the importance pesticide residual studies.

2. Material and Methods

The material and methods used for this study were almost exactly the same as those used during the diurnal bioassay conducted a year earlier (See Chapter 4). The main differences were the pesticides that were tested, the pesticide foliar nutrients cocktail and the nocturnal hours during which the trials were conducted.

In total, three different pesticides were examined, i.e. Sevin®, Endosulfan® and Decis®. Each pesticide was studied in its own trial, followed by three trials where each of the pesticides were mixed with foliar nutrients and tested for efficacy. Thus, in total, six trials were conducted during the study.

Foliar nutrients used in this study consisted of the same substances and mixture properties as those currently deployed by GVN. The foliar nutrients are applied together in a mixture with the pesticides by means of mist blowers directly to the leaves of the trees. The pre-prepared mixture of foliar nutrients used for this study was 97.5 g $CaNO_3$, 15 g $ZnSO_4$ and 22.5 g H_3BO_3 (boric acid) per 15 liter water in a mist blower tank.

The same number of trees were used during the nocturnal bioassay as was used during the diurnal bioassay and trials were initiated at sundown. Every trial occurred over a time period of 20 hours. During this time, the trial was further subdivided into two phases. Phase 1 consisted of the first 12 hours and phase 2 of the following 8 hours. Monitoring took place every 2 hours during the second phase. A trial could therefore be conducted every day and if a trial terminated at 16:00, preparations could be made for the following trial to commence at 19:00 of the same day. The study was conducted over six days and material sampling was done in between trials. Ten adult insects were placed on every tree and 18 trees were used. Six trials were conducted meaning 1080 insects were used during the entire study.

Trees of each cultivar were obtained from the GVN nursery. During the first two trials (Sevin® and Sevin® + foliar nutrients (FN)), the trials were conducted in a greenhouse. However, the last four trials were conducted under an open storage canopy, in order to avoid very high mid-day temperatures that could influence the results and in the process to protect the trial trees from rain. For a period after application rain washes away any pesticides on trees causing irregularities in the results. Temperatures under the storage canopy were significantly lower than those found in the greenhouse simply on account of better free air flow. The trial setup was the same for each trial and the trees were assembled in the same manner as described for the diurnal bioassay.

All adult *A. raptoria* specimens were sampled on trees occurring in the GVN orchards. Sampling was done by hand while scouting the orchard and blocks that had not been recently sprayed with pesticides were selected. As was the case with the diurnal bioassay, captured insects were placed inside glass terrariums for containment.

The method used with the application of the pesticides was conducted in the same manner for all six trials and pesticide dosages were mixed according to a pre-determined recipe (Table 1). During each trial 15 ml of buffering liquid (Aquarite®) was added to each mixture of 15 liters in order to optimize the Ph of the water of the pesticide mixtures. During the first three trials, the Aquarite® was first added to water, followed by the pesticide concentrate. During the last three trials the Aquarite® was still added first, followed by the foliar nutrients (FN), followed by the pesticide. Regular tap water was used.

Each trial started at 20:00 on the trial day. This was seen as time 0 at the start of the first phase. Trees were sprayed at approximately 19:00 every evening.

Table 1: Specified dosages of pesticide and foliar nutrient mixtures used during nocturnal bioassays on *Atelocera raptoria.*

Product	Dosage (ml) / 100 L	Dosage (ml) / 15 L
Sevin®	225	33.75
Endosulfan®	100	15
Decis®	25	3.75
Sevin® + FN	225 + 900g	33.75 + 135 g
Endosulfan®+ FN	100 + 900g	15 + 135 g
Decis® + FN	25 + 900g	3.75 + 135 g

After placement of 10 stinkbugs on sleeved trees, the first reading was taken. This was noted as time 0 and hour 0 at which point all insects were still alive. This was also noted as the start of phase 1. Monitoring was conducted at the conclusion of phase 1, after 12 hours. Thus phase 1 would run throughout the night, from 20:00 until 08:00 the following morning. During phase 2, monitoring was done every two hours up until termination of the trial at 16:00. Monitoring was done by visual inspection through the gauze netting. The number of specimens alive and the number of dead specimens were noted. Inspection of the trees was done in the same sequence in which insects were placed on the trees. Inspection on every tree continued until all 10 insects were spotted and accounted for.

- All specimens still alive after the completion of a trial were removed and killed in 70% ethanol. The nets were removed from the trees after the completion of each trial and washed. The trees used were taken back to the nursery at GVN and replaced with new trees in preparation for the next trial. The same procedures were followed for all six trials until the completion of the study.

3. Results and Discussion

3.1. Temperature and relative humidity (RH)

After the completion of the study, weather data were obtained from one of the weather monitoring stations at GVN (Figs. 1 & 2).

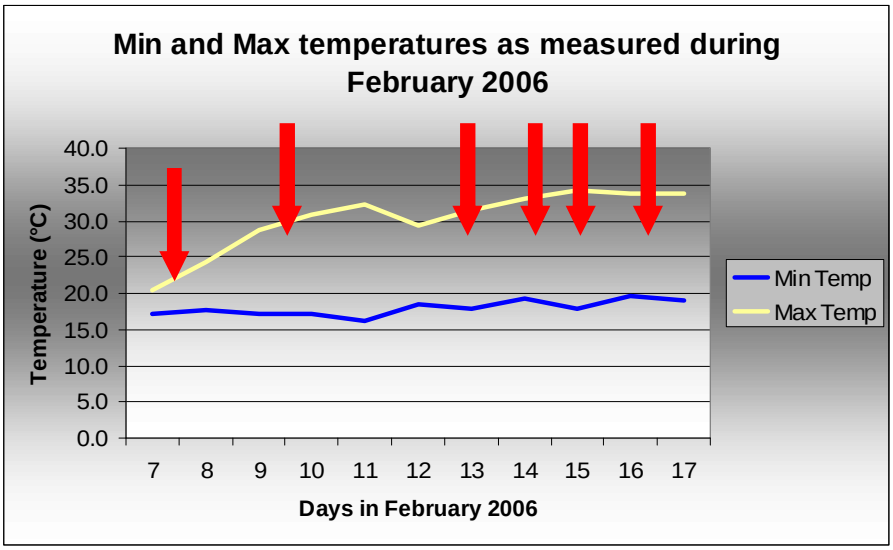


Figure 1: Minimum and Maximum temperatures measured during 7 – 17 February 2006. (Arrows indicate days on which nocturnal bioassay trials on *Atelocera raptoria* were conducted).

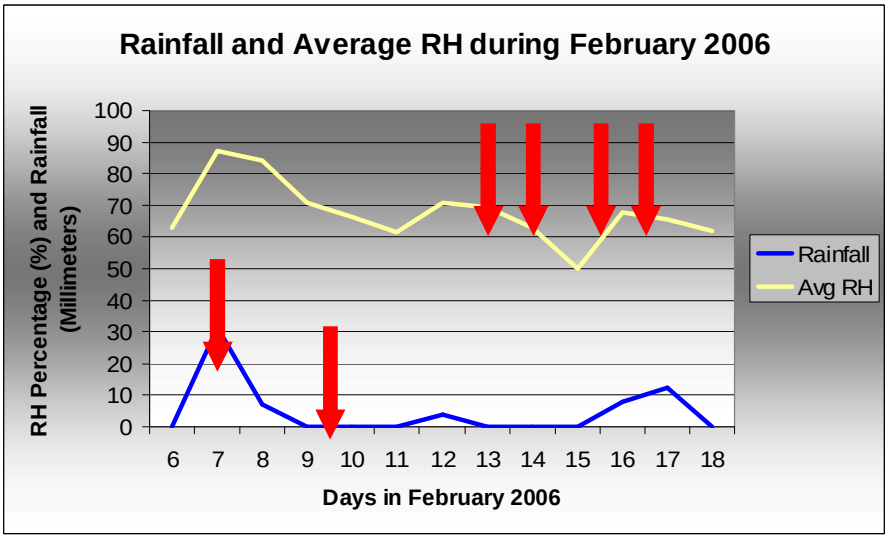


Figure 2: Temperature and relative humidity (RH) taken over 12 days from 6 - 18 February 2006. (Arrows indicate days on which nocturnal bioassay trials on *Atelocera raptoria* were conducted).

The first two trials (Sevin® and Sevin® + FN) were conducted in the greenhouse. Temperatures rose to a high level at mid-day within the greenhouse. However, heavy rain occurred during the first Sevin® trial, resulting in low temperatures. The second Sevin® trial was conducted on a sunny day, which caused temperatures to rise significantly. Thus, irregularities could be seen in the results when these two were compared. Temperatures did not rise as high as those experienced during the 2005 trials however. The other four trials were conducted in the open storage canopy, where temperatures were not so high. Some rain also occurred during the final trial (Decis® + FN), although this did not influence the temperature greatly.

3.2. Pesticide bioassay

One of the concerns during this study, was that the insects which are active during the day would not feed during the night. Also, temperatures during night time would be lower, causing the insects to be less active.

By recording all data sampled over the 20 hours of every trial, a complete data table could be compiled which included total specimen mortality. The process was followed on all 108 trees from all six trials during the entire study. Due to the fact that only 10 specimens were placed on every tree, the number of live specimens per trial tree could easily be calculated.

As was the case during the 2005 study, charts from every tree used in the study could be compiled. These are easier to read and understand and also point out several important events during the pesticide activity process Appendix 1, Figs. 1 - 18).

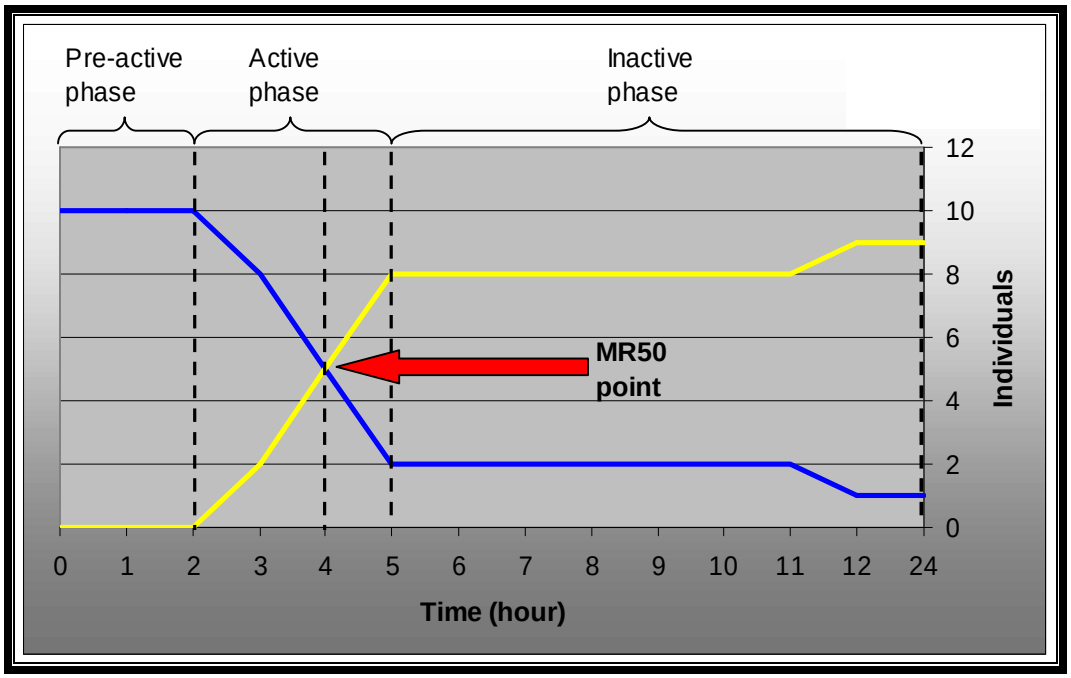


Figure 3: Graphical representation of pesticide activity applying to trials 1 – 6.

Figure 3 shows the process of pesticide activity. The first phase that occurs has been named the pre-active phase. This phase commences at the time of pesticide application. It represents the time preceding the point where pesticide activity can be noticed. Some pesticides would show a much shorter pre-active phase. This time is probably dependant on the active ingredient, mode of transfer and the concentration of the pesticide. The second phase can be referred to as the active phase. This is the phase in which the effect of the pesticide could clearly be seen. This is also the time in which most mortalities would take place. It can be assumed that nearly all mortalities occurring in this phase are due to exposure to the pesticide. In most cases, the mortality rate 50% (MR50) point also falls within this phase. This represents the point where 50% of the trial insects are dead and 50% remain alive. The third and final stage can be referred to as the inactive phase. Theoretically no mortalities due to pesticide exposure occur during this phase. All deaths occurring in this phase should be due to desiccation on account of high temperatures or other diverse influences. This phase is of special significance to the control trials.

The maximum percentage mortalities over all five trial trees from each cultivar of every trial were also examined. These figures were compared to the maximum percentage mortalities of the control trees of the same cultivar in each trial. These charts clearly show the difference between the mortality rates found on trial trees versus control trees (Figs. 4 – 11)

3.2.1. Sevin® (Trial 1)

(A) Shufra

The first cultivar, Shufra, showed fairly average results. On most of the trees, most of the mortalities occurred during phase 1. This phase runs throughout the night. Only one of the trees (Shufra 1) showed an MR50 point, meaning that on none of the other trees more than 50% mortalities occurred. On one of the trees (Shufra 5), only two mortalities occurred in the final two hours of the trial. The control tree suffered no mortalities throughout the trial.

(B) Ariyeh

Results on the Ariyeh cultivar were much the same as on Shufra. Most mortalities occurred within the first 14 hours of the trial. However, none of the trees showed an MR50 point, with the most mortalities occurring on Ariyeh 5, where four mortalities occurred. Two of the trees had mortality rates of only 20%. Again, the control tree showed no mortalities.

(C) Sirora

Of the three cultivars tested using Sevin®, Sirora showed the weakest results. On three of the trial trees (Sirora 1, 4 & 5) only one insect died within the 20 hour trial. Only one tree showed significant results, with 50% mortality occurring. As was the case during with the other two cultivars, the control tree suffered no mortalities.

The maximum mortality percentage of all the trial trees was only 29% against the 0% mortalities of the control trees (Fig. 4). When this is compared to the study done in 2005, it can be seen that mortalities are significantly lower (Fig.

5). In the 2005 trial, 93% mortality occurred, with the control trees showing 37% mortality. However, it should be noted that heavy rain did fall during the duration of this trial, which caused temperatures to be very low compared to those recorded in the 2005 study. These low temperatures would cause the trial insects to be far less active, inhibiting their feeding activities.

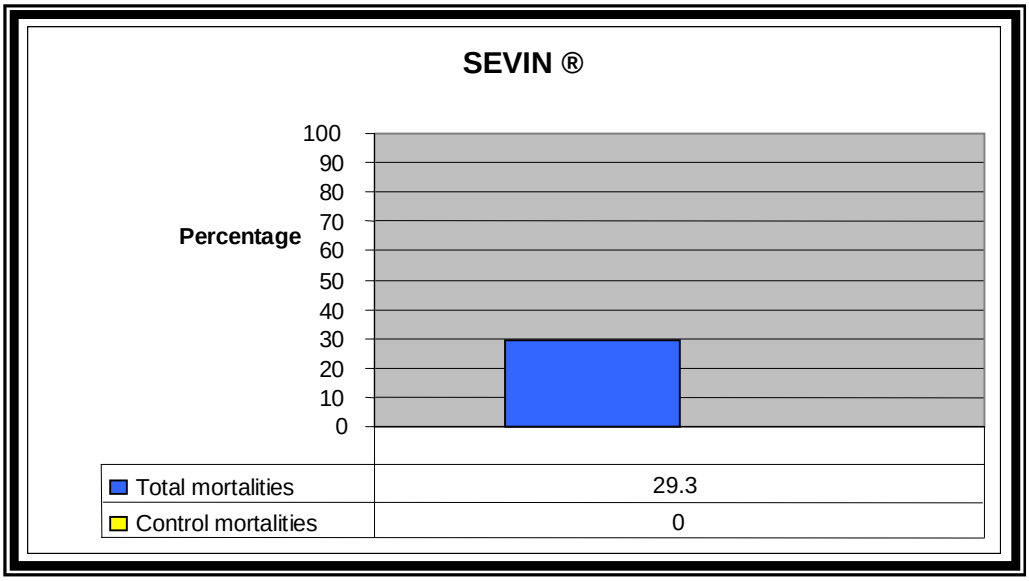


Figure 4: Total trial tree mortalities versus control tree mortalities using Sevin® in a *Atelocera raptoria* bioassay (Figures from the 2006 study).

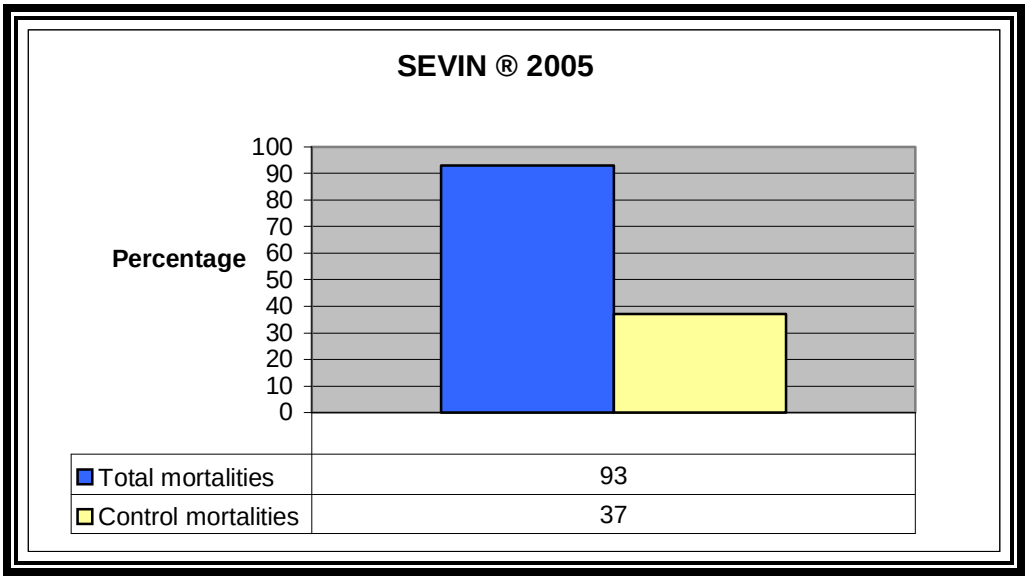


Figure 5: Total trial tree mortalities versus control tree mortalities using Sevin® in a *Atelocera raptoria* bioassay (Figures from the 2005 study).

3.2.2. Sevin® + Foliar Nutrients (Trial 2)

(A) Shufra

During this trial the results on the Shufra trees were better than in the previous trial. In this trial, Sevin® was applied in combination with the foliar nutrients (FN). Three of the trees showed a MR50 point, with 50% mortalities occurring on two of these. One tree (Shufra 1) showed 60% mortality. The other two trees (Shufra 3 & 4) had only 30% mortality each. The control tree showed no mortalities whatsoever.

(B) Ariyeh

Even better results were obtained on the Ariyeh cultivar. All trial trees showed a MR50 point. It would appear that this point mostly occurs between 16 and 18 hours, i.e. between 12:00 and 14:00. Therefore, it would appear that the higher temperatures found later in the day, indeed increases the feeding activity of the insects. Most mortalities also occurred during the later stages of the trial. Again, the control tree showed no mortalities.

(C) Sirora

On the Sirora trees promising results were obtained with four of the five trial trees reaching the MR50 point. The other tree (Sirora 1), showed 40% mortality during the trial. Hundred percent mortality was found on one of the trial trees. No mortalities were observed on the control tree.

In total, insect mortality rates were considerably higher than those found in the Sevin® trial (29%) (Fig. 6). These figures also compare well to those found in the 2005 study. It should, however, be noted that both of these trials were conducted in a greenhouse where mid-day temperatures can rise considerably. However, even if this was the case, no control mortalities were observed in either of the Sevin® trials. Results also show that mortalities were much higher during the Sevin® + FN trial, than those seen on the first Sevin® trial. In all probability this is due to the higher temperatures recorded during the second trial.

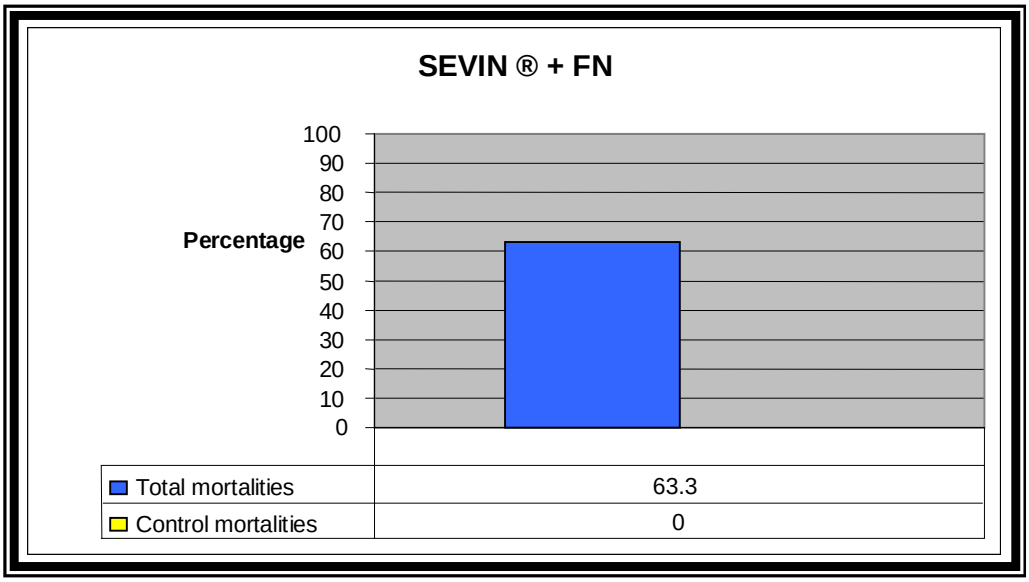


Figure 6: Total trial tree mortalities versus control tree mortalities using Sevin® + Foliar nutrients (FN) in a *Atelocera raptoria* bioassay (Figures from the 2006 study).

3.3.3. Endosulfan® (Trial 3)

(A) Shufra

Conflicting results were obtained during this trial. In the first of the trees (Shufra 1) no mortalities could be observed throughout the entire trial. In contrast, three of the other trial trees (Shufra 2, 3 & 4) reached the MR50 point, with exactly 50% mortalities occurring in each of these. As was the case during the previous trials, most mortalities occurred during phase 2 of the trial. This is when temperatures are higher. No control mortalities could be observed.

(B) Ariyeh

Comparatively weak results were obtained on the Ariyeh cultivar. None of the trees had an MR50 point, with 30% recorded as the highest mortality rate. The other trees showed almost no result, with mortality rates of only 20%. Also, mortalities occurred sporadically throughout the trial, giving no indication of active phases. One mortality (10%) occurred on the control tree during the final two hours of the trial.

(C) Sirora

The Sirora trees showed slightly better results as those found on the Ariyeh trees. As was the case on the Ariyeh trees, none of the Sirora trees crossed the MR50 point. Again, no definite active phases could be distinguished. Two of the trees (Sirora 4 & 5) suffered only one mortality, with the highest mortalities (40%) occurring on Sirora 2. Overall, low mortality rates were noted during this trial. The control tree suffered no mortalities.

As was the case during the Sevin® trials, significantly lower mortalities were obtained during the 2006 than in 2005 (Figs. 7 & 8). Only 25% average trial tree mortalities could be observed during this trial. Conversely, the control trees showed only 3% mortalities.

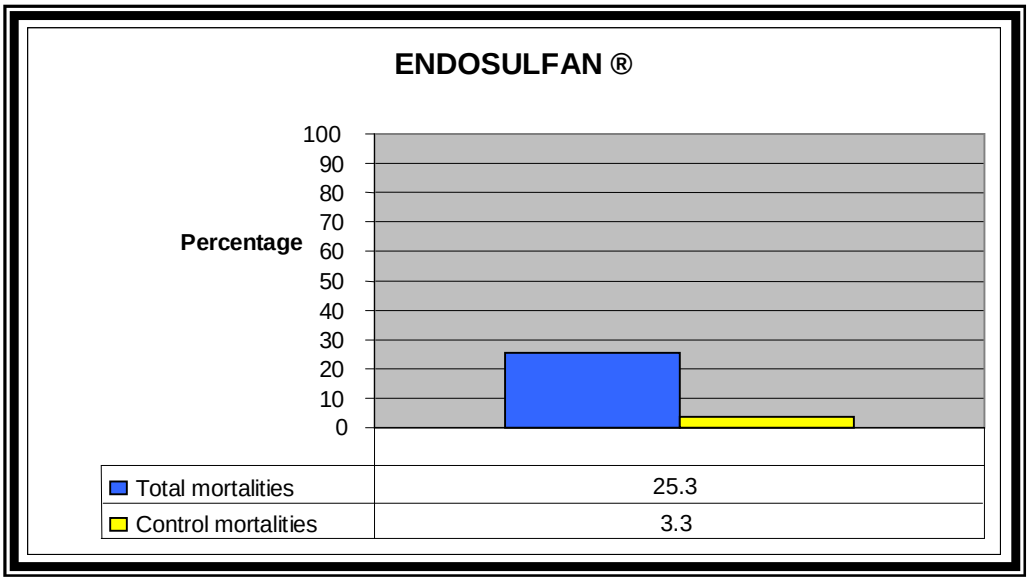


Figure 7: Total trial tree mortalities versus control tree mortalities using Endosulfan® in a *Atelocera raptoria* bioassay (Figures from the 2006 study).

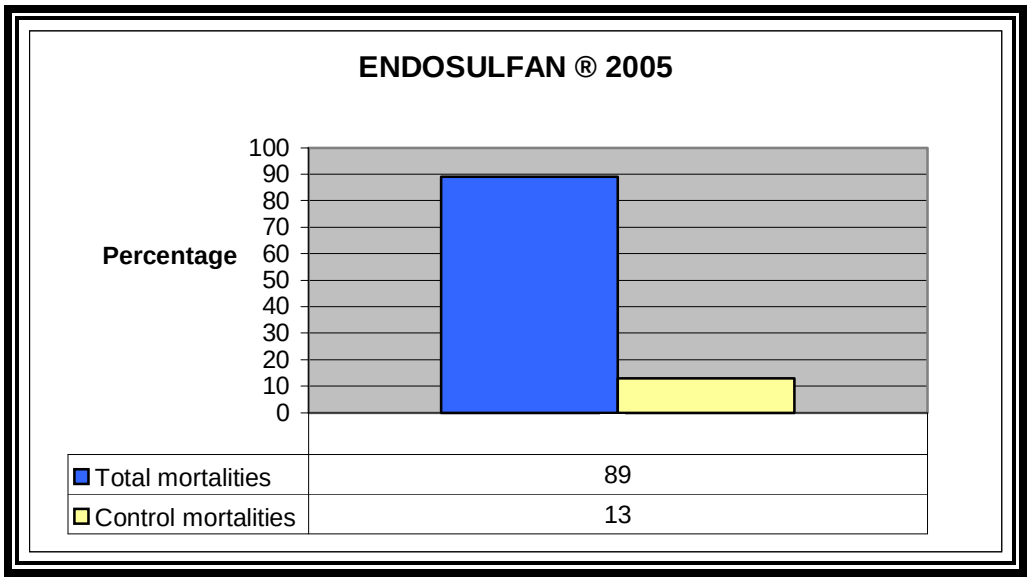


Figure 8: Total trial tree mortalities versus control tree mortalities using Endosulfan® in a *Atelocera raptoria* bioassay (Figures from the 2005 study).

3.3.4. Endosulfan® + Foliar Nutrients (Trial 4)

(A) Shufra

During this trial, the Shufra cultivar displayed slightly weaker results than those seen during the Endosulfan® trial. None of the trees reached the MR50 point and only one mortality occurred on Shufra 5. On all of the other trees, 40% mortality could be seen at the conclusion of the trial. Again, it appears that more mortalities occurred during the second phase of the trial. Again, in all probability, this is ascribed to the high temperatures. No control mortalities were observed.

(B) Ariyeh

Weak results were observed on the Ariyeh trees. Three of the trees (Ariyeh 3, 4 & 5) showed only 20% mortality, with Ariyeh 1 showing only 10%. The only tree showing significant results was Ariyeh 2, where 60% mortality was recorded. No control mortalities were found.

(C) Sirora

The Sirora trees showed much the same results as those seen on the Ariyeh trees. As was the case on the Ariyeh trees, only one tree (Sirora 3) crossed the MR50 point, showing 70% mortality at the conclusion of the trial. Two of the trees (Sirora 2 & 5) showed weak results, with only 10% mortality each. The control tree suffered one mortality after phase 1, and this remained constant throughout the trial.

Almost the same average trial tree mortality percentage was recorded during as was found during the Endosulfan® trial, with the control percentage remaining the same (Fig. 9). As was the case during the Endosulfan® trial, these mortality rates were much lower than those found during the 2005 trials.

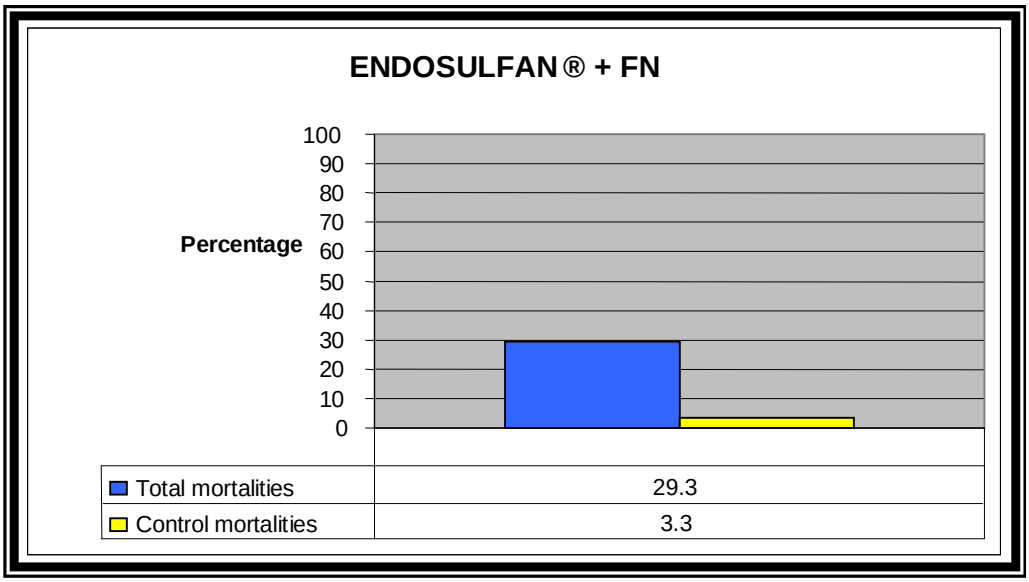


Figure 9: Total trial tree mortalities versus control tree mortalities using Endosulfan® + FN in a *Atelocera raptoria* bioassay (Figures from the 2006 study).

3.3.5. Decis® (Trial 5)

Decis® was a new pesticide not yet deployed by GVN in their pesticide spraying program. This pesticide was also not tested during the study conducted during 2005. Consequently, nothing was known about efficacy of this pesticide.

The results showed that Decis® has almost no effect on *Atelocera raptoria*. During the trials conducted, mortalities were only recorded on single trees of all the cultivars. The highest mortality percentage was noted on the Ariyeh cultivar (Ariyeh 2), with only 20% mortality occurring. Thus, none of the trees from any of the cultivars crossed the MR50 mark. However, the control tree from the Ariyeh cultivar also suffered one mortality.

Almost no mortalities occurred on either the trial or the control trees, with only about 3% mortality recorded (Fig. 10). This pesticide is clearly ineffective for use as a control measure against *Atelocera raptoria*.

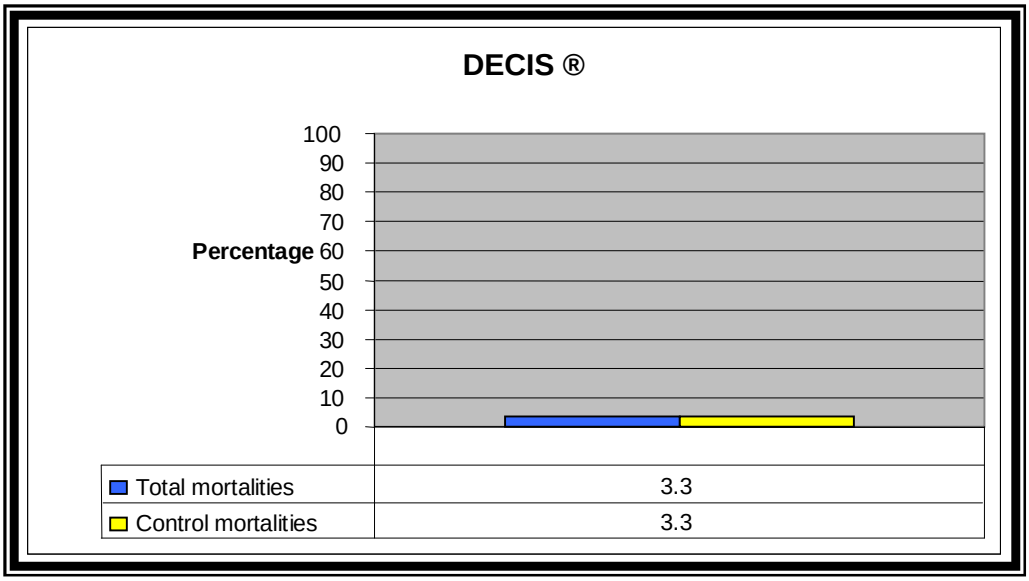


Figure 10: Total trial tree mortalities versus control tree mortalities using Decis® in a *Atelocera raptoria* bioassay.

3.3.6. Decis® + Foliar Nutrients (Trail 6)

As was the case during the pure Decis® trials, almost no mortalities occurred in either the trial trees or the control trees. Again, none of the trees reached the MR50 point, with the highest mortality percentage only at 20% (Ariyeh 5).

Even lower mortality rates were recorded in the Decis® + FN trial, with only 2.7% mortality (Fig. 11). This emphasizes the opinion that Decis® is not suitable for use as a control measure for *Atelocera raptoria.*

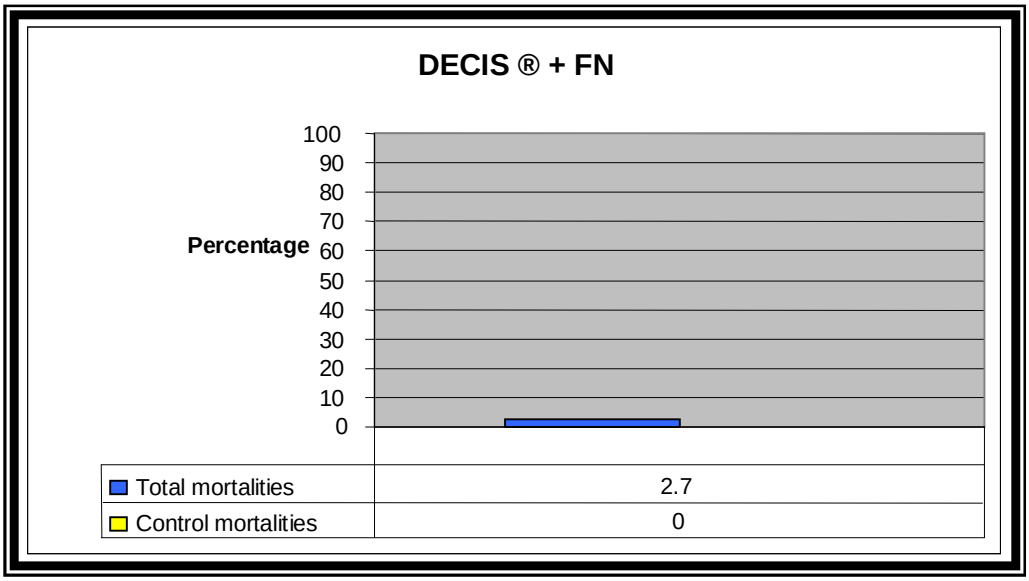


Figure 11: Total trial tree mortalities versus control tree mortalities using Decis® + FN in a *Atelocera raptoria* bioassay.

4. Conclusion

When comparing this study to the 2005 study, much weaker results were obtained. However, from the 2005 study, it was apparent that the high temperatures in the greenhouse did have an effect on the survival of the study insects. It can thus be assumed that overall mortality rates were lower than those shown by the results.

Contrary to the 2005 study, almost no control mortalities could be observed in any of the trials during this study. Thus it could be assumed that temperature did not cause any significant mortalities during this study. All in all, however, mortalities were much lower compared to the 2005 study. This shows that the insecticides are indeed less effective during the night. As the insects are not directly sprayed with the pesticides during any stage of the trials, they must feed for the pesticide to have an effect. Thus, the lower temperatures found during this study, and the fact that most of trial time span was done

throughout the night, did have a significant effect on the mortality percentages.

As was the case during the 2005 trials, Sevin® performed the best overall. This is followed by Endosulfan® and Decis®. Decis® had almost no effect on the trial insects and is not recommended for use as a control measure against *Atelocera raptoria.* The added foliar nutrients used during trials 2, 4 and 6, also did not appear to influence results to any great extent. Although mortalities found were much higher during the Sevin® + FN trial than the normal Sevin® trial, it is believed that this was caused by the significantly higher temperatures recorded during the Sevin® + FN trial, and not by the addition of foliar nutrients.

The inconsistent results of this trial compared to the results found during the diurnal bioassay, lead to the inclusion of a third study, the residual effect study.

5. Residual effect study

5.1. Material & Methods

During this study, conducted on site at GVN the residual effect of the pesticides Sevin® and Endosulfan® were tested. These two pesticides were demonstrated to be the most effective regarding the control of *A. raptoria.* The methods used during the diurnal and nocturnal bioassays were also applied during this study, with a few key differences.

When Sevin® and Endosulfan® were tested, this was done as a single trial. The foliar nutrients used during this study were exactly the same as those used during the bioassay studied and were added in the same manner during the mixing process.

The experimental setup differed from the one used during the bioassays. As the trial would run for an extended period of time, there was decided to deviate from the procedure followed during the bioassays. During this study

only Sirora was used, since it was deemed logistically difficult to conduct a long-running study on all three cultivars. Also, no noteworthy difference between the cultivars could be detected during either of the bioassays. This cultivar was a random choice.

Only 15 trees, obtained from the GVN nursery, were used during this study. These were arranged in basically the same manner as during the bioassays. However, the trees were set up in three rows of five trees each. Of these three rows, the first was sprayed with Sevin®, the second with Endosulfan® and the final row was left untreated as control (Fig. 12).

The trial was run in February 2006 and pesticide application was only done once. Monitoring was conducted once every week over a period of four weeks.

Figure 12: Tree setup used during the pesticide residual effect study on *Atelocera raptoria*.

Every tree was supplied with 10 adult insects. At every monitoring event after a week had elapsed, all the trial insects were monitored, and mortality rates were noted. After each monitoring event, all insects were removed and replaced with fresh insects sampled in the GVN orchards. This was repeated another three times.

Pesticides were applied in exactly the same manner as described in the nocturnal bioassay. As was the case during the nocturnal bioassay, foliar nutrients were also added to both the pesticides that were used.

5.2. Results and Discussion

The results of the residual effect study is summarized in Fig. 13. As was the case during the bioassays, Sevin® again performed the best. During Phase 1 (first week after application), mortality rates of 66% could be noted on the trees treated with Sevin®. The Endosulfan® trees shows mortality rates of only 39%, while 20% of the trials insects died on the control trees.

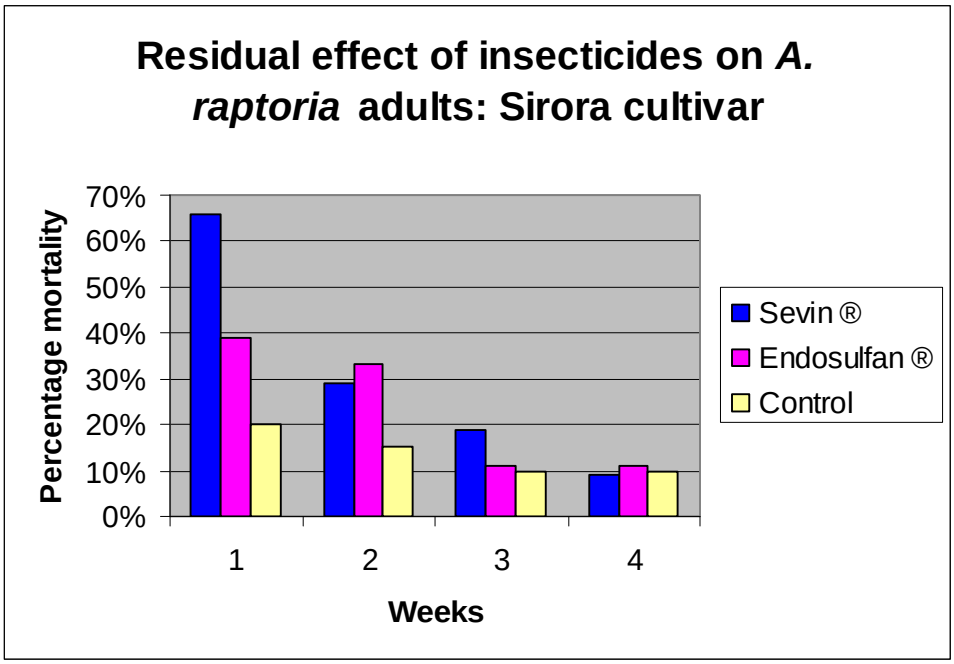


Figure 13: Residual effect of two pesticides, Sevin® and Endosulfan®, on *Atelocera raptoria* over four weeks.

During Phase 2 (second week after application), mortality rates were significantly lower, with around 30% of insects dying on both the Sevin® and Endosulfan® treated trees. Around 15% of the control insects died as well.

This downward trend continues during Phase 3 (third week after application), with only 19% of the insects dying on the trees treated with Sevin® and around 10% dying on the Endosulfan® and control trees. Finally, during Phase 4 (four weeks after application), very low mortality rates were recorded, with all treatments showing around 10% mortality.

5.3. Conclusion

Results recorded during the pesticide residue study, compares well with those recorded during the bioassay studies. Results of the nocturnal bioassay show that Sevin® + FN had mortality rates of 63.3% during the first week after application. During this study mortality rates of 66% could be seen. Furthermore, Endosulfan®+ FN trials conducted during the nocturnal bioassay showed a mortality rate of 29.3%. Again, this compares fairly well with the 39% mortality found during this study.

By examining the recorded data (Fig. 13), it can be observed that the residual effect of both Sevin® and Endosulfan® weakened considerably after 2 weeks. This is verified by the fact that the control trees showed the same number of mortalities as those seen on the treated trees. Even during the second week following application, mortality rates of especially Sevin® dropped drastically. Realistically this illustrates that pesticides should be applied on a two-weekly basis as far as a pesticide application program is concerned.

6. References

Blasco, C., Font, G. & Pico, Y. 2006. Evaluation of 10 pesticide residues in oranges and tangerines from Valencia (Spain). *Food Control* **17:** 841 - 846.

Blazquez, C.H. 1973. Residue determination of Ethylenethiourea (2-Imidazolidinethione) from tomato foliage, soil, and water. *Journal of Agricultural Food Chemistry* **21:** 330 - 346.

Borror, D.J., Triplehorn, C.A. & Johnson, N.F. 1992. *An Introduction to the Study of Insects, Sixth Edition.* Saunders College Publishing, New York.

Bostanian. N.J. & Racette, G. 1997. Residual toxicity of lambda-Cyhalothrin on apple foliage to *Amblyseius fallacis* and the tarnished plant bug, *Lygus lineolaris. Phytoparasitica* **25(3):** 193 - 198.

Chen, Z-M & Wang, Y-H. 1996. Chromatographic methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods and environmental samples. *Journal of Chromatography* **754:** 367 - 395.

Chiang, T., Dean, M.C. & McDaniel, C.S. 1985. A fruit fly bioassay with phosphotriesterase for detection of certain organophosphorus insecticide residues. *Environmental Contamination and Toxicology* **34:** 809 - 814.

Mellanby, K. 1939. Low temperature and insect activity. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **127 (849):** 473 - 487.

Ng, K.Y., Schonlau, H.J. & Weber, P. 1986. Integrated pest control in oil palm (*Elaeis guineensis)* with ®Alsystin. *Pflanzenschutz-Nachrichten Bayer* **39:** 30 - 46.

Rojas de Arias, A., Lehane, M.J., Schofield, C.J. & Fournet, A. 2003. Comparative evaluation of pyrethroid insecticide formulations against *Triatoma infestans* (Klug): Residual efficacy on four substrates. *Memoras do Instituto Oswaldo Cruz* **98(7):** 975 - 980.

Torres, C.M., Pico, Y. & Manes, J. 1996. Determination of pesticide residues in fruit and vegetables. *Journal of Chromatography* **754:** 301 - 331.

Zacharda, M. & Hluchy, M. 1991. Long-term residual efficacy of commercial formulations of 16 pesticides to *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) inhabiting commercial vineyards. *Experimental and Applied Acarology* **13:** 27 - 40.

Zoebelein, G. & Kniehase, U. 1985. Laboratory, greenhouse and field trials on the effect of nikkomycins on insects and mites. *Pflanzenschutz-Nachrichten Bayer* **38:** 203 – 304.

Appendix 1

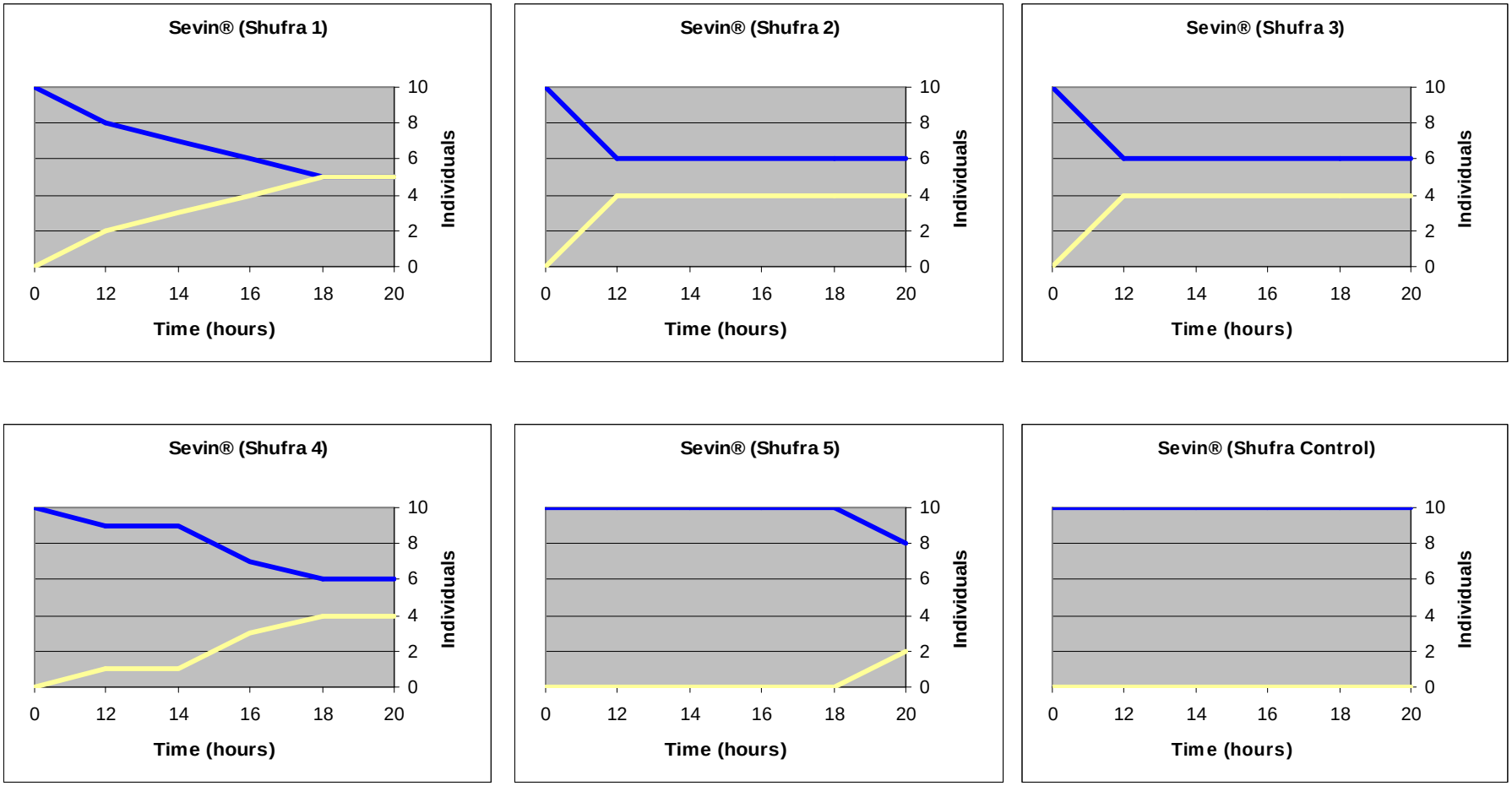


Figure 1: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® on pistachio, Cultivar Shufra, trees.

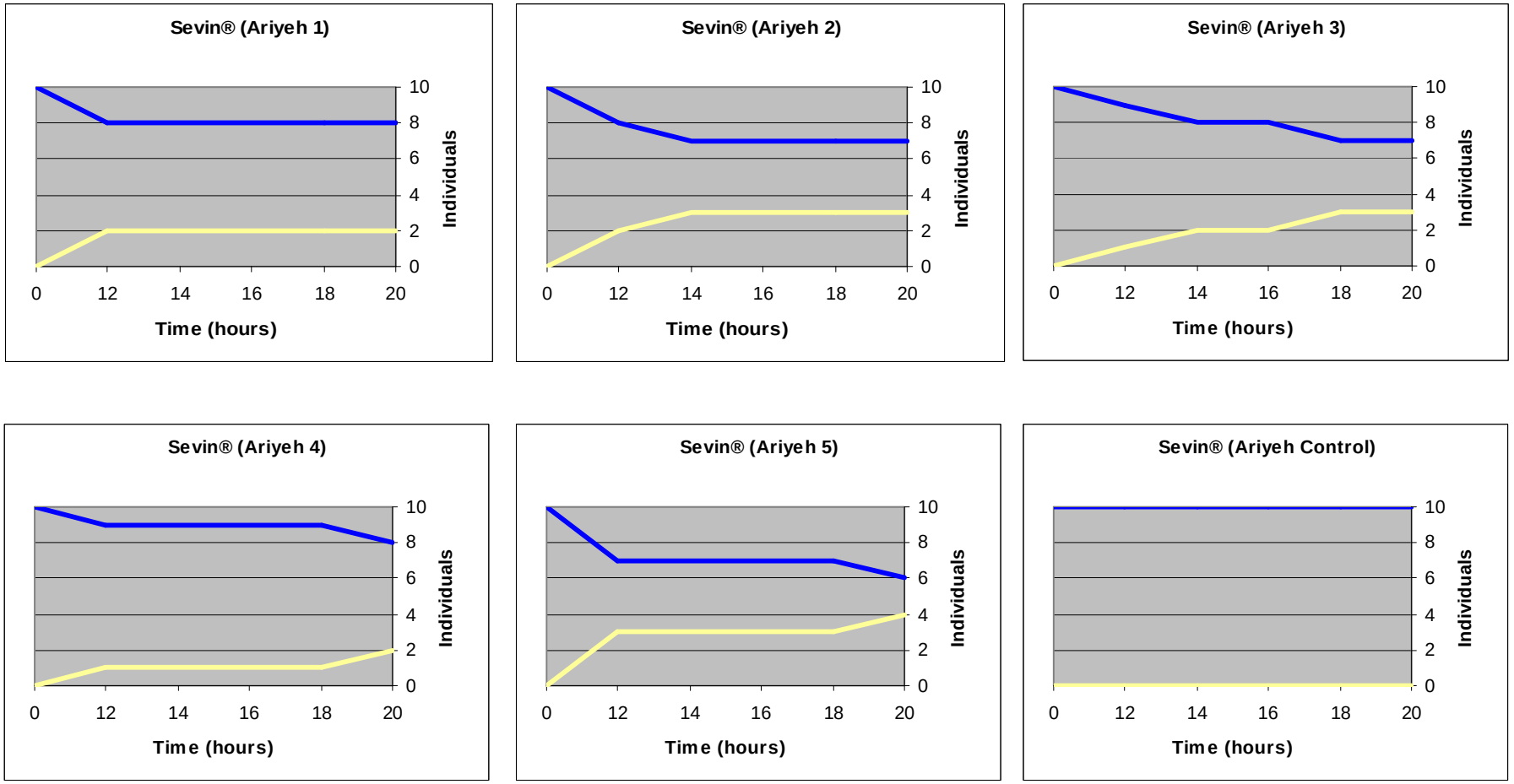


Figure 2: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® on pistachio, Cultivar Ariyeh, trees.

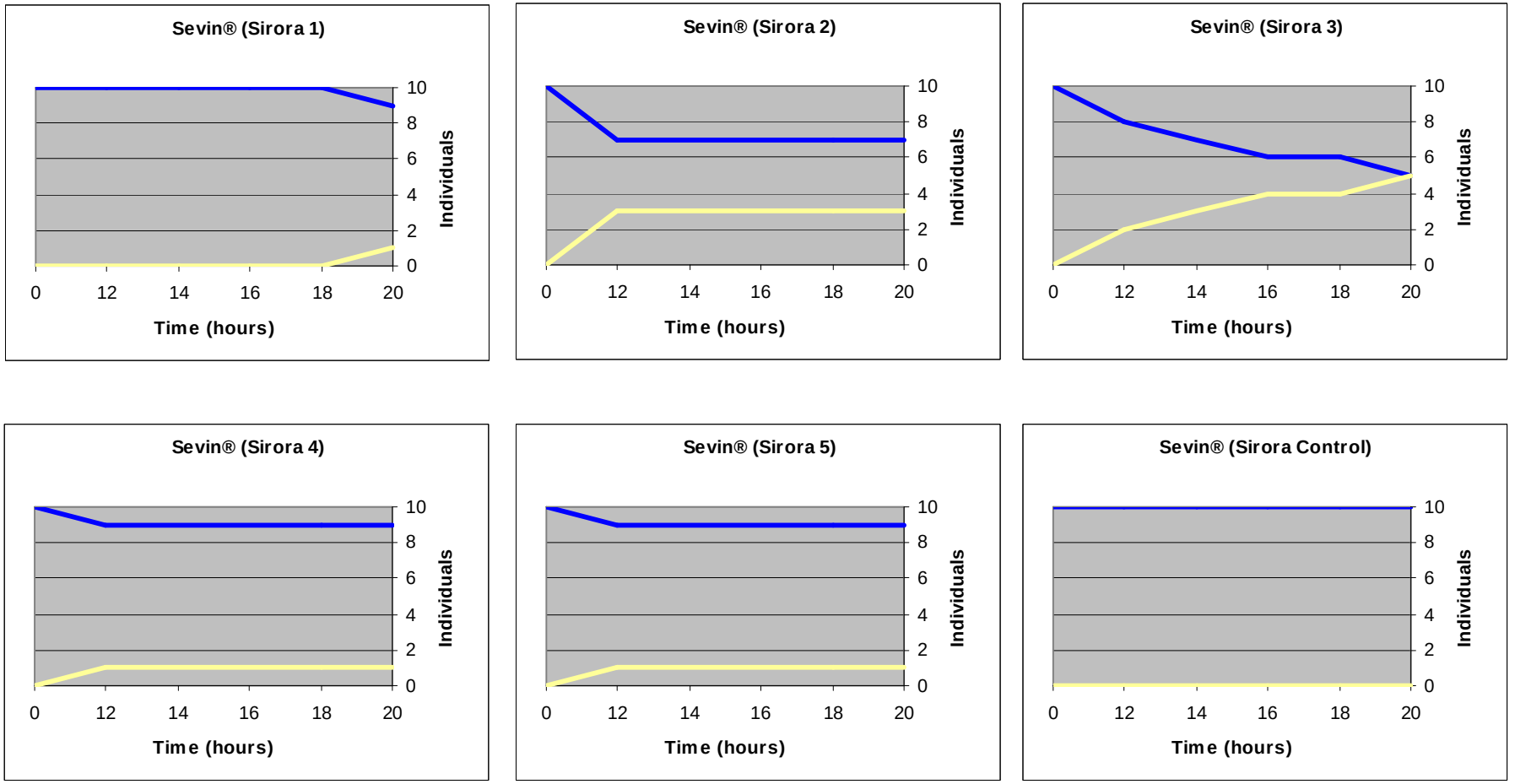


Figure 3: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® on pistachio, Cultivar Sirora, trees.

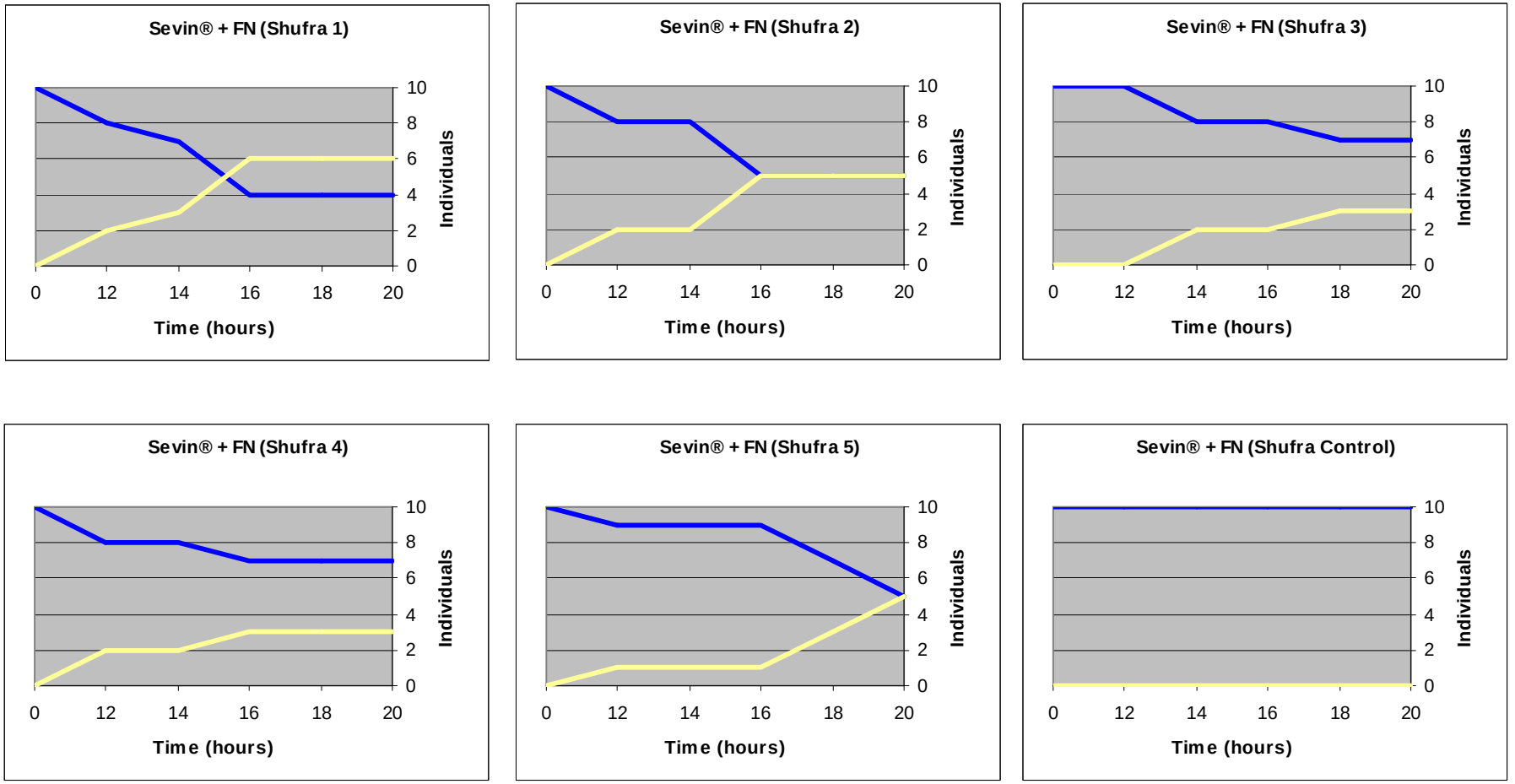


Figure 4: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® + foliar nutrients (FN) on pistachio, Cultivar Shufra, trees.

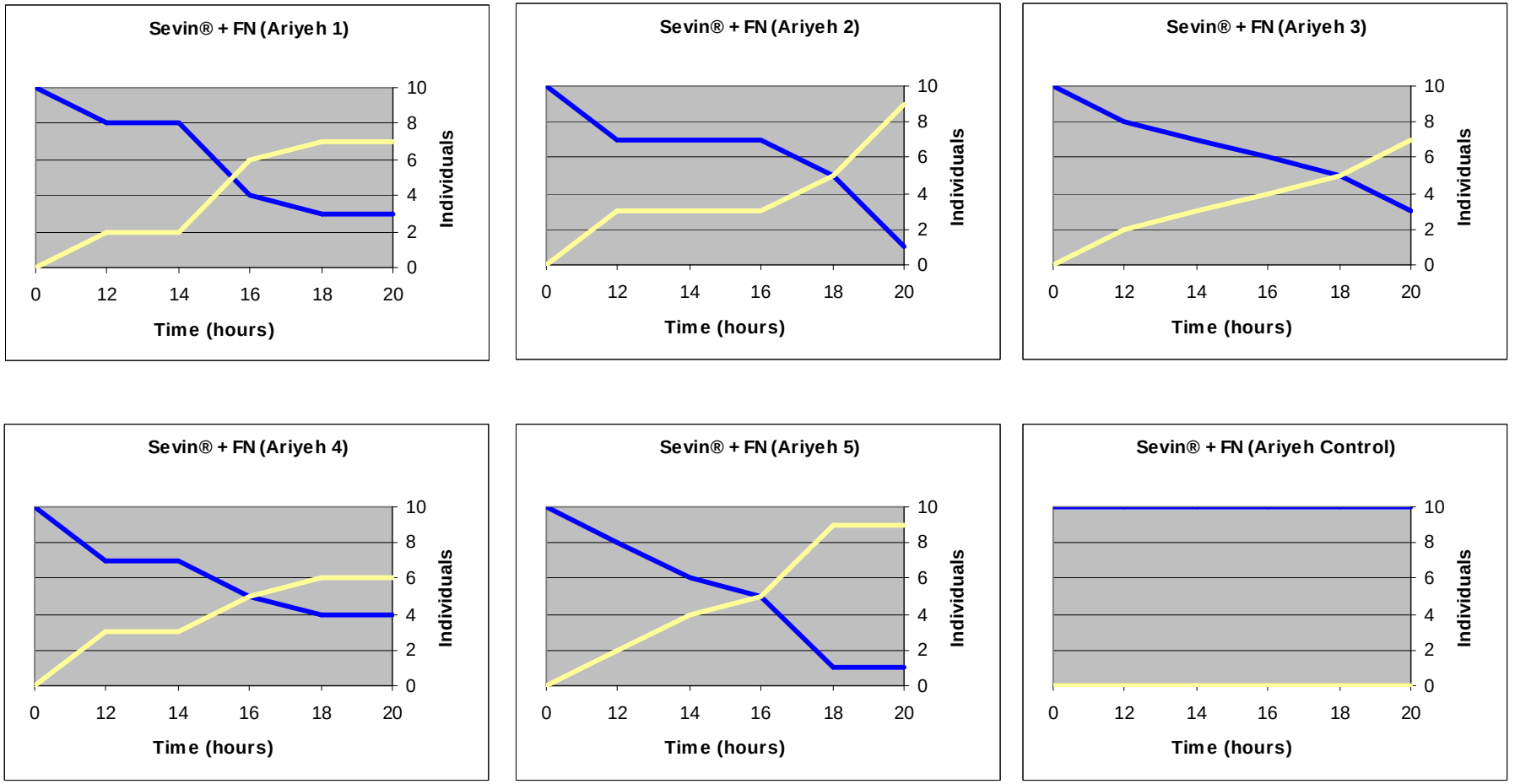


Figure 5: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® + FN on pistachio, Cultivar Ariyeh, trees.

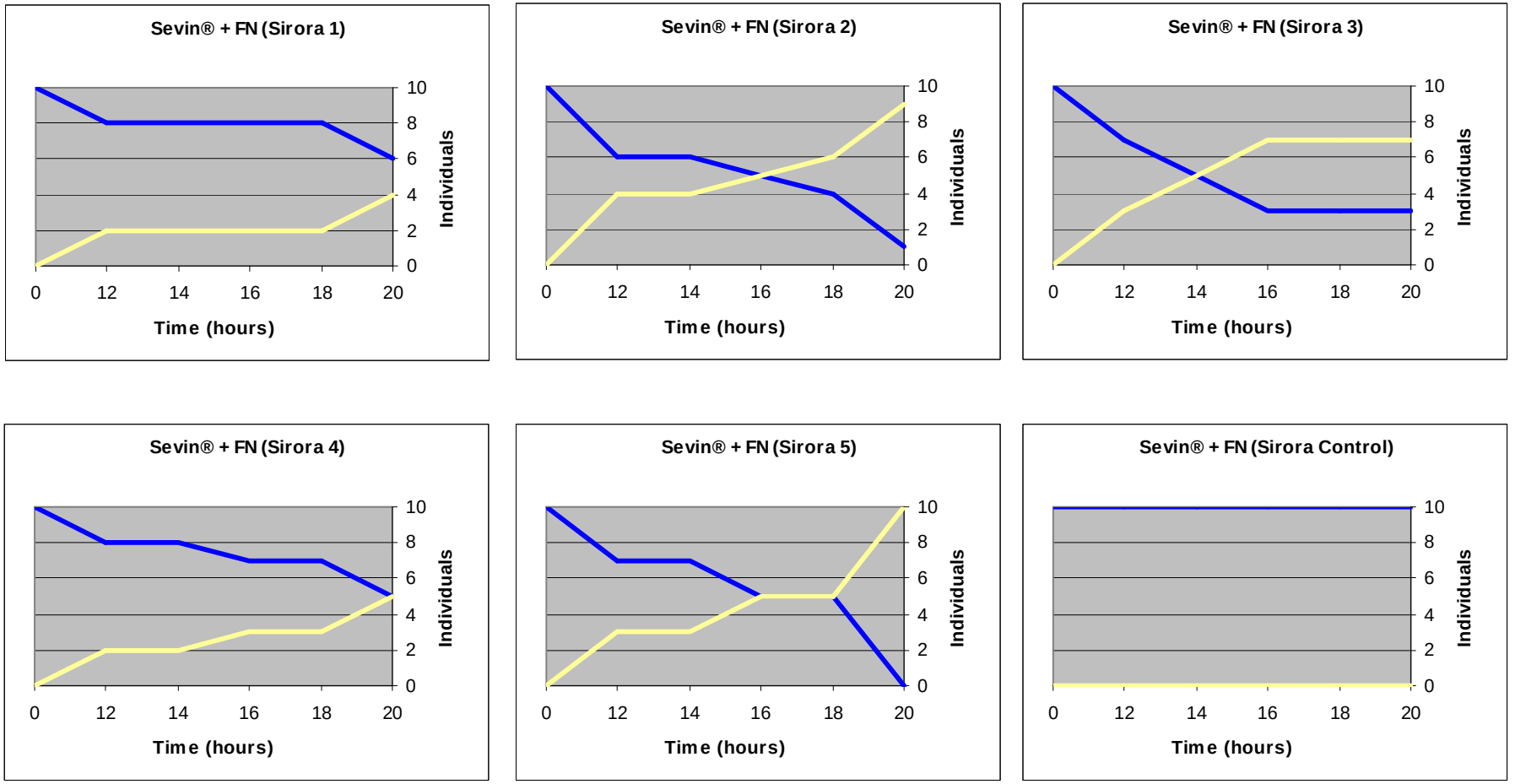


Figure 6: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Sevin® + FN on pistachio, Cultivar Sirora, trees.

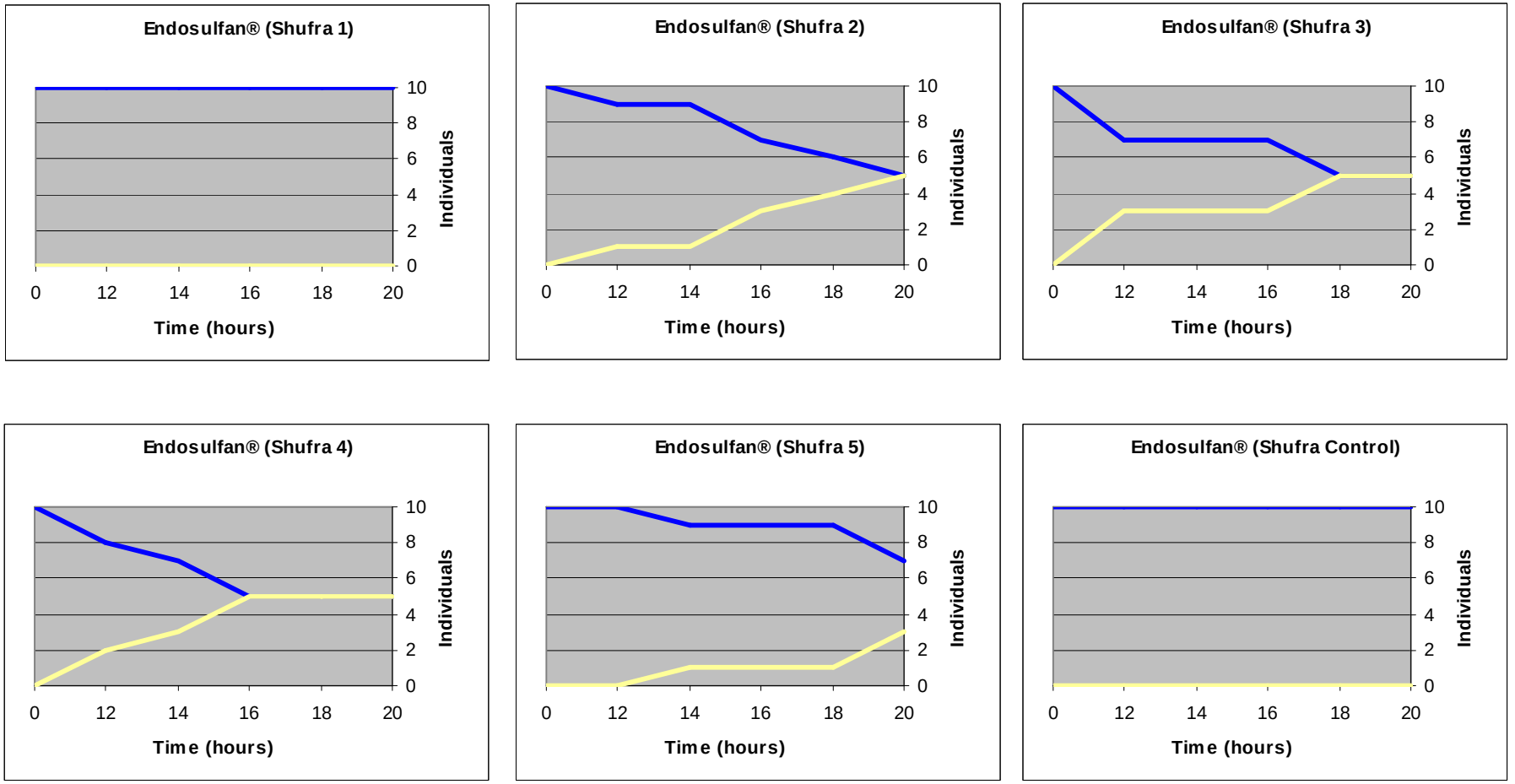


Figure 7: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® on pistachio, Cultivar Shufra, trees.

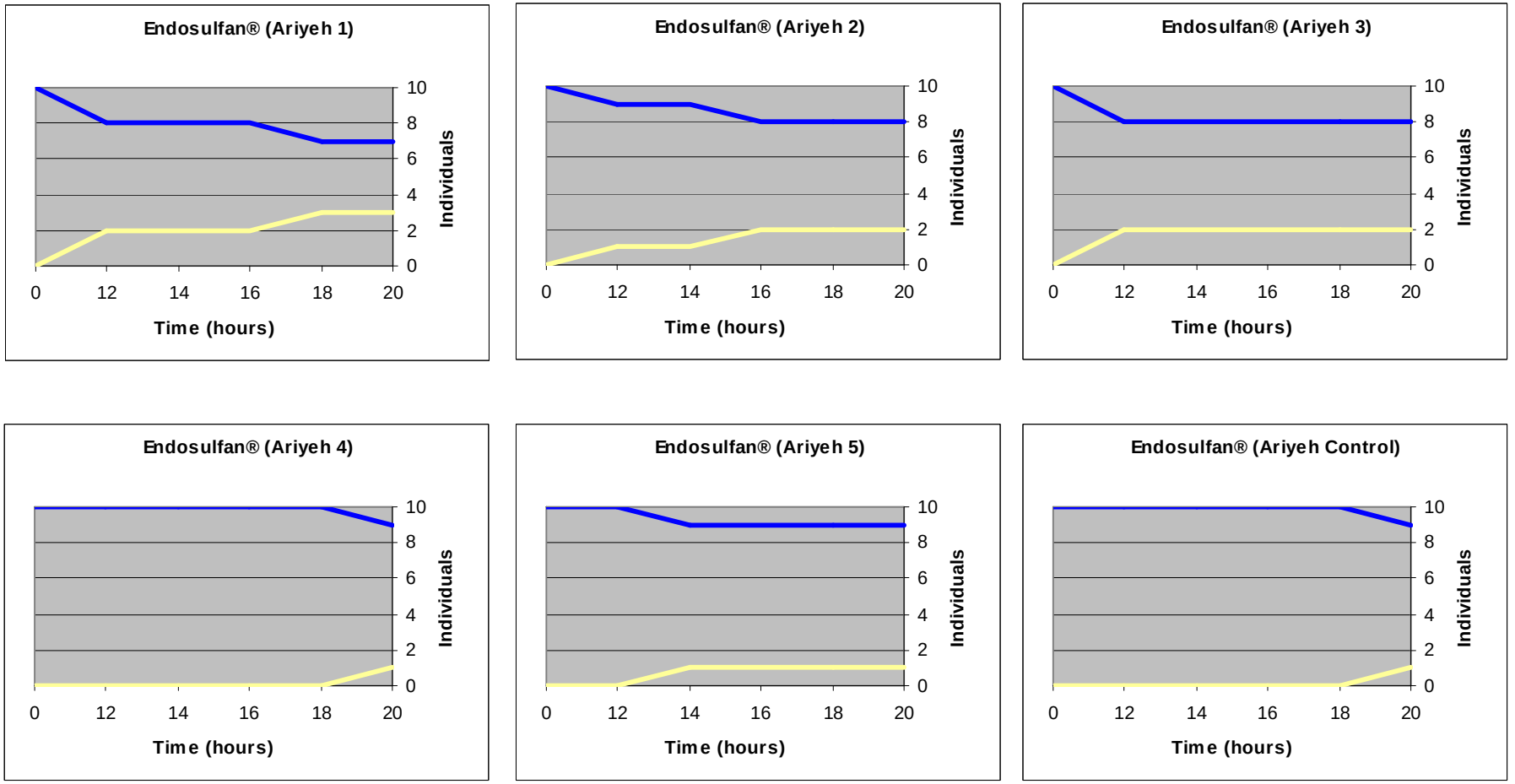


Figure 8: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® on pistachio, Cultivar Ariyeh, trees.

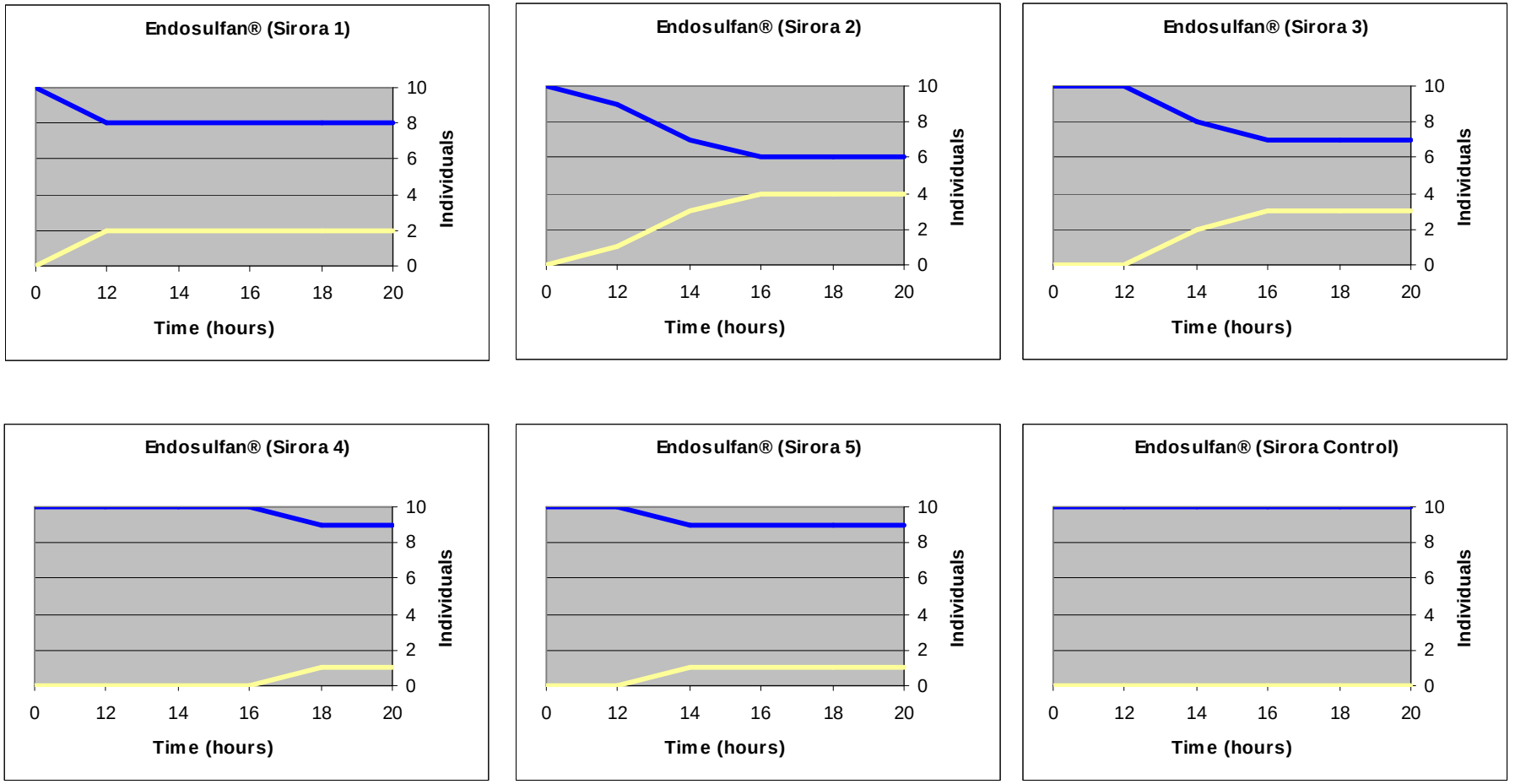


Figure 9: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® on pistachio, Cultivar Sirora, trees.

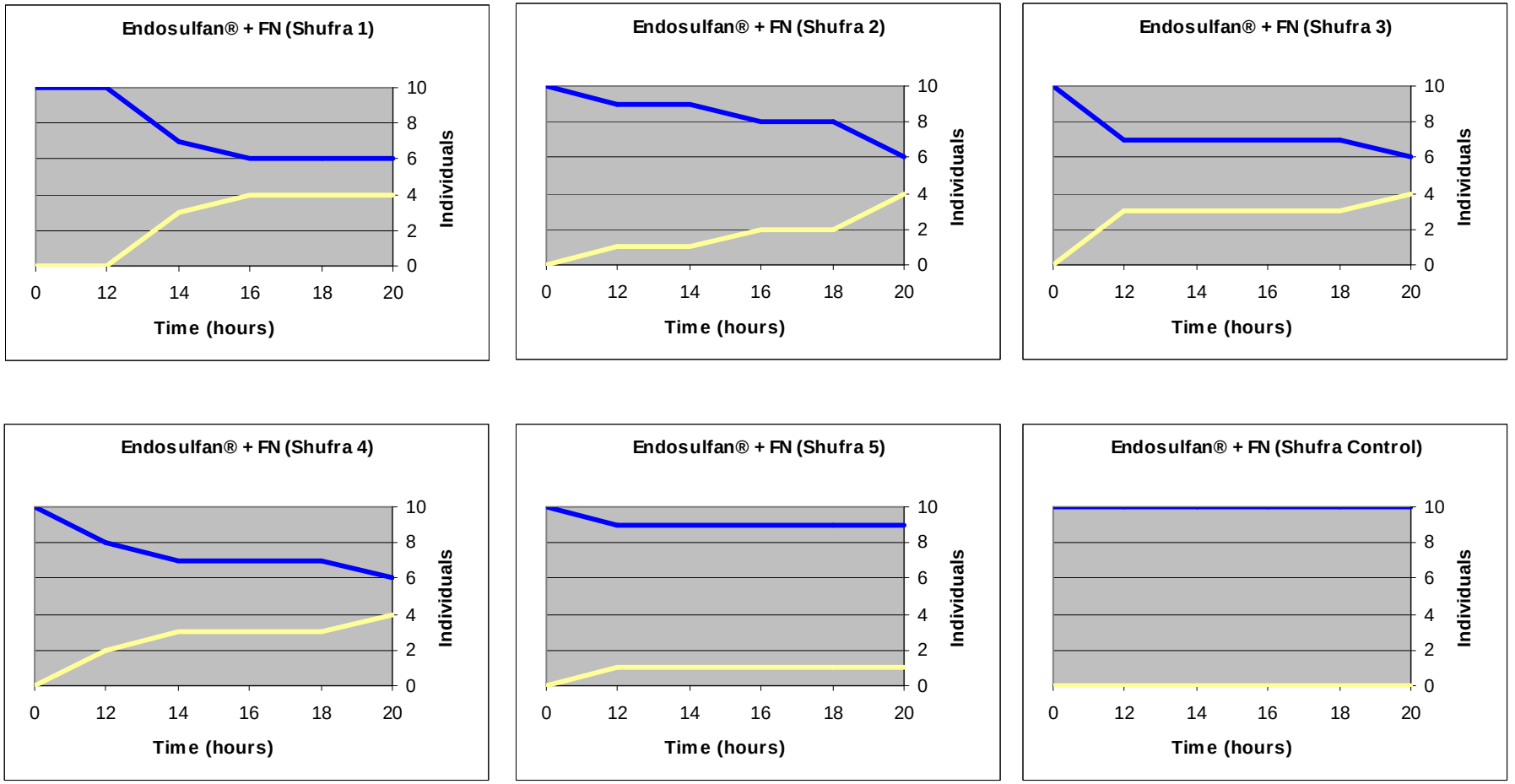


Figure 10: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® + FN on pistachio, Cultivar Shufra, trees.

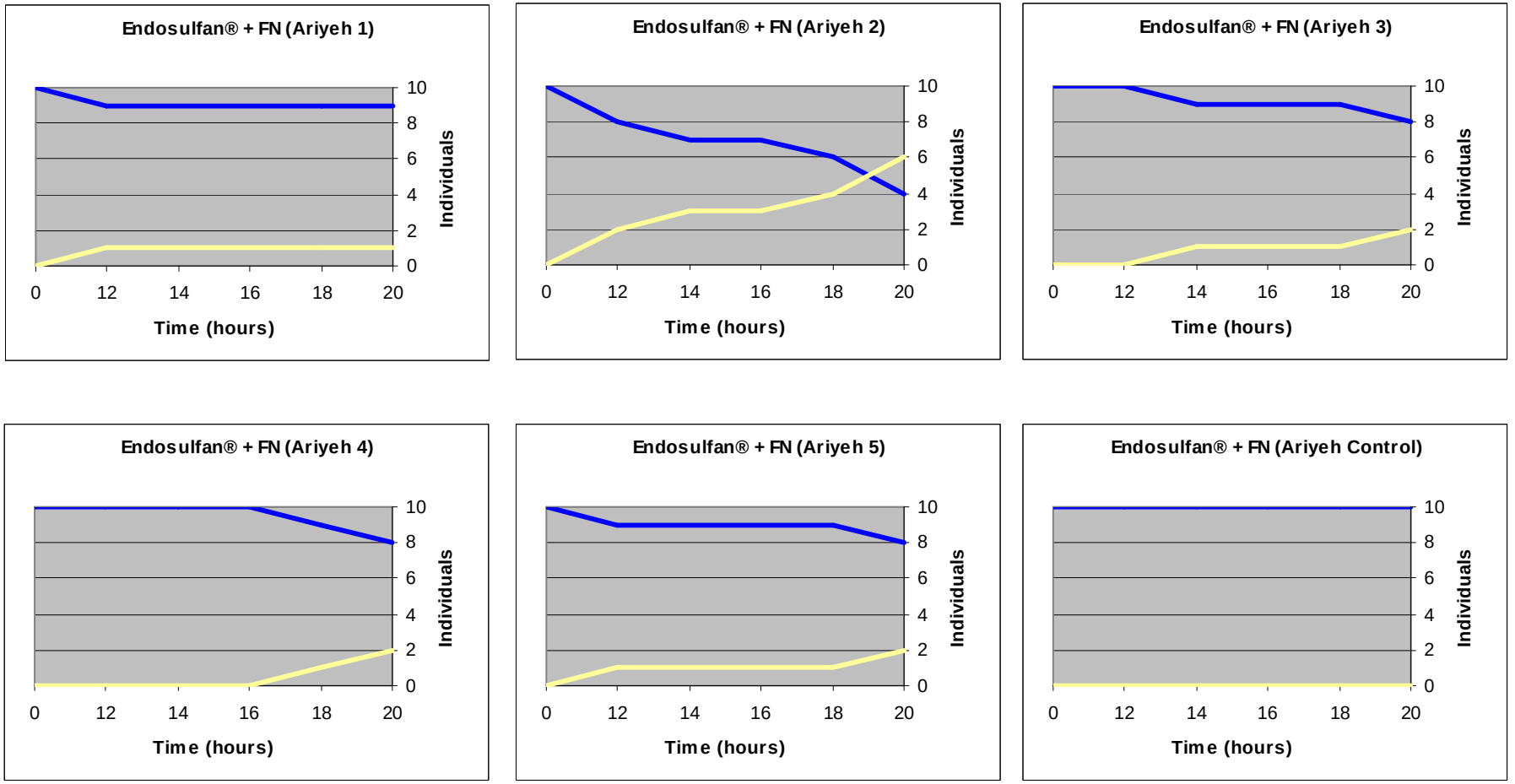


Figure 11: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® + FN on pistachio, Cultivar Ariyeh, trees.

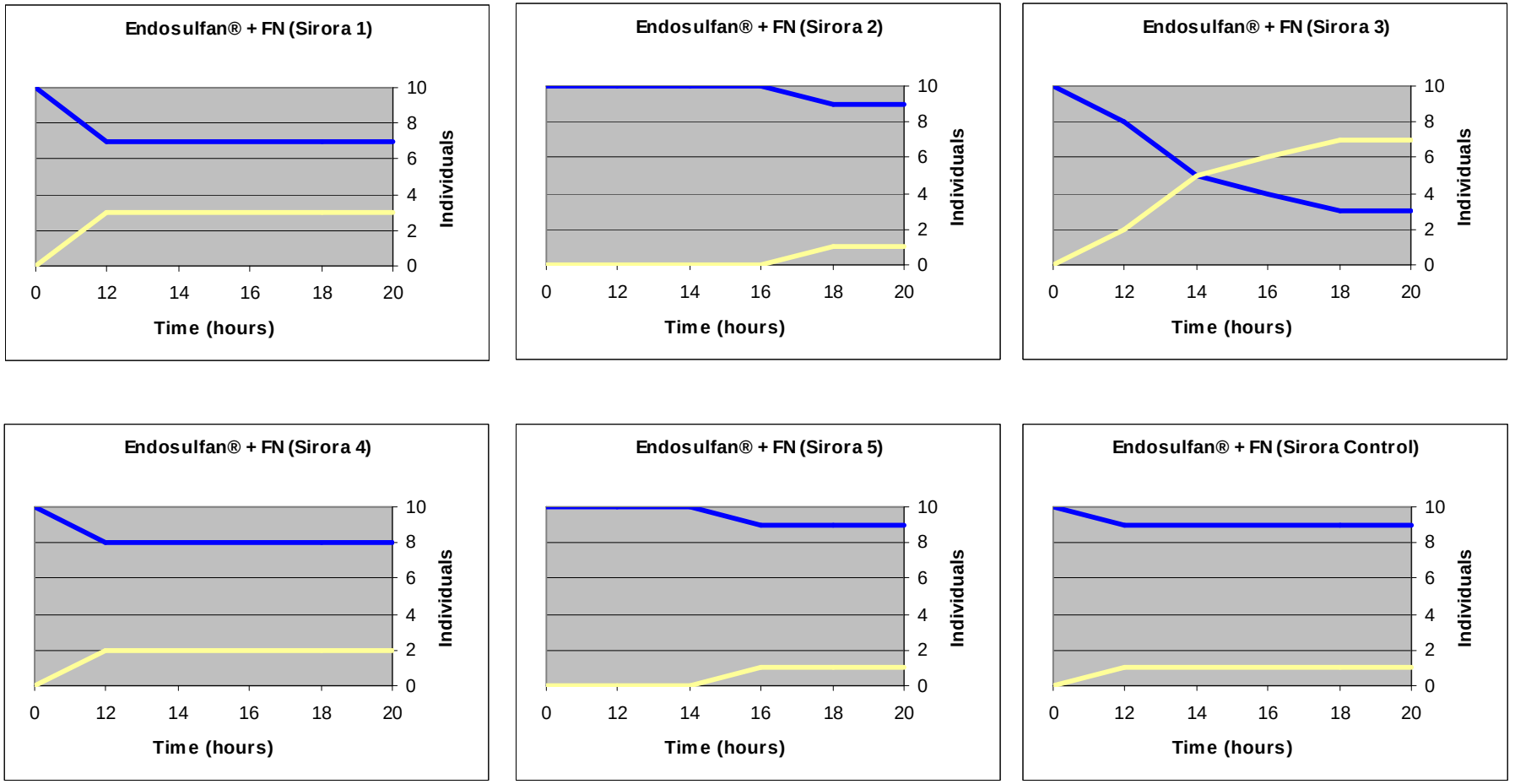


Figure 12: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Endosulfan® + FN on pistachio, Cultivar Sirora, trees.

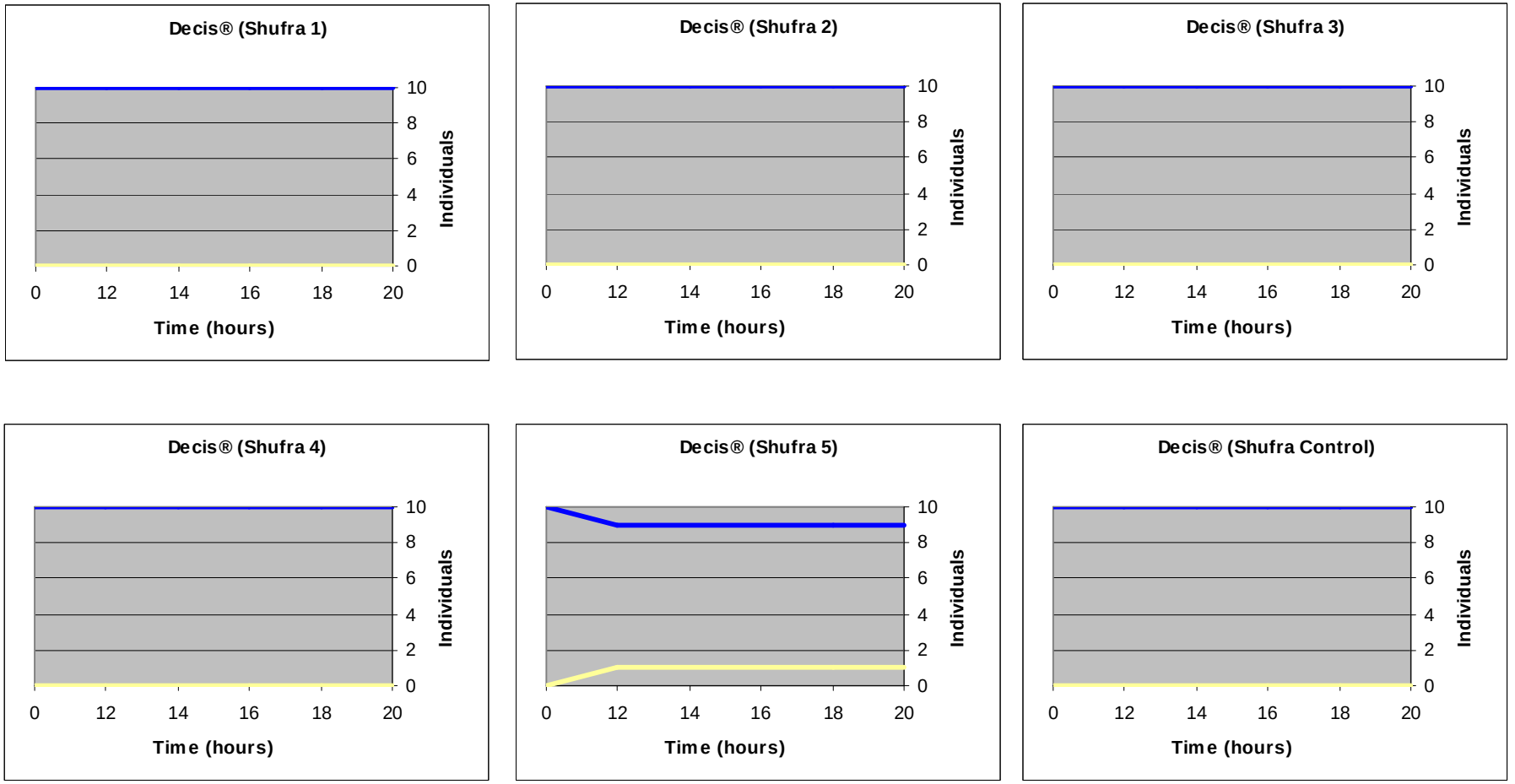


Figure 13: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® on pistachio, Cultivar Shufra, trees.

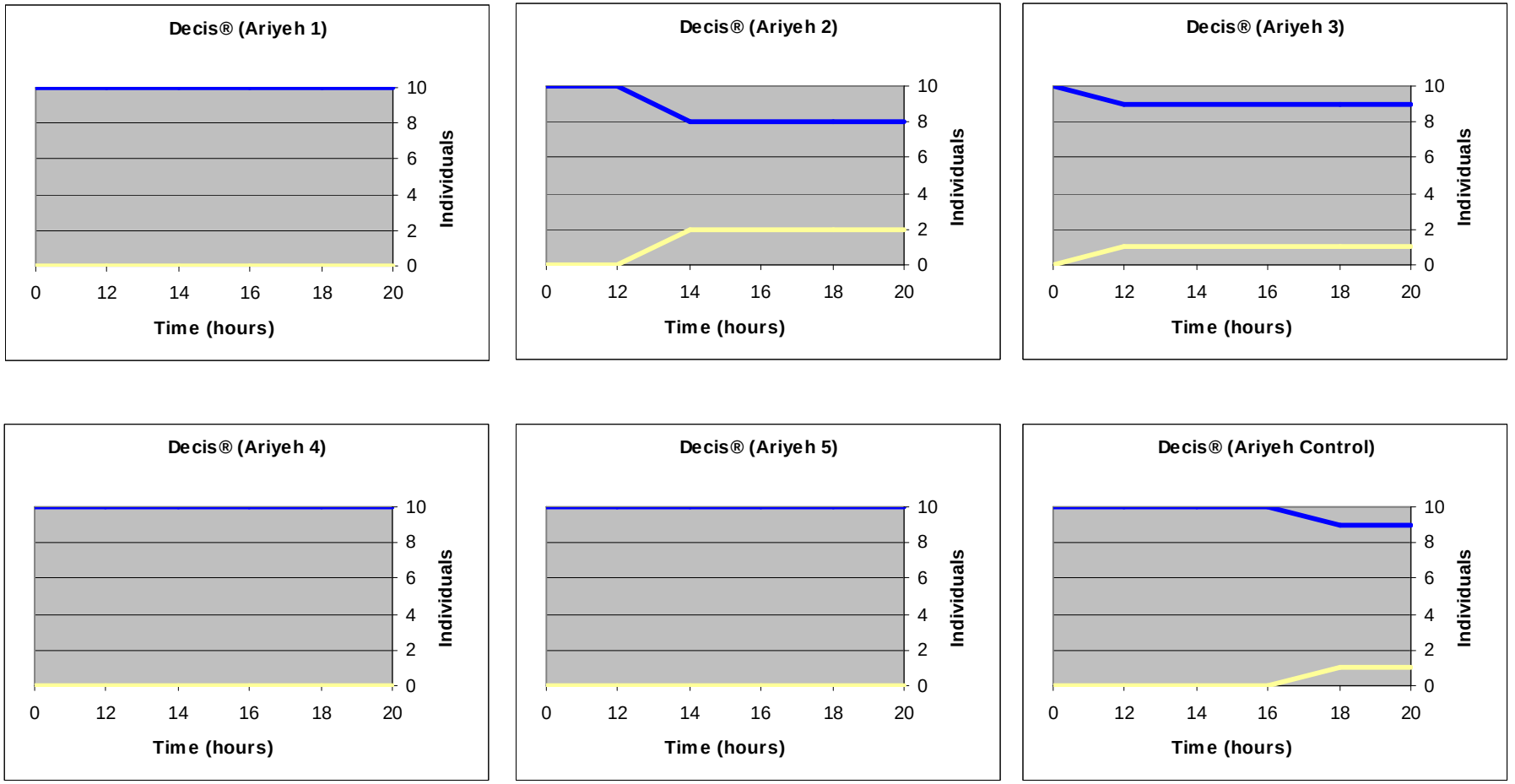


Figure 14: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® on pistachio, Cultivar Ariyeh, trees.

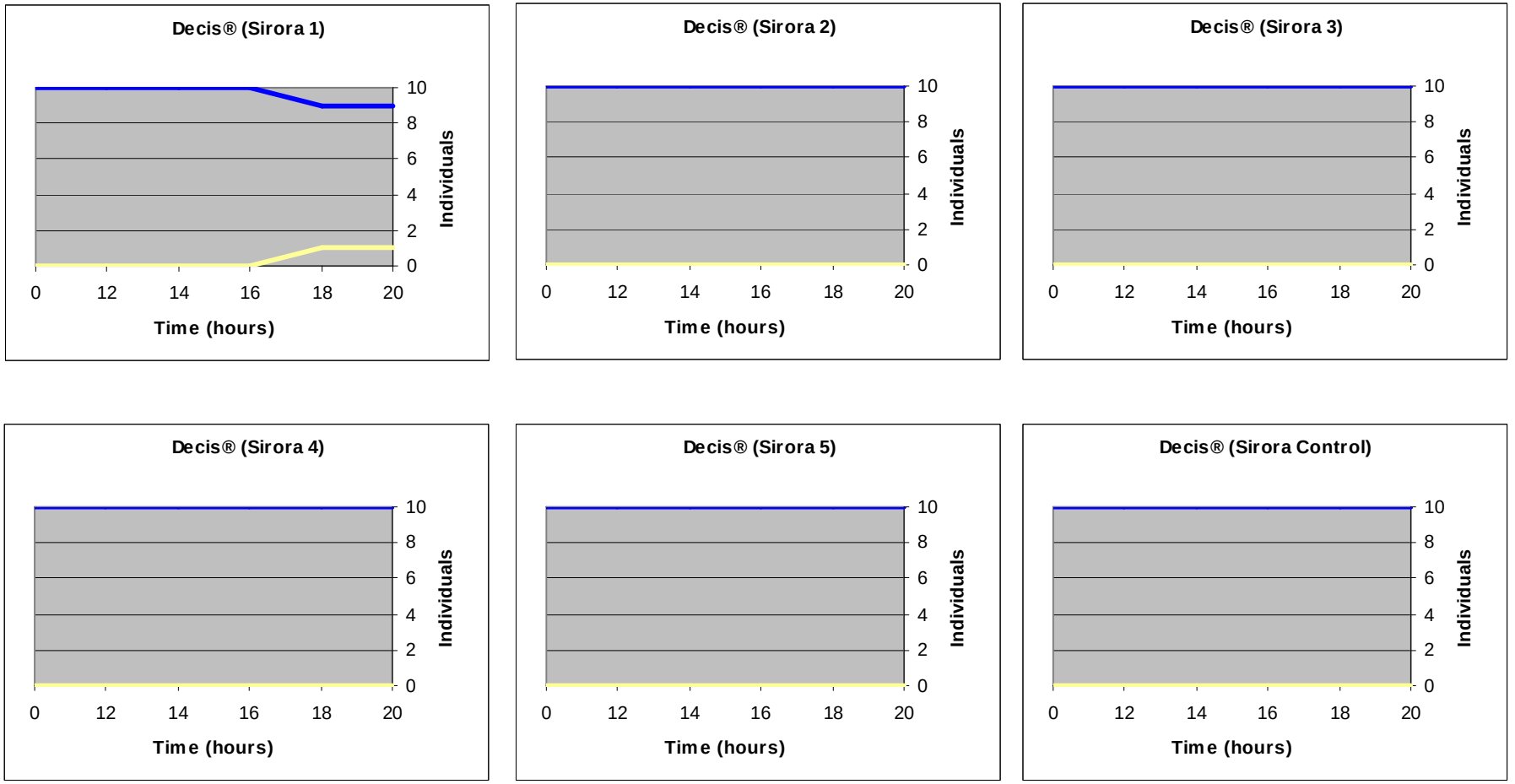


Figure 15: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® on pistachio, Cultivar Sirora, trees.

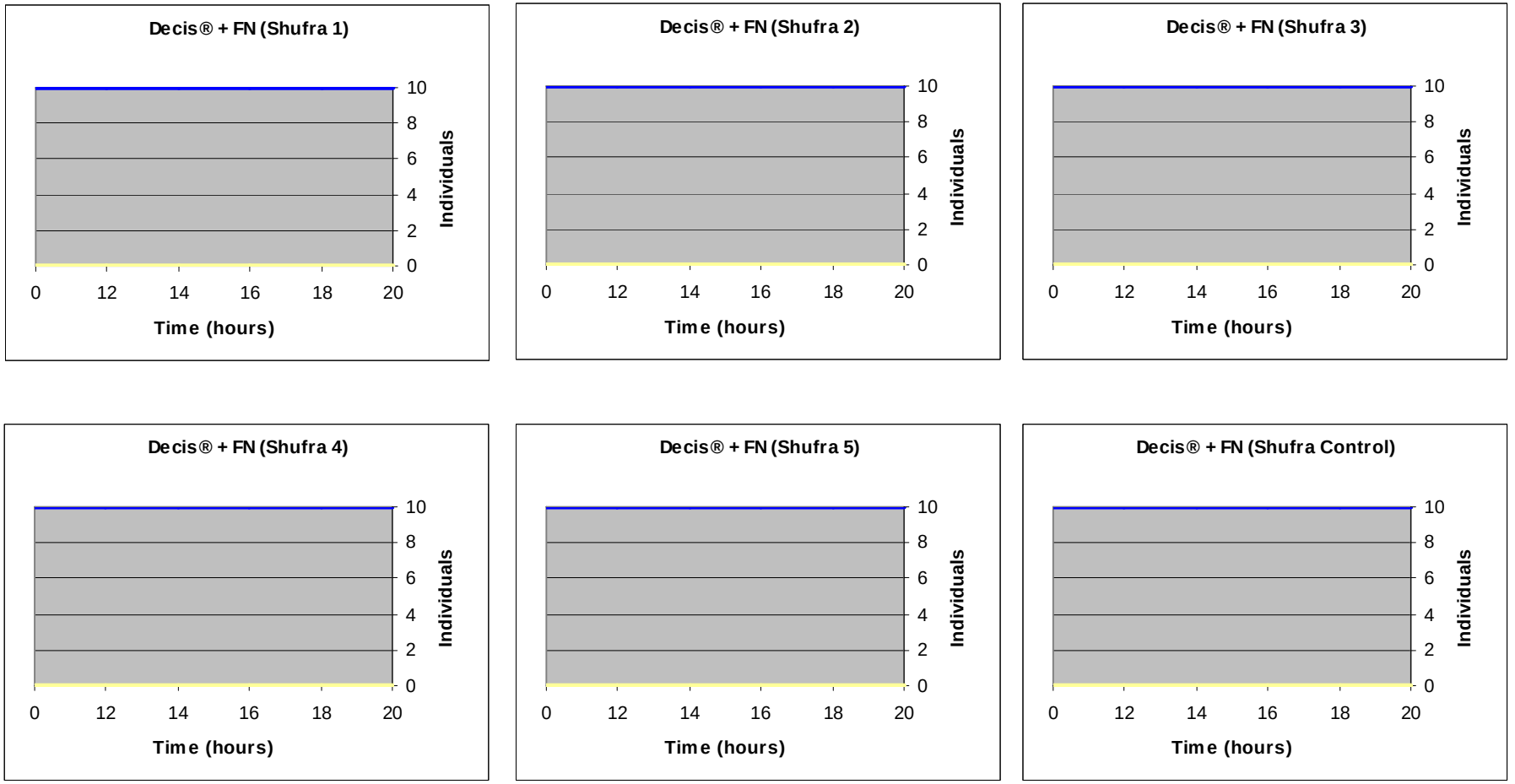


Figure 16: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® + FN on pistachio, Cultivar Shufra, trees.

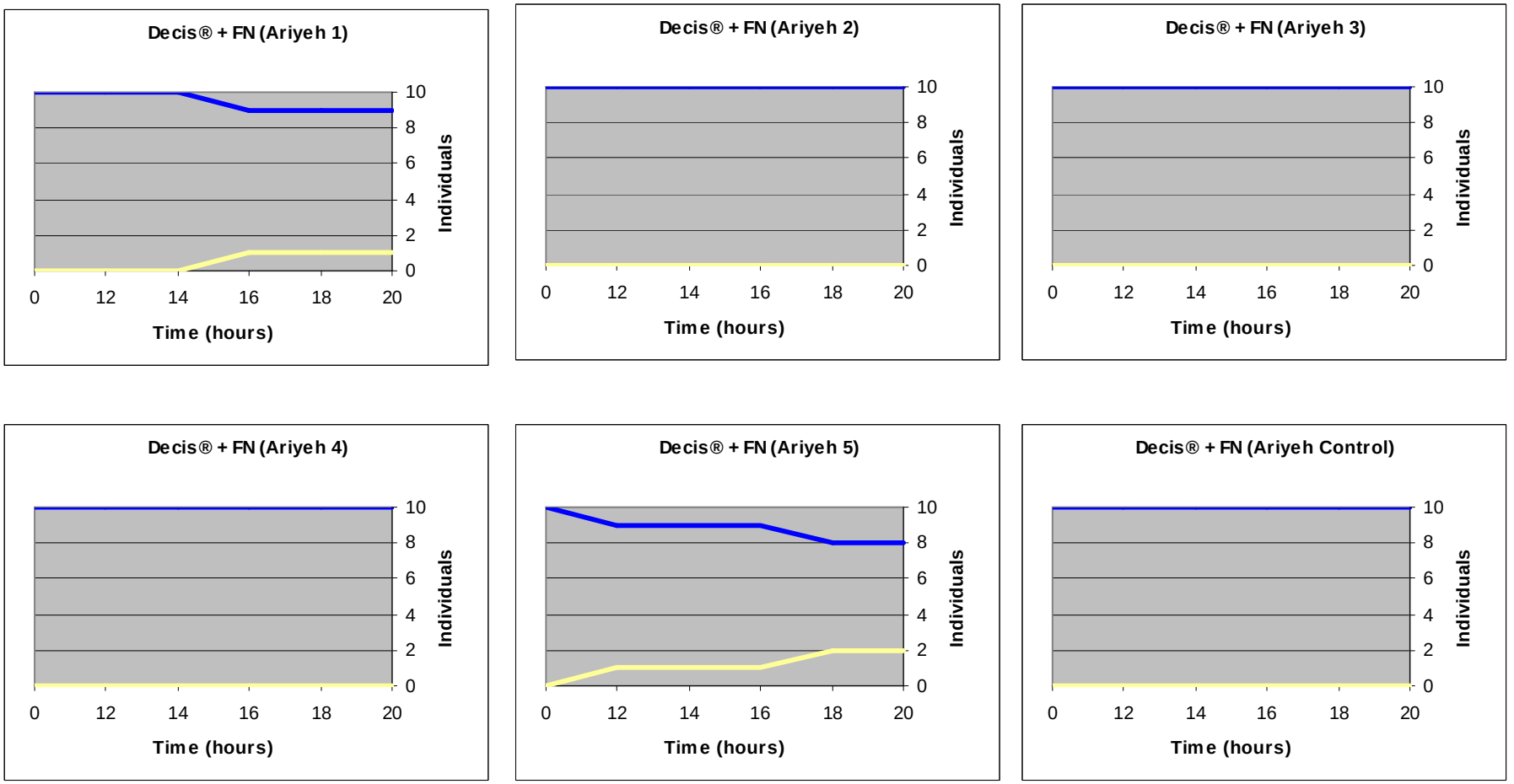


Figure 17: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® + FN on pistachio, Cultivar Ariyeh, trees.

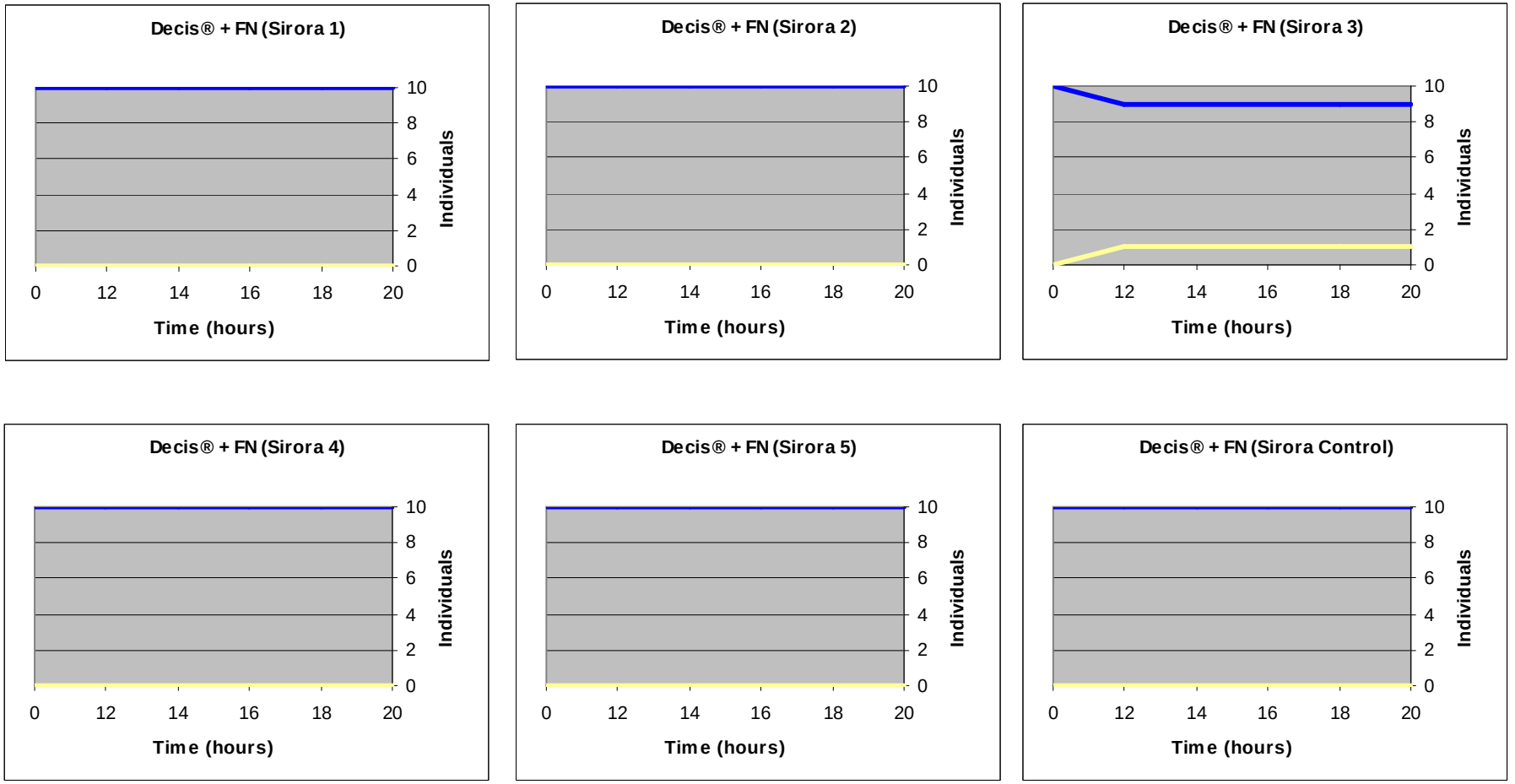


Figure 18: Results of a pesticide bio-assay conducted on *Atelocera raptoria* using Decis® + FN on pistachio, Cultivar Sirora, trees.

CHAPTER 6

Laboratory pesticide bioassay on *Atelocera raptoria* (Hemiptera, Pentatomidae)

1. Introduction

In conjunction with the two field bioassays on *Atelocera raptoria* that were conducted (Chapters 4 & 5), a direct contact laboratory study was also done. This study examined the direct contact capabilities of the pesticides when applied to *A. raptoria* and was conducted at the University of the Free State. In theory, higher mortality rates should be obtained in this manner. All insect material used for this study was obtained at Green Valley Nuts (GVN).

When examining other examples of bioassays on insects performed in the past, it becomes clear that in many of the cases direct contact was the method of application. Many of these bioassays are conducted under natural conditions, usually meaning that the target crop is divided into plots for the purpose of the bioassay. Application of pesticides will then occur in exactly the same manner that applies for natural field conditions. Monitoring is then done within certain time periods and mortalities are noted. Relevant examples include: studies on various pests, including cabbage root fly and turnip root fly, on turnips and swedes (Kelly & Miller, 1978), comparative efficacy studies of *Beauveria bassiana* and *Bacillus thuriengiensis* for the control of the Colorado potato beetle (Lacey, Horton, Chauvin & Stocker, 1999), the efficacy of Chinaberry extracts and certain pesticides against the pea leafminer (Abou-Fakhr Hammad, Nemar & Kawar, 2000), and the insecticide efficacy of several pesticides for the control of *Frankliniella occidentalis* (Broughton & Herron, 2007). During the bioassays that were conducted at GVN, the trial insects were not contact spayed, since the focus was on the secondary contact efficacy of the tested pesticides. It was therefore deemed meaningful to evaluate the direct contact effectiveness of the pesticides as well.

Greene & Capps (2003, 2004) conducted very relevant bioassays on pentatomids using a variety of pesticides. Trials were conducted on three predominant phytophagous stink bug species attacking cotton bolls in Arkansas, USA. These species were the green stink bug, *Agrosternum hilare*, the well known southern green stink bug, *Nezara viridula*, and the brown stink bug, *Euschistus servus*. In these cases application of the pesticide was done

in a slightly different fashion than in the case *A. raptoria.* According to Greene & Capps (2003, 2004), the pesticide formulation was directly applied to the ventral abdominal segments of each insect and the study lasted 96 hours in all trials. During the laboratory trials conducted on *A. raptoria*, the insects were wetted using a handheld sprayer and trials only lasted 16 hours in total. However, this should not influence the results found. Even though mortalities found during the trials done by Greene & Capps (2003, 2004), did show an increasing trend towards 98 hours, so did the untreated control. It is thus concluded that 16 hours should be enough time to accurately measure the efficacy of a contact pesticide.

Several other examples of laboratory based bioassays can also be found in the literature. But, as was the case with the field trial bioassays and residual efficacy studies, each is adapted to the particular requirements of the study. Some relevant examples include studies like the laboratory and field evaluation of Cropopex® on various mite species (Zoebelein, Dörntlein, & Hammann, 1980), trials conducted on the effect of nikkomycins on various insects and mites (Zoebelein, & Kniehase, 1985), a laboratory-based method to measure pesticide efficacy against broad mite (*Polyphagotarsonemus* latus) (Herron, Jiang, & Spooner-Hart, 1996), insecticide efficacy studies on *F. occidentalis* (Thysanoptera: Thripidae) on many horticultural crops (Herron, Rophail, Gullick, 1996), the efficacy of *Ocimum basilicum* and *Ocimum gratissimum* essential oil extracts for the control of *Callosobruchus macalatus* (Keita, Vincent, Schmit, Arnason, & Belanger, 2001), the effect of various insecticides on *Orius insidiosus* (Studebaker & Kring, 2003), and lastly the comparison of insecticide susceptibility of *Geocoris punctipes* and *Lineolaris* for the control of the tarnished plant bug (Tillman, Mulrooney, & Snodgrass, 2003).

2. Material and methods

2.1. Pesticides and experimental setup

The laboratory study that was conducted used the same pesticides as those used during the diurnal bioassay study (Chapter 4). These were Sevin®, Endosulfan®, Pencap®, Karate®, Calypso® and Klartan®.

As in the previous bioassays (Chapters 4 & 5) healthy *A. raptoria* adults were used to conduct this trial. The insects were captured at Green Valley Nuts (GVN) and transported to Bloemfontein where the study was conducted under laboratory conditions at the University of the Free State. The insects were kept in prepared terrariums with pistachio leaves provided for sustenance. The study consisted of 12 trials and each pesticide was tested in a separate trial with a single repeat trial. Every trial conducted required 20 insects, divided into 10 trial insects and 10 control insects and in total 240 insects were used for the entire study. No trees were used in this study and insects were placed inside gauze covered plastic containers for each trial period.

Trial and control insects were placed in different containers and kept apart for the duration of each trial. A third identical container was used to monitor temperature and relative humidity (RH) during the trials. This was done by placing a thermometer and a hygrometer inside the particular container. The containers were fitted with a slightly wetted tissue paper base to provide moisture for the insects and prevent desiccation. All containers were placed in a secluded, ventilated cabinet during the trial. The ventilation ensured that any lingering pesticides were quickly extracted from the cabinet. All containers were thus subjected to the same conditions in terms of external temperature and other elements.

2.2. Preparation and application of pesticides

The method used with the preparation and application of the pesticides was conducted in the same manner for all six trials. Pesticides were prepared according to the specifications of each individual pesticide. To spray the trial insects, a 1 liter handheld spray bottle was used. However, to mix the pesticides, a 5 liter plastic container was used. This was done because of the difficulty of measuring such low quantities accurately. The left-over pesticide mixture from the 5L bottle was then disposed of. The pesticides were accurately measured using a 10ml measuring cylinder and a pipette accurate up to 5ml. The pesticides were measured and then added to the 5 liter container filled with water.

This mixture was shaken well and then poured into the spray bottle used to dispense the pesticide. Each pesticide quantity used was calculated using the specified dosage recommendations provided by the pesticide distributor (Table 1). After the preparation of the pesticide used for the specific study was complete, it was dispensed onto the trial insects. Due to the fact that this was a direct-contact bioassay study, the pesticides were sprayed directly onto the insects. The control insects were first removed from the room to prevent any possibility of pesticide exposure. The spraying was conducted in the same manner during all trials. This was done by setting the spray nozzle to its smallest drop size and spraying 20 direct sprays onto the trial insects. In this way, the insects were thoroughly wetted with the trial pesticide. For protection, a face mask and rubber gloves were worn throughout the procedure.

Table 1: Specified dosages of six pesticides for three different mixtures used during laboratory pesticide bioassay conducted on *Atelocera raptoria*.

Product	Dosage(ml) / 100 L	Dosage(ml) / 15 L	Dosage(ml) / 5 L
Sevin®	225	33.75	11.25
Endosulfan®	100	15	5
Pencap®	70	10.5	3.5
Karate®	20	3	1
Calypso®	15	2.25	0.75
Klartan®	30	4.5	1.5

Every trial lasted 16 hours in total. Again, every trial was divided into two separate phases. Phase 1 lasted six hours and monitoring was done every 10 minutes from the time the pesticides are applied. This was done for 360 minutes. Continuing directly from this phase 2 runs on with another one-off monitoring done at the end of a 10 hour period.

Monitoring was done by visual inspection using prepared data-capture sheets. All dead and live insects were counted and noted. This was done to ensure that the slower working pesticides were accurately monitored and to ensure that the so called “knockdown-effect” (i.e. temporary incapacitation) did not influence the results. Trials were conducted in the order Endosulfan®, Sevin®, Klartan®, Karate®, Calypso®, Pencap®, Endosulfan® (Repeat) , Sevin® (Repeat), Klartan® (Repeat), Karate® (Repeat), Calypso® (Repeat) and Pencap® (Repeat) and numbered sequentially.

3. Results and discussion

3.1. Temperature and relative humidity (RH)

During this study very constant temperature and relative humidity (RH) values were recorded. The temperature remained between 21°C and 23°C during all the trials. Very high RH readings of 93 - 99% were recorded during phase 1 of the trials. Only at the termination of the 10 hour phase 2 did RH drop to around 70%. Basically, the same temperature and RH was noted during all trials. The mild temperatures and high RH did not influence the pesticide evaporation or cause desiccation of the insects and it is thus unlikely that the results were influenced in any way.

3.2. Bioassay

Accordingly graphs were constructed in the same manner as those used for the two previous bioassays (Chapters 4 & 5). These graphs were constructed for every container in the 12 trials (Appendix 1, Figs. 1 - 6)

3.2.1. Endosulfan®

During both Trial 1 and Trial 7 a very long pre-active phase could be noted. In trial one, insects only began dying after 170 minutes and during Trial 7 only after 300 minutes. Both trials showed slow mortality rates toward the completion of phase 1. Both trials did, however, reach the MR50 point. After the completion of phase 2, 100% mortality was recorded. It would thus appear that this is an effective, albeit slow-working, contact pesticide. In the controls, only one insect died in the Trial 1 control at the completion of phase 2. In the Trial 7 control, two insects were dead after the completion of the 16 hour trial. Figure 1 shows maximum mortality percentages for this pesticide and it can be seen that 100% of the trial insects died during both trials. The two controls showed 10% and 20% mortality respectively. Good contact effects were therefore obtained using this pesticide. The fact that both of the trials show much the same results indicates a high confidence level in pesticide efficacy

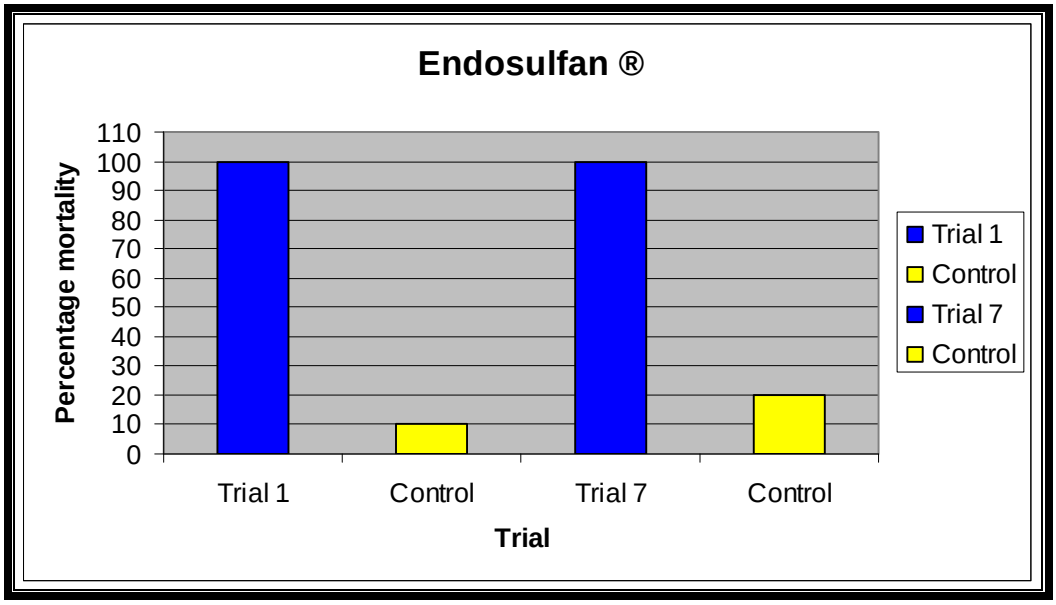


Figure 1: Maximum percentage values obtained during a direct contact bio-assessment study on *Atelocera raptoria* using Endosulfan®.

3.2.2. Sevin®

Even better results were obtained during the Sevin® trials. Much shorter pre-active phases could be seen in both Trial 2 and Trial 8. In both cases, the pre-active phase lasted 90 minutes. Fairly short active phases could be noticed, with both trials showing an MR50 point. Both the controls showed good results with only one insect dying during phase 1 of the Trial 2 control. No mortalities were recorded in the other control.

As was the case with Endosulfan®, 100% of the trial insects were dead after the completion of phase 2 (Fig. 2). Only the first control showed any mortalities, with 10% of the insects dying. This pesticide showed excellent contact activity, with a fairly abbreviated active period.

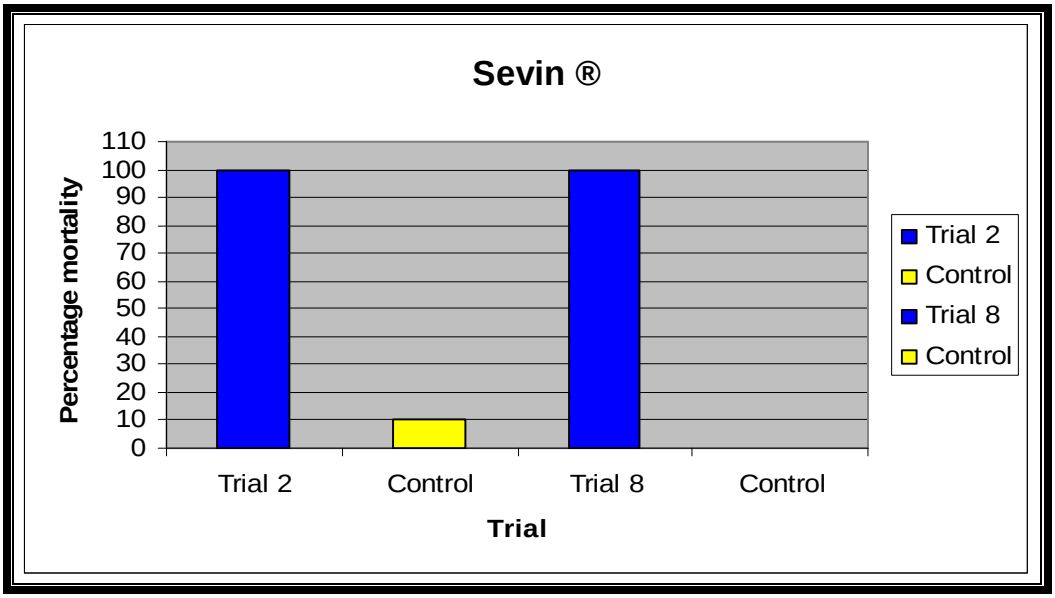


Figure 2: Maximum percentage values obtained during a direct contact bio-assessment study on *Atelocera raptoria* using Sevin®.

3.2.3. Klartan®

Good results were obtained from the Klartan® trial as well. Pre-active phases ranging from 150 minutes during Trial 3 and only 40 minutes in Trial 9 were recorded. Both trials showed a MR50 point fairly early in the trial. The active phase during Trial 3 lasted only 60 minutes, resulting in 100% mortality. In Trial 9, the active phase lasted slightly longer, i.e. 160 minutes in total. Neither of the control containers showed any mortalities.

Looking at the maximum percentage graph (Fig. 3), it is immediately apparent that this trial showed excellent results. In both Trial 3 and the repeat trial (Trial 9), a 100% mortality rate was recorded. Neither of the controls showed any mortalities.

3.2.4. Karate®

The best results during the entire study were obtained from Karate®. Almost no pre-active phase could be distinguished in either of the trials. Exceptionally short active phases could also be seen. In the first of the trials, 100% mortality

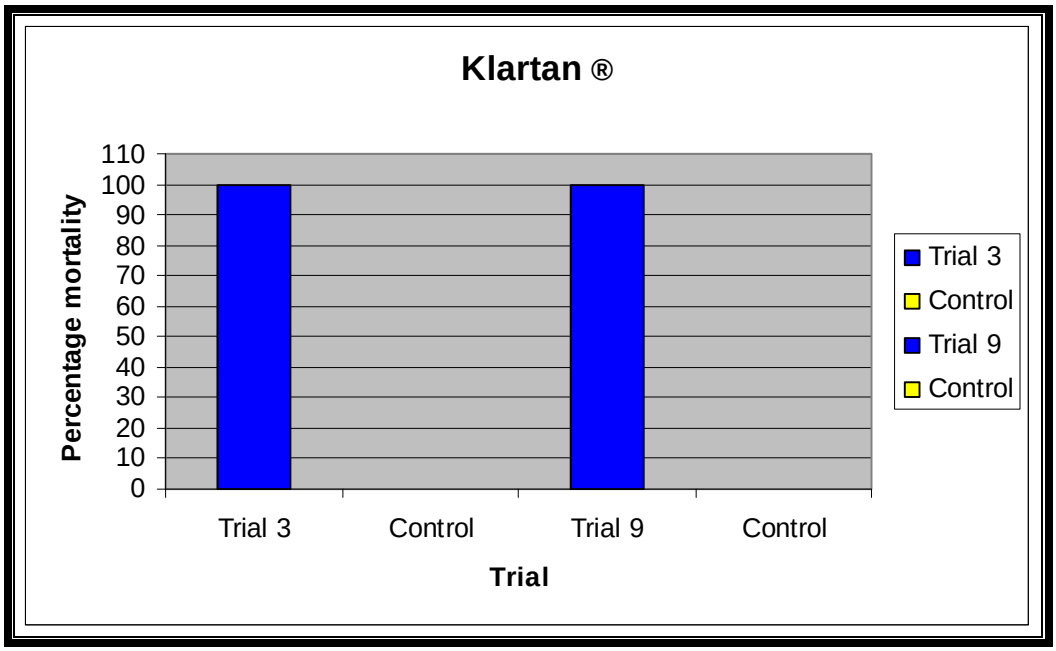


Figure 3: Maximum percentage values obtained during a direct contact bio-assessment study on *Atelocera raptoria* using Klartan®.

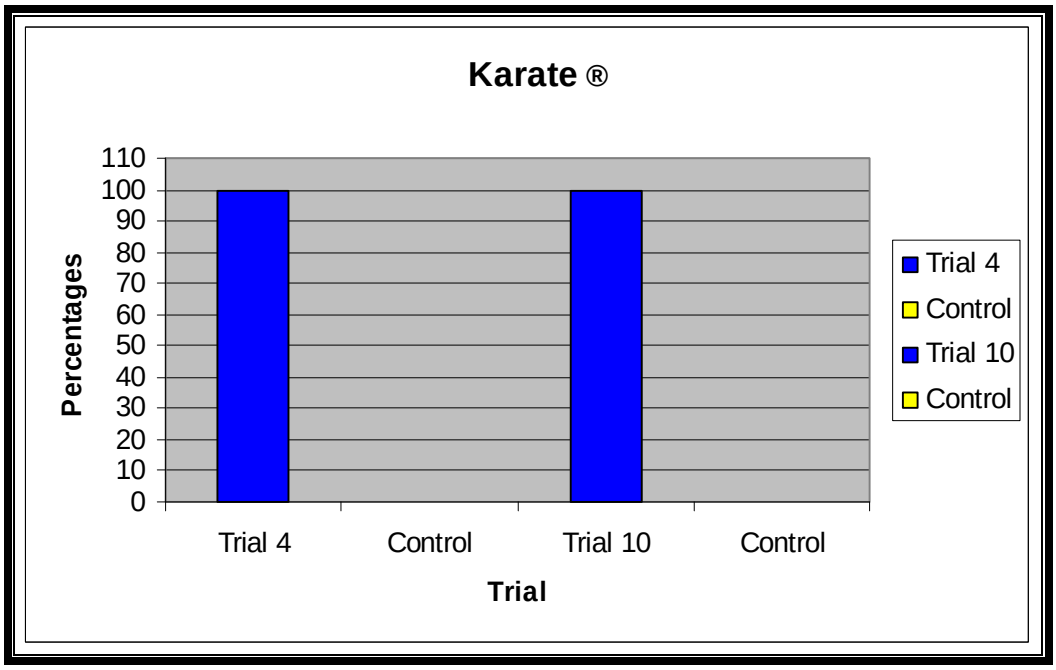


Figure 4: Maximum percentage values obtained during a direct contact bio-assessment study on *Atelocera raptoria* using Karate®.

was recorded after only 70 minutes. In Trial 10 all insects were dead after 200 minutes. Conversely, neither of the control trees showed any mortalities. The graph in Fig. 4 demonstrates that Sevin® has the best overall direct contact effect during the study. Similar results as the previous trial (Klartan®) were obtained during this trial, with both trials showing 100% mortality of trial insects and no control mortalities)

3.2.5. Calypso®

Reasonably good results were obtained during the Calypso® trial as well. Fairly short pre-active phases could be noted during this trial and both trials reached the MR50 mark. Longer active phases of between 140 and 200 minutes could also be noted. For the first time during the study, a trial failed to reach 100% mortality. Only nine of the trial insects died during Trial 5 (Fig. 5). On the other hand, Trial 11 showed 100% mortality. Of the two control trees, only one insect died (Fig. 5).

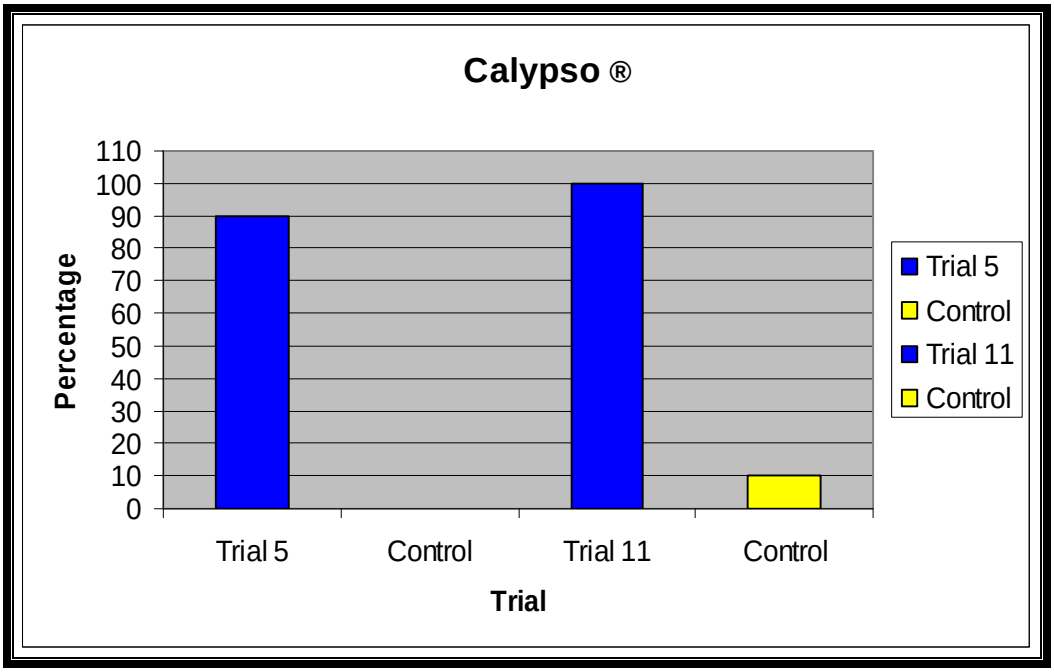


Figure 5: Maximum percentage values obtained during a direct contact bio-assessment study on *Atelocera raptoria* using Calypso®.

3.2.6. Pencap®

Pencap® showed the worst results during this study, with no mortalities recorded during the entire trial period. No pre-active phase, active phase or MR50 point could be distinguished in either of the trials.

4. Conclusion

A maximum percentage mortality table was compiled to show a summary of the pesticide results. The pesticides were ranked in descending order from best to worst in terms of activity (Table 2).

Table 2: Percentage mortalities found during a bio-assessment done on six different pesticides used in a direct contact bioassay on *Atelocera raptoria*.

Pesticide	Percentage first trial mortalities	Percentage first control mortalities	Percentage repeat trial mortalities	Percentage repeat control mortalities
Karate®	100	0	100	0
Klartan®	100	0	100	0
Sevin®	100	10	100	0
Endosulfan®	100	10	100	20
Calypso®	90	0	100	10
Pencap®	0	0	0	0

The pesticides all performed well, with the definite exception of Pencap®. The Calypso® trial also only delivered 90% mortalities. Overall it would appear that Sevin®, Karate®, Endosulfan® and Klartan® performed the best. All four these pesticides also performed well during the diurnal bioassay (Chapter 4). Clearly, Calypso® and Pencap® cannot be recommended for *A. raptoria* management, since both showed disappointing results in both the studies that were conducted. The fact that Pencap® showed no results in the direct contact study and the poor performance in the diurnal bioassay study shows that the

active ingredient of this pesticide does not affect *A. raptoria* activity whatsoever and should not be considered in Hemiptera control in general.

Also, the very short active phase of the most successful pesticides examined, in the case of Karate®, less than an hour, implicates a short residual lifetime, which may be important when considering residue levels left on nuts after pesticide application. This is important when considering fruit for the export market, where pesticide residue levels are of great concern.

5. References

Abou-Fakhr Hammad, E.M., Nemer, N.M. & Kawar, N.S. 2000. Efficacy of Chinaberry tree (Meliaceae) and certain insecticides against the pea leafminer (Diptera: Agromyzidae). *Journal of Agricultural Science* **134:** 413 – 420.

Broughton, S. & Herron, G. A. 2007. *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) chemical control : Insecticide efficacy associated with three consecutive spray strategies. *Australian Journal of Entomology* **46:** 140 -145.

Greene, J.K. & Capps, C.D. 2003. Efficacy of selected insecticides for control of stink bugs – 2003. *Summaries of Arkansas Cotton Research 2003, AAES Research Series* **521:** 227 – 232.

Greene, J.K. & Capps, C.D. 2004. Efficacy of selected insecticides for control of stink bugs – 2004. *Summaries of Arkansas Cotton Research 2004, AAES Research Series* **533:** 177 – 185.

Herron, G., Jiang, L. & Spooner-Hart, R. 1996. A laboratory-based method to measure relative pesticide and spray oil efficacy against broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Experimental and Applied Acarology* **20:** 495 – 502.

Herron, G. A., Rophail, J. & Gullick, G.C. 1996. Laboratory-based, insecticide efficacy studies on field-collected *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implication for its management in Australia. *Australian Journal of Entomology* **35:** 161 - 164.

Keita, S. M., Vincent, C., Schmit, J., Arnason, J.T. & Belanger, A. 2001. Efficacy of essential oil of *Ocicum basilicum* L. and *O. gratissimum* L. apllied as an insecticidal fumigant and powder to control *Callosobruchus macalatus* (Fab.) [Coleoptera: Bruchidae]. *Journal of Stored Products Research.* **37:** 337 – 349.

Kelly, J.R. & Miller, M.A. 1978. Effectiveness of ®Curaterr against pests of swedes and turnips in the British Isles. *Pflanzenschutz-Nachrichten Bayer* **31:** 258 - 282.

Lacey, L.A., Horton, D.R., Chauvin, R.L. & Stocker, J.M. 1999. Comparitive efficacy of *Beauveria bassiana, Bacillus thuringiensis* and Aldicarb® for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. *Entomologica Experimentalis et Applicata* **93:** 189 – 200.

Studebaker, G.E. & Kring, T.J. 2003. Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae), measured by field, greenhouse and Petri dish bioassays. *Florida Entomologist* **86(2):** 178 – 185.

Tillman, P.G., Mulrooney, J.E. & Snodgrass, G.L. 2003. Comparison of susceptibility of *Geocoris punctipes* and *Lygus lineolaris* to insecticides for control of the tarnished plant bug. *Southwestern Entomologist* **28 (1):** 1 – 8.

Zoebelein, G., Dörntlein, D. & Hammann, I. 1980. Results of laboratory and field evaluation of ®Cropotex, an acaricide of a new compound group. *Pflanzenschutz-Nachrichten Bayer* **33:** 169 - 184.

Zoebelein, G. & Kniehase, U. 1985. Laboratory, greenhouse and field trials on the effect of nikkomycins on insects and mites. *Pflanzenschutz-Nachrichten Bayer* **38:** 203 - 304.

Appendix 1

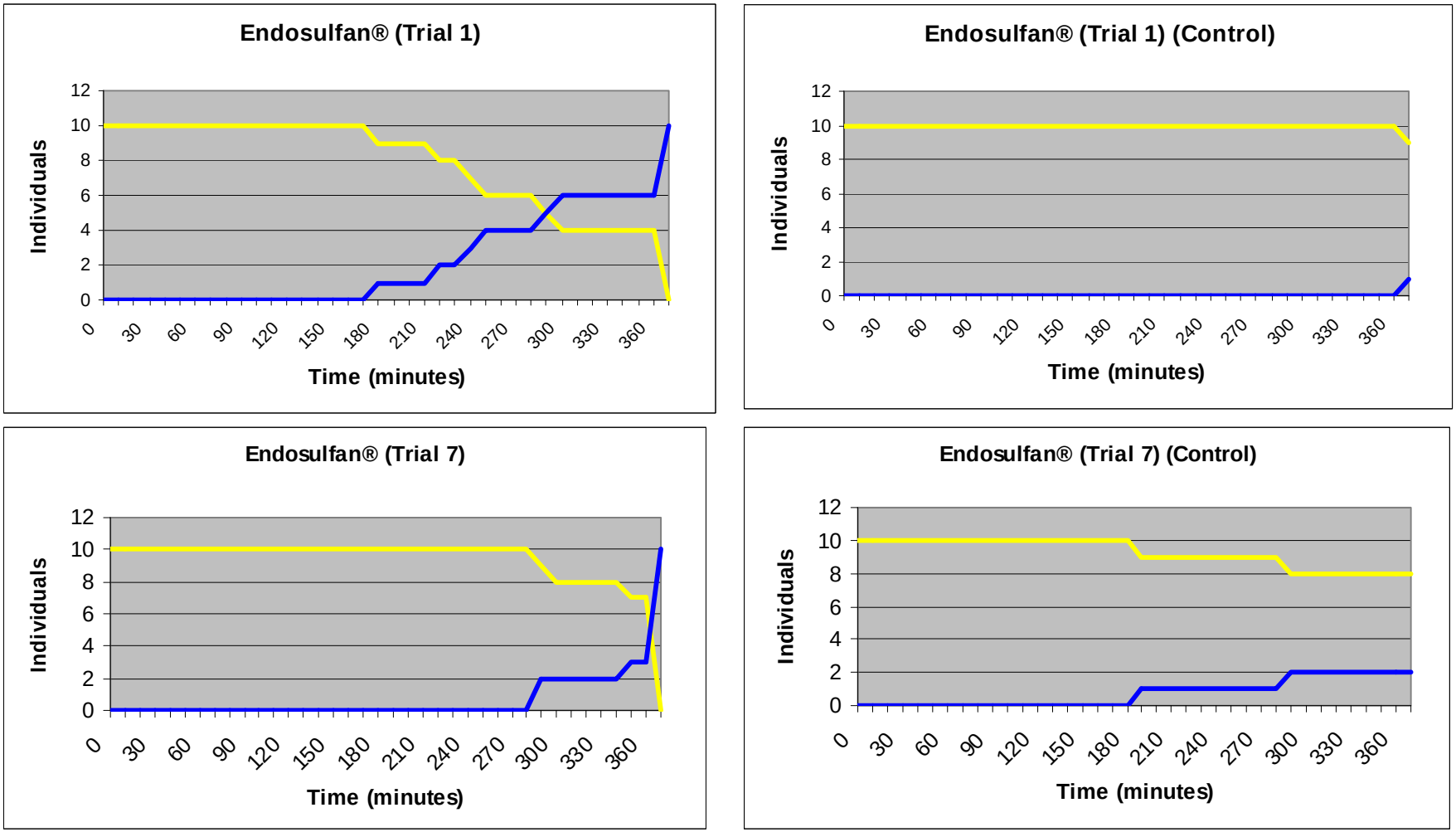
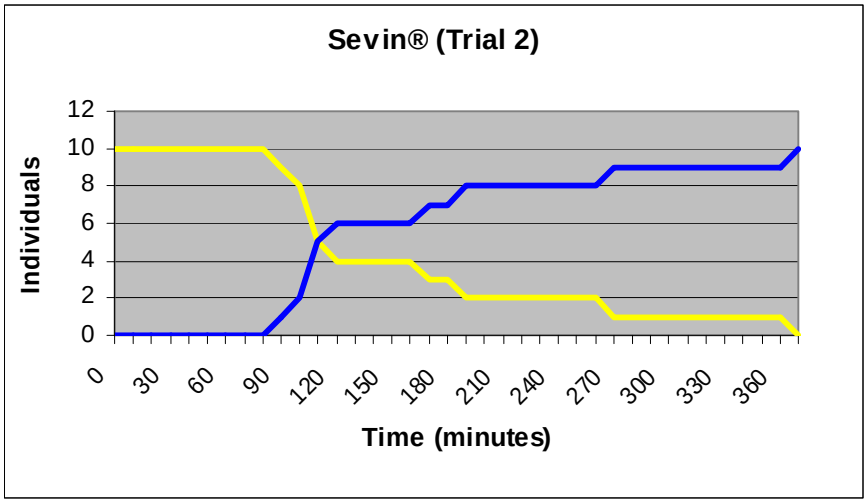
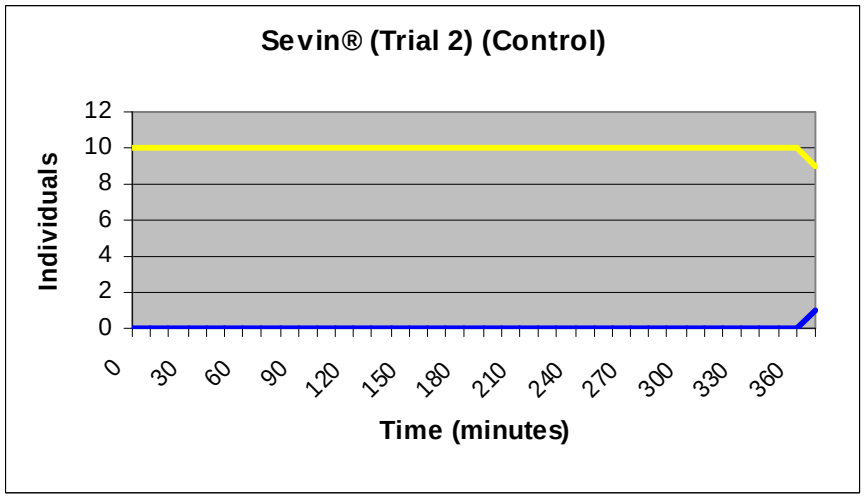
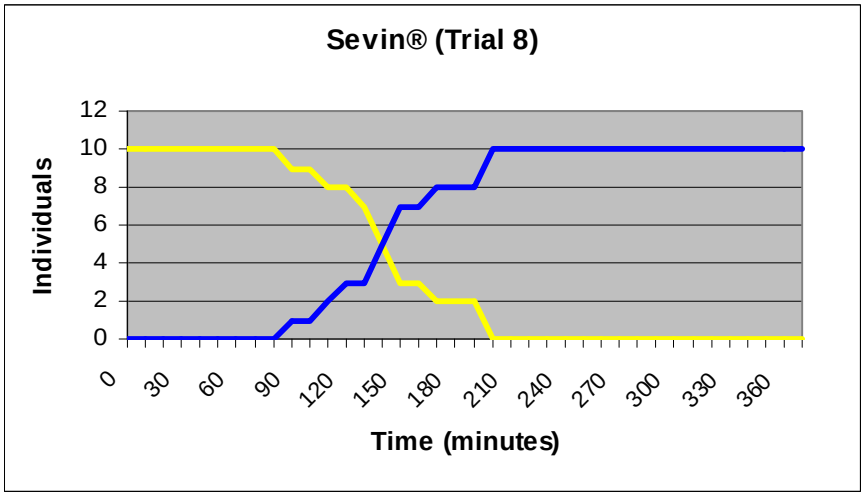


Figure 1: Direct contact pesticide bio-assay on *Atelocera raptoria* using Endosulfan SC®.







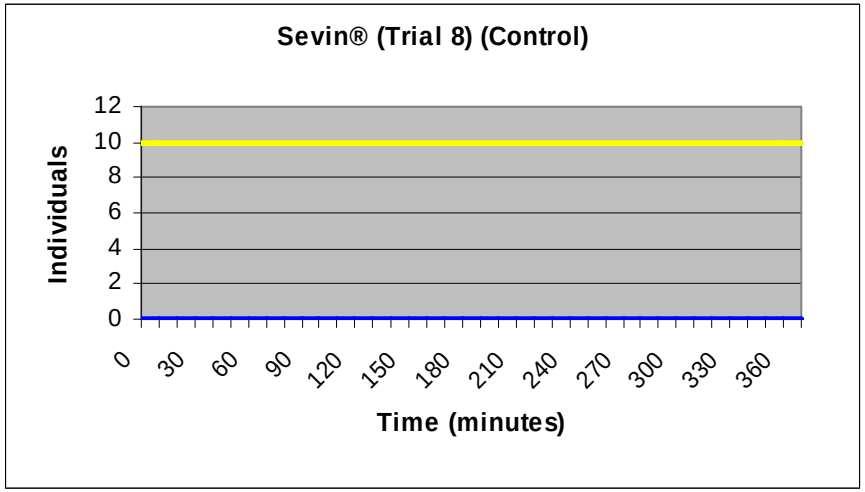


Figure 2: Direct contact pesticide bio-assay on *Atelocera raptoria* using Sevin®.

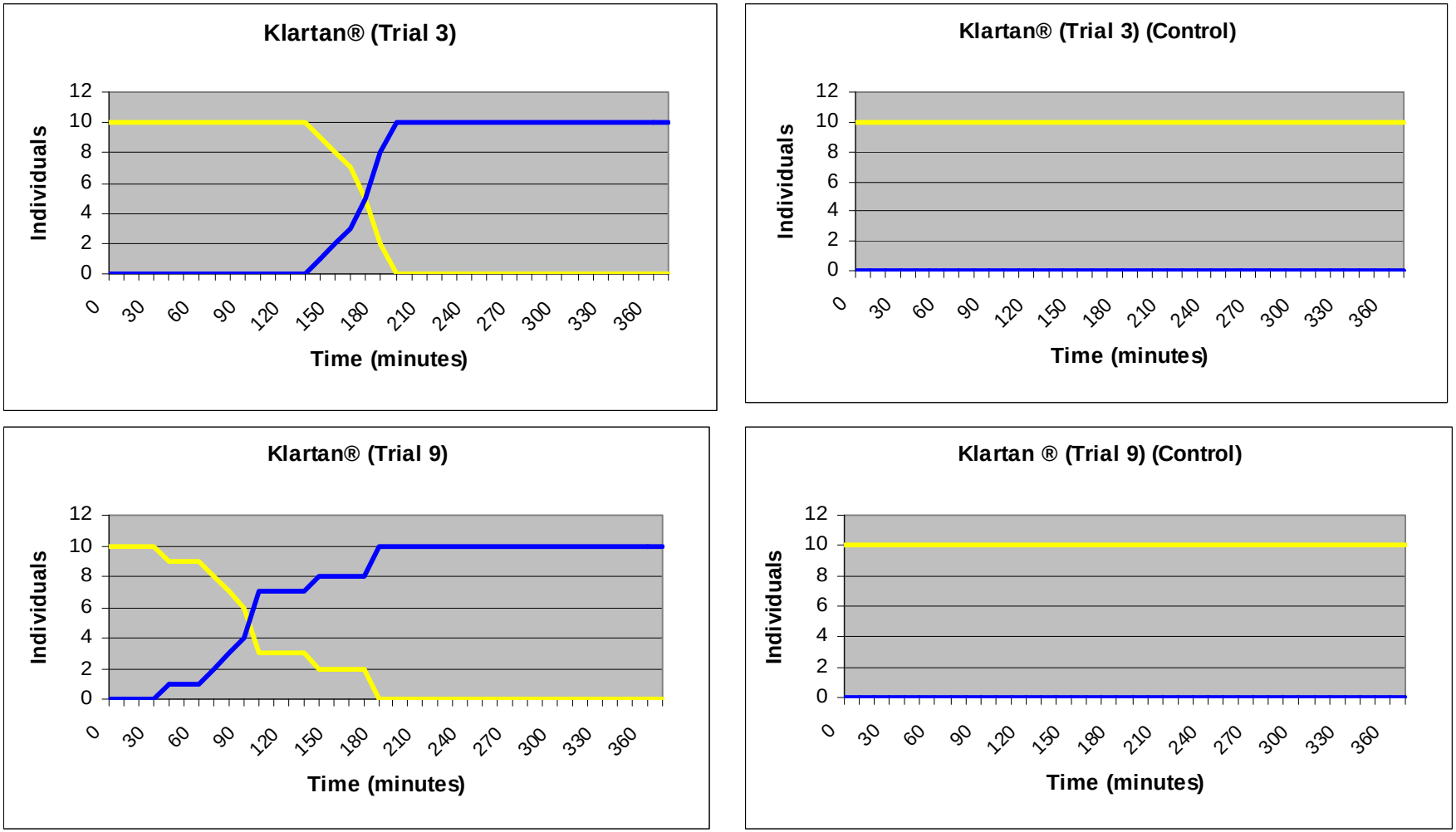
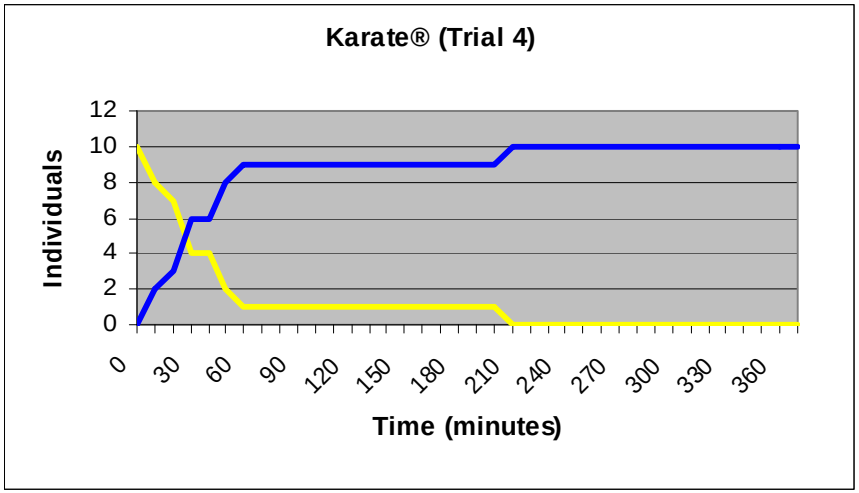
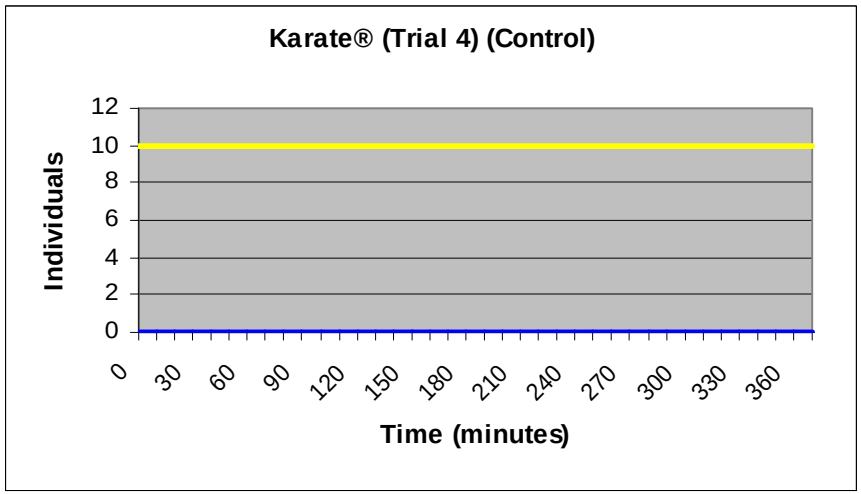
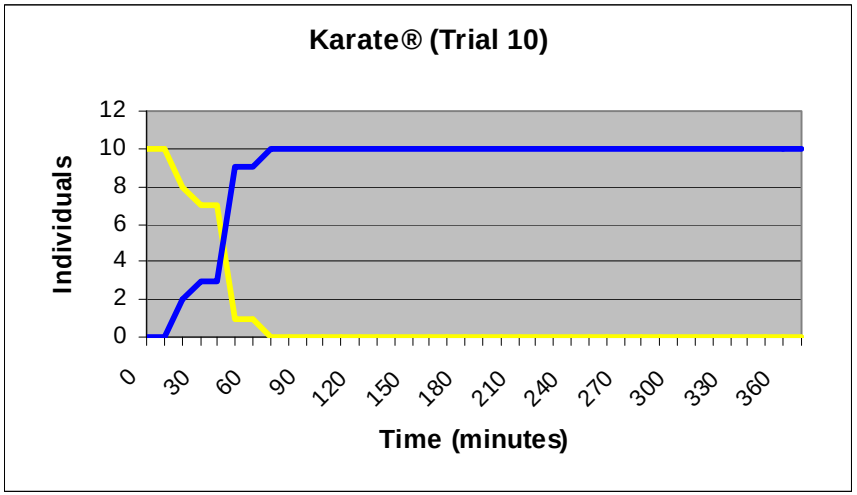


Figure 3: Direct contact pesticide bio-assay on *Atelocera raptoria* using Klartan®.







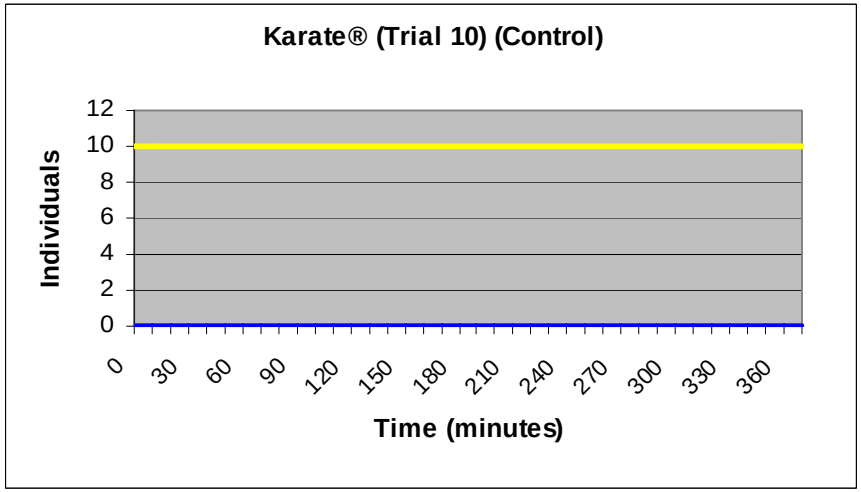


Figure 4: Direct contact pesticide bio-assay on *Atelocera raptoria* using Karate®.

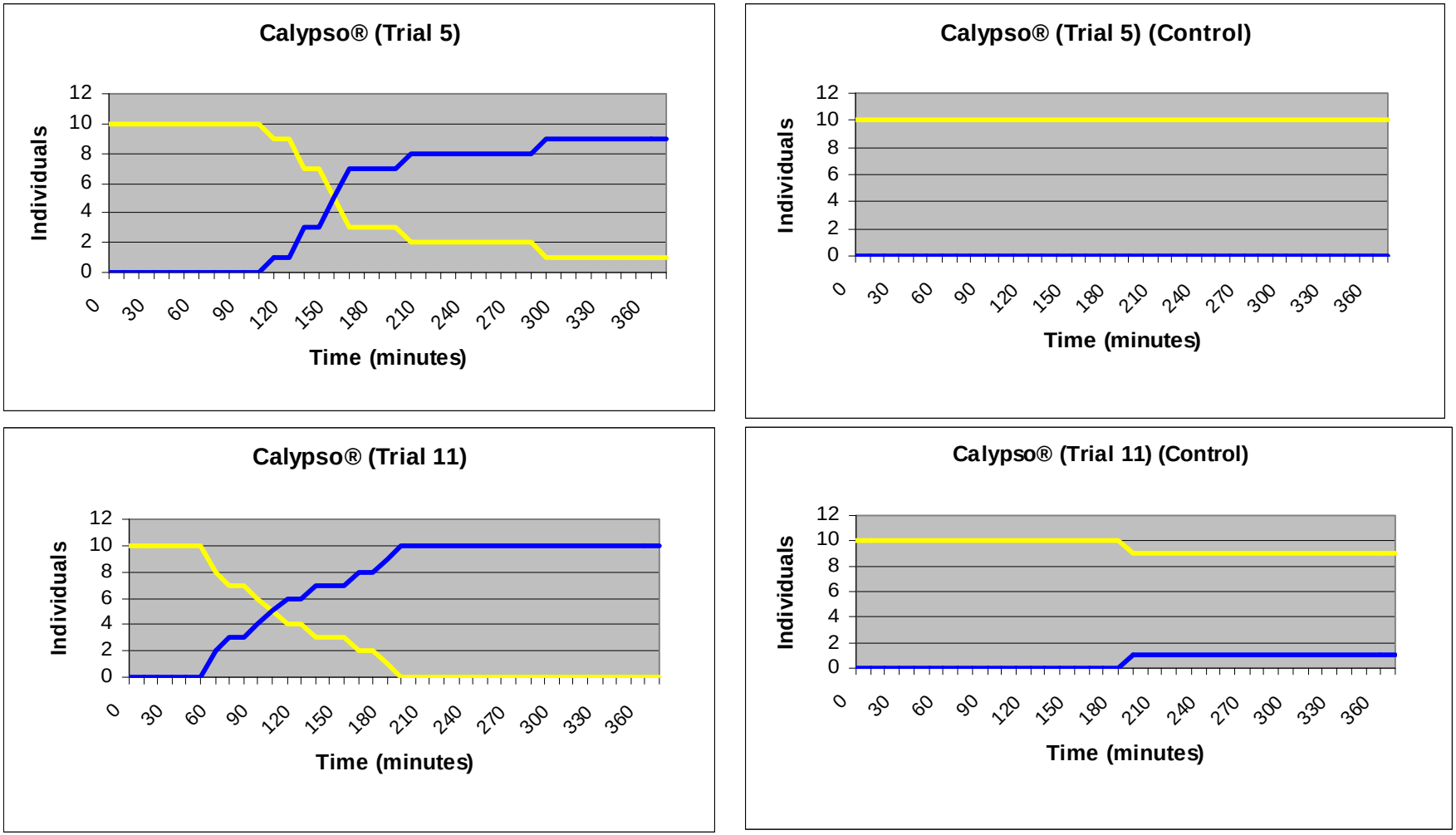


Figure 5: Direct contact pesticide bio-assay on *Atelocera raptoria* using Calypso®.

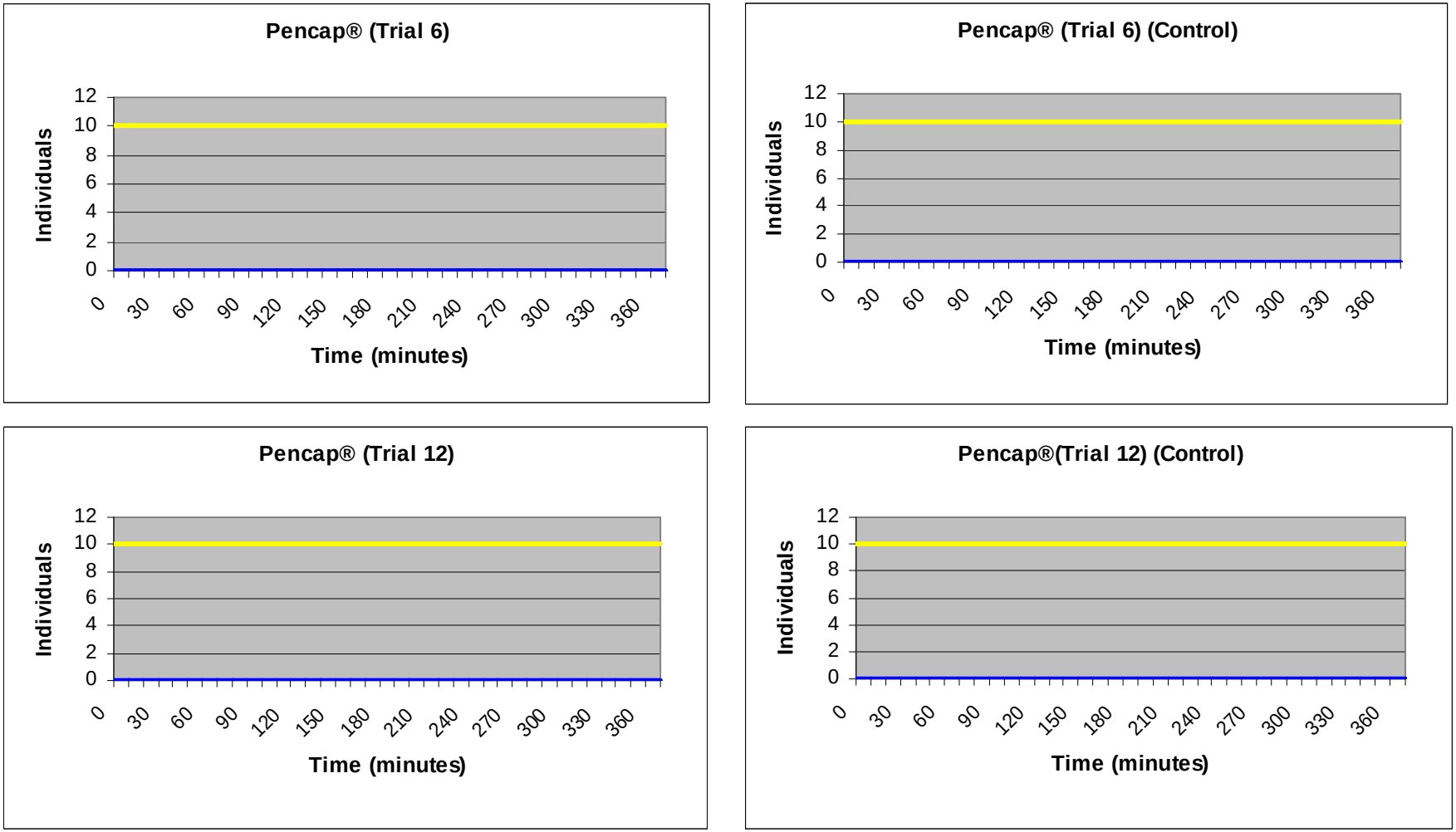


Figure 6: Direct contact pesticide bio-assay on *Atelocera raptoria* using Pencap.

CHAPTER 7

An accumulative degree-day model driven pesticide application program for the management of *Atelocera raptoria* on pistachio

1. Introduction

To successfully apply a degree-day model as a managing practice, in-depth knowledge of the pest is required. The life cycle and number of instars, temperature threshold, optimal developmental temperature and developmental time of instars should all be determined. These aspects are thoroughly discussed in Chapter 2.

As already mentioned in previous chapters, all Arthropods are poikilothermic and depend on temperature in their environment to regulate their metabolism, physiological processes and body temperature (Mellanby, 1939; Borror, Triplehorn & Johnson, 1992). The theory behind degree-day models as management practice is based on the fact that insects require a certain amount of heat-energy to undergo and complete development (Higley & Wintersteen, 1997; Johnson, Bessin & Townsend, 1998; Steffey, 1999; Anon, 2004). When the degree-day requirement of a pest species is determined it can be manipulated towards establishing a management strategy. Such a degree-day model can be used as an integral tool in an Integrated Pest Management (IPM) program. By calculating the accumulation of degree-days, predictions as to egg eclosion, nymph peaks, adult peaks and pest emergence can be made. In the past, the use of degree-day models has contributed significantly towards the understanding of several pest- species and their predators. Examples include studies on the hatching of winter eggs of the European red mite (Acari: Tetranychidae) (Broufas & Koveos, 2000); the spring emergence patterns of *Carposina sasakii* (Lepidoptera: Carposinidae) in apple orchards of Korea (Kim, Lee & Yiem, 2000); and the relevant relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinelidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae) (Roy, Brodeur & Cloutier, 2002). Generation time of a species can also be determined by using degree-days. This can be of great help in determining pesticide spraying calendars, biological agent release dates and the implementation timing of other cultural practices. During this study, the implementation of a pesticide spraying

program based on a degree-day model was specifically investigated. The concept of using degree-days has been around for quite some time. As early as 1956, Lindsey & Newman (1956) proposed a method towards the calculation of accumulated heat units, which would later become known as degree-days. This method was referred to as the single triangle method and functions by drawing a straight line between the minimum daily temperature and the maximum daily temperature. This method assumes the following day's minimum will be the same, and draws another line connecting to that point. This forms a triangle. Degree-days are then estimated by calculating the surface area of the triangle above the lower threshold. A few years later, Arnold (1960) devised a method for estimating accumulated heat units by means of averaging daily maximum and minimum temperatures. This is a very simple method and does not require complex mathematical formulae. This is referred to as the averaging (rectangle) method. Arnold's method, however, did not allow for the introduction of an upper developmental threshold. It was only a few years later that Baskerville & Emin (1968) proposed a computerized system to include both lower an upper developmental thresholds and referred to it as the single-sine method. This technique uses the day's minimum and maximum temperatures to produce a simple sine curve over a period of 24 hours and then calculates the degree-days by calculating the area above the lower developmental threshold and below the curve. This method also assumes that the sine curve is symmetrical around the daily maximum (Anon, 2004). In 1976 Allen proposed a double sine method. This method fits a sine curve from the minimum daily temperature to maximum daily temperature. Following this, a separate sine curve is fitted from the maximum temperature of the day to the minimum temperature of the next day. Thus, degree-days for the day are the sum of two half days (12 hours). Shortly after this, Sevacherian, Stern & Mueller (1977) developed the double-triangle method. This method operates in the same fashion as the double sine method, but only using triangles instead of halved sine curves.

During this study the averaging method of Arnold (1960) was used, whereby only the lower developmental threshold is incorporated. The reason for this was because the upper developmental threshold was found to be high enough so as to not influence degree-day estimates.

In more recent times, a myriad of different techniques and methods for the calculation of degree-days have been developed, mainly in light of several shortcomings concerning the averaging method proposed by Arnold (1960) and others. One of the main points of criticism concerning Arnold's model is the fact that only daily maximum and minimum temperatures are used. This method calculates the daily average temperature by means of daily temperature units (DTU). This is done by adding the minimum daily temperature (Tmin) and the maximum daily temperature (Tmax), taken over a period of 24 hours, and then dividing the sum by two, giving rise to the equation: Avg. Daily Temp. = (Tmin + Tmax) / 2 (Delahaut, 2002). However, using hourly temperature unit (HTU) readings provides a more accurate calculation than the previously mentioned model that is only based on daily averages (Roltsch, Zalom, Strawn, Strand & Pitcairn, 1999; Cesaraccio, Spano, Duce & Snyder, 2001). According to Purcell (2003), several reports have indicated that calculation of thermal units (degree-days) on an hourly basis, and averaging these values over 24 hours, was superior to calculations of thermal units using the average of maximum (Tmax) and minimum (Tmin) temperatures. The HTU method of calculation requires a great deal more calculations, however, making this method much more labour intensive. The mentioned HTU method also requires some fairly complex mathematical calculations, which has to be done using temperature data from every hour. Computer programs and electronic weather data collection devices can be utilized to perform these calculations based on recorded temperature data, even on a minute temperature unit basis (Nugent, 2005). Although using hourly weather data offers the greatest accuracy for estimating degree-days, the daily maximum and minimum data are often used to estimate degree-days by approximating the diurnal temperature trends (Snyder, Spano, Cesaraccio & Duce, 1999). It was therefore decided that for the purposes of the Green Valley Nuts (GVN) degree-day model that this study was intended for, the

averaging method using only daily minimum and maximum would be sufficient. The differences obtained using the two different methods are also not very pronounced and would not greatly influence the results this study was intended for.

Higley, Pedigo & Ostlie (1986) also discussed a range of factors that could influence the predictive capabilities of degree-day models. These included conditions such as nutrition and behavior based thermoregulation. Another point of concern was the fact that basic modeling techniques fail to take into account the behavioral thermoregulation by larval and nymphal stages of the particular insects, which can result in significant increases in body temperature relative to ambient, especially in species which bask (Bryant, Bale & Thomas, 1998). Roltsch *et al.* (1999) also argued that it was of particular concern as to how well available temperature readings reflect the microclimatic temperature an organism actually experiences within a field environment. Consequently, Bryant *et al.* (1998) proposed a method whereby this factor could be included in the calculations. Again, as was the case concerning HTU calculations, this would make calculations much more complex, requiring a computer for data analyses, as well as rendering the process impractical for rapid processing by farmers. These facts, and the labour intensity coupled with calculating the formulae by hand, only confirmed the choice of using the simple averaging method at GVN.

As can be seen from the above, there exists an ongoing argument as to the best method for calculating degree-days and research is ongoing as to the most accurate method. However, due to the difficulties and labour intensity of the more recently proposed modern methods, it was decided that the averaging method would suffice due to it's simplicity and practicality considering the setup at GVN.

2. Material & Methods

2.1. Temperature thresholds of *Atelocera raptoria*

There is a developmental threshold temperature for all insects. First off, one finds the lower developmental threshold. No development occurs when temperatures are below this level. Insects also have an optimum temperature range in which they will grow most rapidly. Finally there is maximum temperature (termed upper cutoff) above which development stops (Johnson, Bessin, & Townsend, 1998). By recording the success rates of hatching and parasitism at different temperatures, it can be determined what the optimum temperature regime, as well as the upper and lower temperature thresholds, for *A. raptoria* encompass.

This study was conducted together with the trials described in Chapter 2 and the sampling of materials and the study setup deployed are therefore the same as those described in Chapter 2 which concern the status of *A. raptoria* egg packages.

2.2. Implementing a degree-day model as management tool

Using the results obtained from the temperature threshold trails, a degree-day model was implemented on site by GVN staff. It was also the responsibility of GVN to implement the degree-day model in conjunction with the pesticide spraying regime to achieve the greatest efficacy against *A. raptoria*.

For the construction of a degree-day model, the minimum developmental threshold had to be determined and the daily maximum (Tmax) and minimum (Tmin) temperatures were required. These temperature values (Tmax & Tmin) were recorded on site at GVN using weather monitoring data and data loggers. As mentioned earlier, it was decided to use the simple averaging or rectangle method proposed by Arnold (1960), whereby Degree-days = Maximum + Minimum temperature / 2 – Minimum Threshold.

2.3. Effect of degree-day accumulation as a management tool

The adding of accumulated degree-days as a management practice was implemented in the latter part of 2005, after the first bioassays were completed (see Chapters 4 & 6). This was possible since it was coupled to the research done on the bio-ecology of *A. raptoria*. The effect of the implemented degree-day driven pesticide spraying calendar was determined by examining the scouting data obtained from GVN. The scouting protocols were suggested by Louw (2003), in response to a completed biomonitoring survey of all insects present in the pistachio orchards.

3. Results & discussion

3.1. Determining the temperature thresholds of *A. raptoria.*

It was determined that the embryos could not withstand temperature as high as 40°C and no successful hatching occurred after this point.. The number of empty eggs at the lower temperatures (10°C and 15°C) indicates that the development of the eggs was inhibited because of the low temperature. No nymph emergence was recorded at 10°C, with only 4 nymphs emerging at 15°C (Fig. 1). (See also Chapter 2)

The graph shows that successful development of the eggs increased towards mid-range temperatures and then again decreased, as temperatures got higher. From this data, approximate lower and upper temperature thresholds for *A. raptoria* egg development could also be determined. These thresholds represent the temperature cut-off points after which no successful development and emergence can take place (Table 1).

The minimum temperature below which no growth occurs is referred to as the minimum developmental threshold. Development will increase with higher temperatures up to a maximum temperature referred to as the maximum developmental threshold. These thresholds are determined experimentally and are different for each species (Higley & Wintersteen, 1997).

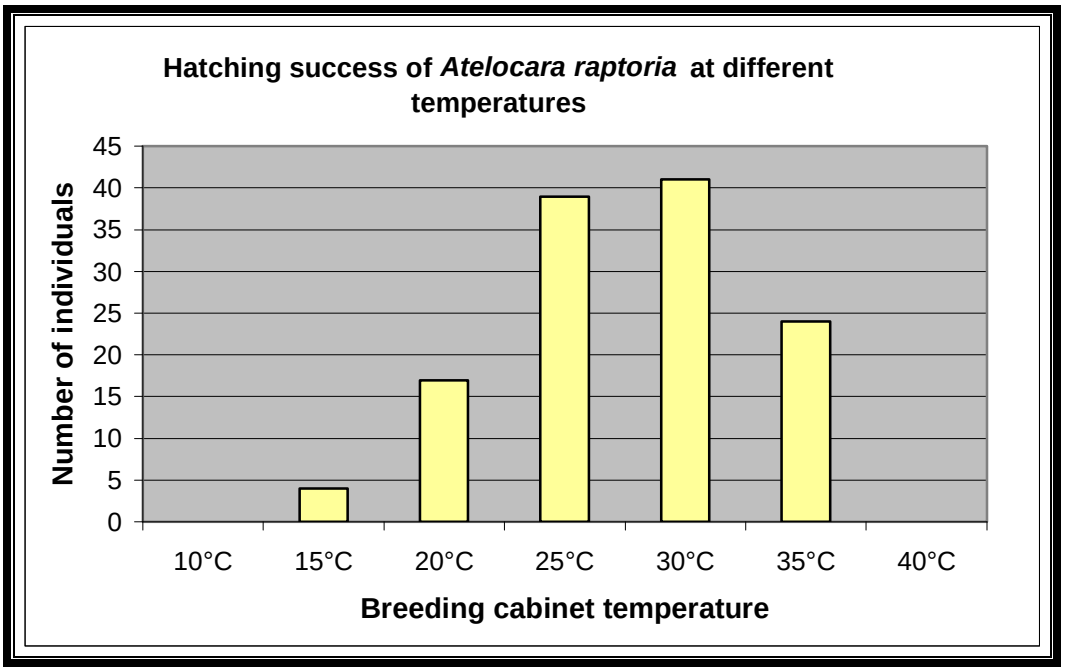


Figure 1: Hatching success of *Atelocera raptoria* eggs at fluctuating temperatures.

More exact studies (between 10°C and 15°C and between 35°C and 40°C) can be done in the future to determine the exact thresholds. However, 10°C is commonly used as a lower developmental threshold when calculating degree-days as this value is representative of many insect species (Pruess, 1983) and consequently it was used when calculating the degree-day values for *A. raptoria* as well.

Table 1: Temperature thresholds for *Atelocera raptoria* egg development

	Lower threshold	**Upper threshold**
Nymphs	**15°C > 10°C**	**35°C < 40°C**

These values can be compared to those of other pentatomids, such as *Nezara viridula.* Culligan (1975) states that *N. viridula* nymphs are not able to rupture the chorion at 15°C, but do live for a further ten days after the completion of development until energy reserves are depleted. Culligan (1975) states that the theoretical developmental zero of *N. viridula* (lower threshold) is 13°C. He also states that the upper lethal limit (upper threshold)

is in the vicinity of 38°C to 40°C. The optimum developmental temperature, which is defined by Uvarov (1931) as the temperature range at which the relatively greatest percentage of individuals accomplish their development within the relatively shortest period, is both of theoretical and practical importance. In *N. viridula* this range is between 23°C and 27°C (Culligan, 1975).

The *N. viridula* values correlate with those found for *A. raptoria.* This is to be expected, as both these stinkbugs prefer sub-tropical to tropical climates. Both of these climates maintain high daily temperatures with average, temperature nights. The temperature will rarely, if ever, fall below 0°C in the summer months. California in the USA, South Africa, central Chile and Southwestern Australia all posses a subtropical climate. Most of the world's subtropical climate falls between 30° north and 40° south latitude (Ritter, 2003). Not surprisingly, these areas are amongst the greatest nut producing areas in the world. Subsequently, many of the same pest species, such as *N. viridula* and *A. raptoria* (in South Africa specifically), hamper production in these areas.

3.2. Implementing a degree-day model as management tool

By using the averaging method discussed above it was possible to compile a generation time for *A. raptoria* under field conditions at GVN. In this regard it was calculated that nymph emergence occurred approximately 105 degree-days after biofix. This was confirmed by interpreting data sampled during extensive monitoring programs run at GVN. These scouting programs would occur on a weekly basis during the peak growth season in the year, in this case throughout spring and summer.

The total generation time of *A. raptoria* was calculated to be between 800 and 900 degree-days. To correlate the accumulation of degree-days with the generation time of *A. raptoria*, a biofix was needed. This is a date chosen on account of a specific activity related to the target species and whereby

accumulation of degree-days commences. This critical date is referred to as the biofix date and logically varies from species to species. Biofix dates are usually based on specific biological events such as planting dates, first trap catch, or first occurrence of the pest (Anon, 2003). The biofix selected for the first generation of *A. raptoria* was the first egg packages to be scouted in the orchards. As mentioned earlier, scouting is conducted throughout the growing season, making it easy to detect the first appearance of *A. raptoria* egg packages. As biofix for subsequent generations, the total maximum degree-day accumulation of the first generation is used, which, in this case, was 900 degree-days. This point would then serve as biofix for the second generation, whereby the management cycle is restarted. Pesticide application for the first generation of A. raptoria is done on 120 degree-days, with a follow-up application 7 days later. This ensures that nymphal eclosion has occurred regarding the majority of the egg packages. Theoretically, the nymphs are highly susceptible to the pesticides enhancing the effect of the applied pesticides. This same procedure is followed with the subsequent generations, after the completion of the first (Fig. 2).

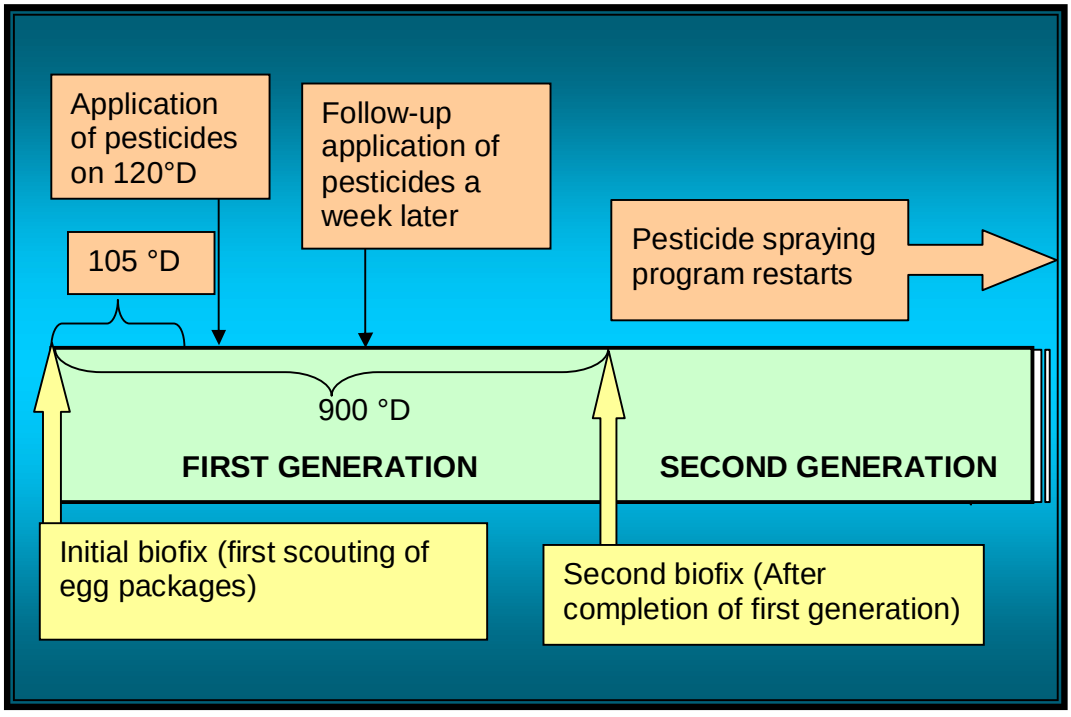


Figure 2: Visual representation of a degree-day driven management program for *Atelocera raptoria*.

3.3. Effect of degree-day model as a management tool

By plotting the previously mentioned scouting data, graphs could be compiled showing the occurrence of *A. raptoria* in pistachio orchards at GVN. Occurrence is expressed as percentage occurrence on trees sampled and is basically the percentage of trees sampled on which *A. raptoria* was recorded. During scouting, individual numbers were not counted due to the high numbers recorded and the labour intensity and time consummation coupled with this. This would have provided more accurate data, and should be done when possible. However, percentage occurrence on the trees still provide a good indication on the population levels of *A. raptoria* occurring in the orchards. This monitoring was done on all the different cultivars currently planted at GVN. As a great many blocks are monitored throughout the year, averages including all monitored blocks were used for the compilation of the graphs. As mentioned earlier, monitoring is done during the main growing period of the trees, in this case from mid-October (week 42) throughout spring and summer up until the end of June of the following year (week 26). Even though monitoring is not done every single week during this period, it provides a clear indication as to the *A. raptoria* population numbers present in the orchards (Figs. 3 – 8).

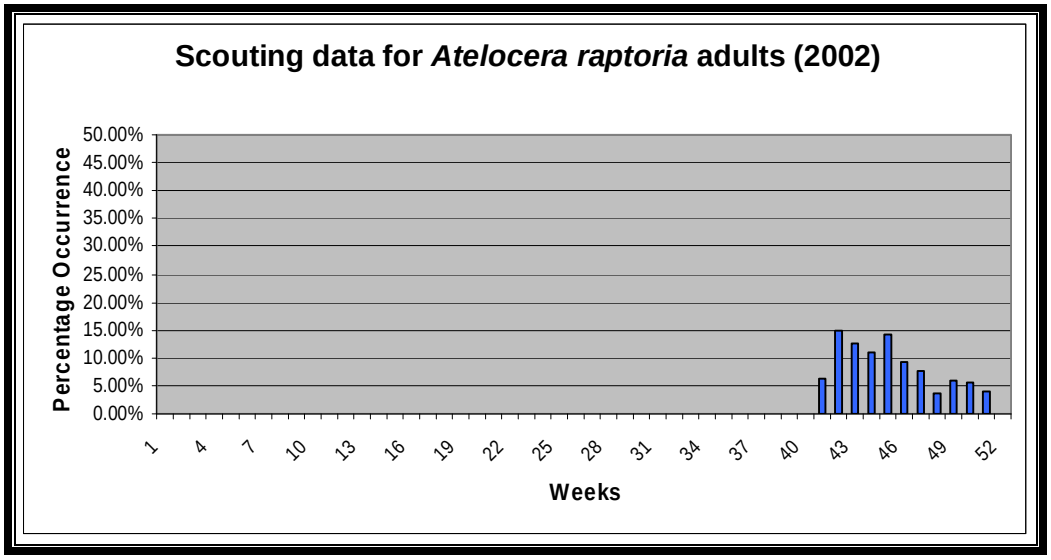


Figure 3: Adult *Atelocera raptoria* scouting data from various cultivars sampled at GVN during the latter part of 2002.

Figure 3 shows the period when monitoring for *A. raptoria* was initiated during the latter part of 2002. Fairly high occurrence, up to 15%, can be noted during the late spring and early summer months of 2002.

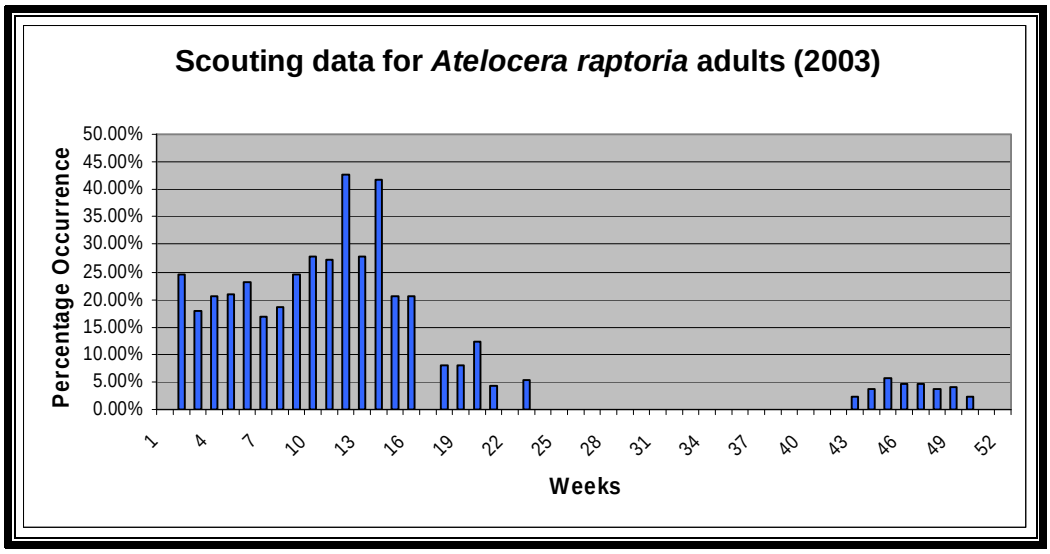


Figure 4: Adult *Atelocera raptoria* scouting data from various cultivars sampled at GVN during 2003.

The highest occurrence of *A. raptoria* could be observed when plotting the sampled data of 2003. Percentages exceeded 40% in some instances with average occurrence being well above 25% overall (Fig. 4). The greatest numbers were found during late summer when all generations of *A. raptoria* had been completed. According to degree-day data accumulated at GVN, the generation time of *A. raptoria* is approximately 2 months. This means that under ideal conditions, perhaps up to four generations of *Atelocera* can be expected during a single growing season. This correlates with the research done on the closely related *Nezara viridula*. Four generations per year have been observed for this species in Florida and Louisiana, while in some instances in Queensland, Australia, only three generations were observed (Todd, 1989; Knight & Gurr, 2007).

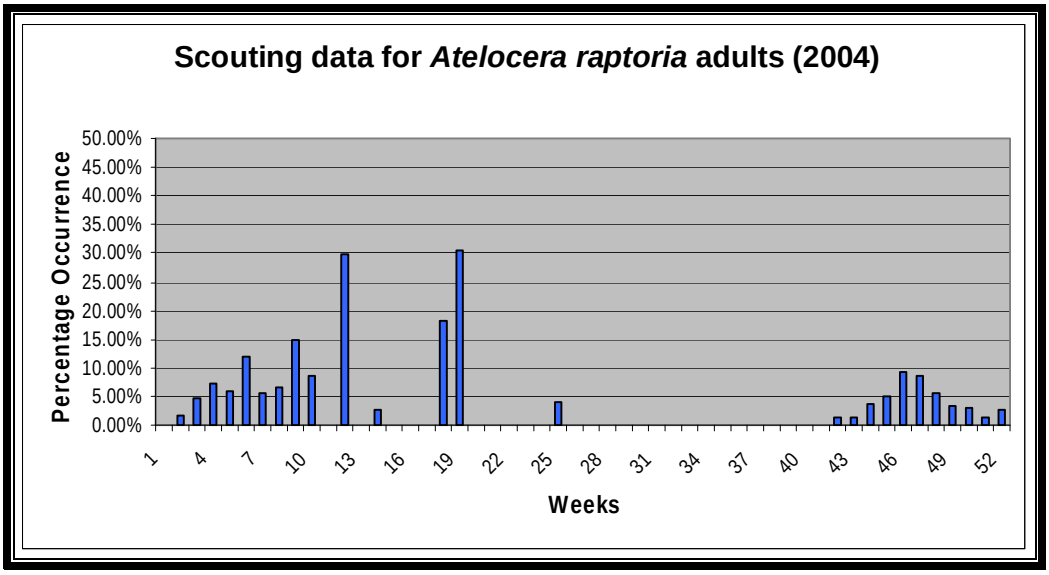


Figure 5: Adult *Atelocera raptoria* scouting data from various cultivars sampled at GVN during 2004.

Data sampled during 2004 showed slightly less numbers than those recorded during 2003. However, percentage occurrence was still found to be above 30% during some weeks (Fig. 5). For unknown reasons, monitoring could not be conducted for several weeks during summer, however, the tendancy of occurrence is still reflected in a satisfactory manner.

In 2005 fairly high occurrence of *A. raptoria* could again be seen earlier in the year. Unfortunately, monitoring was again not conducted during several weeks around March and April. However, the general trend can still be clearly seen, with percentage occurrence frequently rising above 10% average during several weeks. The latter part of 2005 shows a different scenario, however. This period occurs just after the degree-day model was implemented in conjunction with the two most suitable pesticides found during the 2005 bioassays (Chapter 4). The pesticides were applied 120 degree-days after the biofix date. Pesticides were then again applied a week later. This was repeated once a new biofix date was established, in other words, when new egg packages were discovered. Clearly, a notable lower occurrence was recorded during this time, averaging below 5% throughout the latter part of 2005 (Fig. 6).

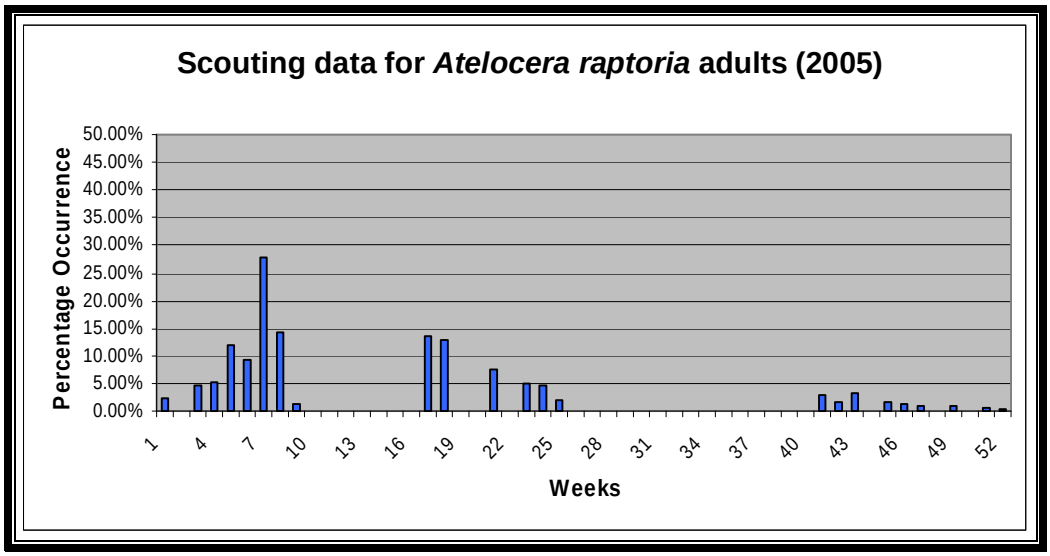


Figure 6: Adult *Atelocera raptoria* scouting data from various cultivars sampled at GVN during 2005.

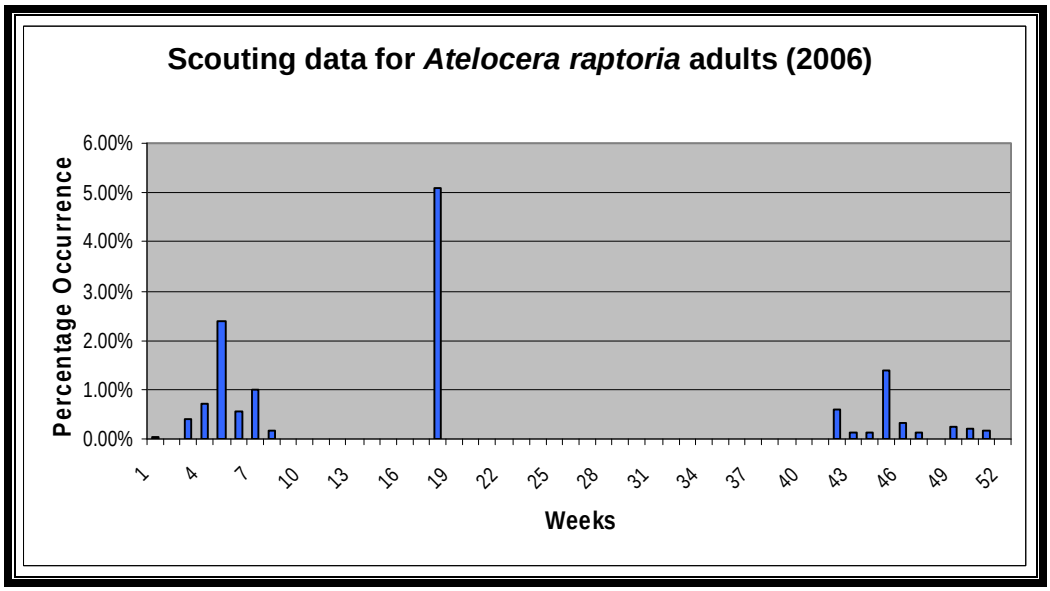


Figure 7: Adult *Atelocera raptoria* scouting data from various cultivars sampled at GVN during 2006.

The effects of the applied degree-day model is observed even better when considering the scouting data obtained during 2006. All scoutings reflected a percentage average occurrence of below 5%, with one week above 3% and the majority below 1% (Fig. 7).

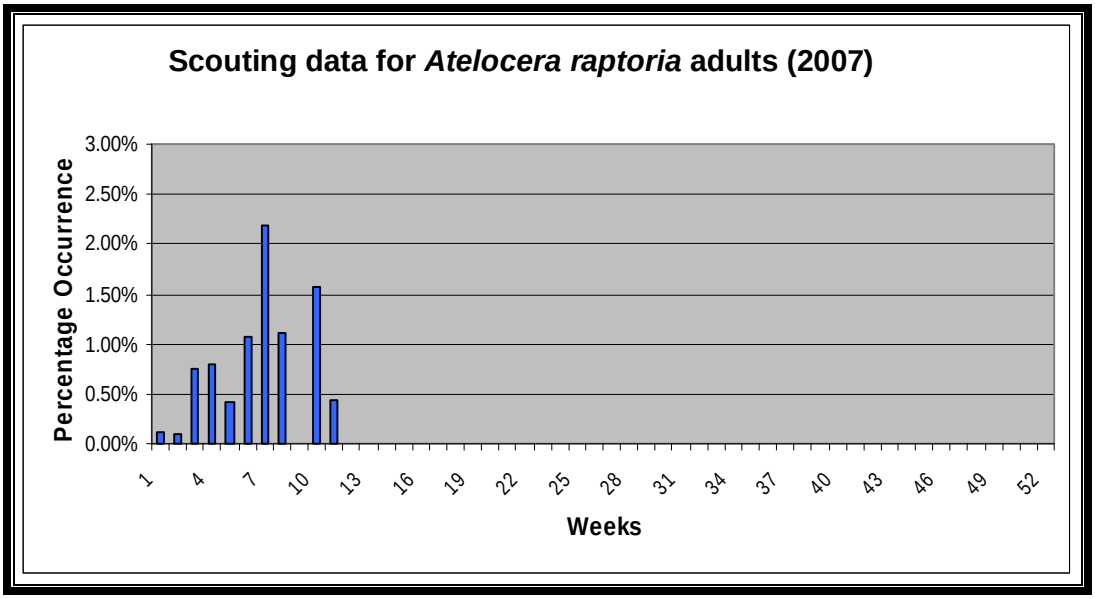


Figure 8: The adult *Atelocera raptoria* scouting data from various cultivars collected at GVN during 2007.

Only data of the first few weeks of 2007 (during peak summer and later) was available (Fig. 8), but it nonetheless showed very low average percentage occurrence of *A. raptoria*. In this case, only one week was higher than 2% occurrence, with the majority again averaging around 1%.

4. Conclusion

The results seemed to overwhelmingly indicate the importance of implementing a degree-day model as a guide towards the compilation of an adapted spraying program. Over the course of approximately two years (late 2005 to early 2007), the average occurrence of *A. raptoria* in the orchards have declined a great deal, almost to the point of becoming insignificant, whilst during 2003 and 2004, occurrence frequently rose to above 30%.

In light of this, the degree-day study can be considered a definite success as far as the management of *A. raptoria* in the GVN orchards is concerned. It has subsequently been reported from GVN that nut kernel damage attributed to *A. raptoria* feeding has dropped to almost zero in the last couple of years.

Even though orchard scouting and monitoring will have to be maintained, as has been the case during the last five years, the presence of *A. raptoria* as a primary pest on pistachio at GVN seems to have been greatly reduced.

5. References

Anonymous, 2004. *About degree-days.* Statewide IPM Program, Agriculture and Natural Resources, University of California. Retrieved from Http://www.ipm.ucdavis.edu/WEATHER.

Allen, J.C. 1976. A modified sine wave method for calculating degree-days. *Environmental Entomology* **5:** 388 – 396. (Original article not seen)

Arnold, C.Y. 1960. Maximum-minimum temperatures as a basic for computing heat units. *Proceedings of the American Society of Horticultural Science* **76:** 682 – 692. (Original article not seen)

Baskerville, G.L. & Emin, P. 1968. Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology* **50(3):** 514 – 517.

Borror, D.J., Triplehorn, C.A. & Johnson, N.F. 1992. *An introduction to the study of insects.* 6th Edition. Saunders College Publishing, New York.

Broufas, G.D. & Koveos, D.S. 2000. Threshold temperature for post diapause development and degree-days to hatching of winter eggs of the European red mite (Acari: Tetranychidae) in Northern Greece. *Environmental Entomology* **29(4):** 710 – 713.

Bryant, S.R., Bale, J.S. & Thomas, C.D. 1998. Modification of the triangle method of degree-day accumulation to allow for behavioural thermoregulation in insects. *Journal of Applied Ecology* **35:** 931 – 927.

Cesaraccio, C., Spano, D., Duce, P. & Snyder, R.L. 2001. An improved model for determining degree-days values from daily temperature data. *International Journal of Biometeorology* **45:** 161 – 169.

Culligan, G.A. 1975. *Structure and physiology of the egg of Nezara virudula (L.) (Pentatomidae) with notes on oviposition and hatching.* M.Sc. Dissertation, University of Pretoria, Pretoria. 104 pp.

Delahaut, K. 2002. *Degree day calculation*. UW-Madison IPM program, University of Wisconsin.
Retrieved from http://wihort.uwex.edu/gardenfacts/X1086.pdf.

Higley, L.G., Pedigo, L.P. & Ostlie, K.R. 1986. DEGDAY: A program for calculating degree-days, and assumptions behind a degree-day approach. *Environmental Entomology* **15:** 999 – 1016.

Higley, L. & Wintersteen, W. 1997. *Using Degree Days in an Integrated Pest Management Program.* Iowa State University, University Extension. Retrieved from Http://www.extension.iastate.edu/Publications.

Johnson, D., Bessin, R. & Townsend, L. 1998. *Predicting insect development using degree days.* University of Kentucky Entomology, University Extension.
Retrieved from Http://www.uky.edu/Agriculture/entomology

Kim, D-S., Lee, J-H. & Yiem, M-S. 2000. Spring emergence pattern of *Carposina sasakii* (Lepidoptera: Carposinidae) in apple orchards in Korea and its forecasting models based on degree-days. *Environmental Entomology* **29(6):** 1188 – 1198.

Knight, K.M.M. & Gurr, G.M. 2007. Review of *Nezara viridula* (L.) management strategies and potential for IPM in field crops with emphasis on Australia. *Crop Protection* **26:** 1 – 10.

Lindsey, A.A. & Newman, J.E. 1956. Use of official weather data in spring time: Temperature analysis of an Indiana phenological record. *Ecology* **37(No. 4.):** 812-823.

Louw, S. vd M. 2003. *Pistachio Entomology at IDC – Green Valley Nuts: Present knowledge and future focus.* Report to Industrial Development Corporation, Green Valley Nuts, Prieska. 27 pp.

Mellanby, K. 1939. Low temperature and insect activity. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **127(849):** 473 – 487.

Nugent, J. 2005. *Calculating growing degree-days.* Northwest Michigan Horticultural Research Station, Michigan Agricultural Experiment station. Retrieved from http://www.maes.msu.edu/nwmihort/gdd_calc.html.

Pruess, K.P. 1983. Day-degree methods for pest management. *Environmental Entomology* **12:** 613 – 619.

Purcell, L.C. 2003. Comparison of thermal units derived from daily and hourly temperatures. *Crop Science* **43:** 1874 – 1879.

Ritter, M. 2003. *Mediterranean or Dry Summer Subtropical Climate. The Physical Environment.* Retrieved from Http://www.uwsp.edu/geo/faculty.

Roltsch, W.J., Zalom, F.G., Strawn, A.J., Strand, J.F. & Pitcairn, M.J. 1999. Evaluation of several degree-day estimation methods in California climates*. International Journal of Biometeorology* **42:** 169 – 176.

Roy, M., Brodeur, J. & Cloutier, C. 2002. Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinelidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). *Environmental Entomology* **31(1):** 177 – 187.

Sevacherian, V., Stern, V.M. & Mueller, A.J. 1977. Heat accumulation for timing *Lygus* control measures in a safflower-cotton complex. *Journal of Economical Entomology* **70:** 399 – 402. (Original article not seen).

Snyder, R.L., Spano, D., Cesaraccio, C. & Duce, P. 1999. Determining degree-day thresholds from field observations. *International Journal of Biometeorology* **42:** 177 – 182.

Steffey, K. 1999. *Estimating development of insects with accumulation of degree-days.* University of Illinois, University Extension. Retrieved from Http://www.ag.uiuc.edu/cespubs/pest/articles.

Todd, J.W. 1989. Ecology and behaviour of *Nezara viridula*. *Annual Review of Entomology* **34:** 273 – 292. (Original article not seen)

Uvarov, B.P. 1931. Insects and climate. *Transactions of the Entomological Society of London* **79:** 1 – 247. (Original article not seen).

CHAPTER 8

General discussion and summary

1. Introduction

The present study was conducted to implement a management strategy towards the control of *Atelocera raptoria* (Hemiptera: Pentatomidae) in pistachio orchards at Green Valley Nuts (GVN), a division of Industrial Development Corporation of South Africa (Ltd), near Prieska in the Northern Cape Province, South Africa. Pest management strategies need baseline information regarding the biotic and abiotic environment of the target species in order to implement a credible launching pad for the investigation and to create confidence levels with the particular clients. General insect biomonitoring in relation to environmental variables has been conducted at GVN since 1999, providing the necessary sound basis for further, more detailed investigations. The present study, which has demonstrated that the effective, sustainable management of this primary pest species is very feasible under the localized conditions at GVN, is such a case in point. Whilst first and foremost attempting to establish a management strategy for *A. raptoria* at GVN, it was also envisaged that at least some of the outcomes of this study could serve as recommendations and reference points regarding the management of comparable primary pests elsewhere.

2. New crop development

Pistachio was only recently (ca. 1980) introduced to South Africa as an economically viable tree crop. This fact immediately raises many questions as to the continued well-being of the newly introduced crop. It has been well documented that plant species introduced to a new habitat, albeit a new country or just an area with a different climate, will be subjected to an unknown and unique range of pressures. Especially if the newly created agro-ecosystem is a mono-culture in structure, as is the case with most agricultural crops. Such a venture creates new niches for oligo- and polyphagous insects in the area, offering alternatives to the usual, naturally existing host-ranges.

New crop introductions into an area implies that possible pests, beneficial organisms and possible pathogens within the newly created agro-ecosystem are all unknown. Besides localized phytophagous species creating problems, pests normally associated with the new crop, can be translocated into new areas and become established. Such phytophagous species can become primary pests due to the absence of both inter-specific competition and natural enemies. *A. raptoria* to a certain extent demonstrates this point. This polyphagous stinkbug species is endemic to South Africa and has not been reported to be damaging on pistachio before. The newly established pistachio monoculture at GVN has provided ideal conditions for this widespread species to, in turn, establish in the orchards where it has become a primary pest.

3. Bio-surveys and sustained monitoring

As briefly referred to earlier, when a crop is new to an area, it is of principal importance to find out more about all the insects (both beneficial and detrimental) associated with this newly created area. The surest method of achieving this is by conducting an extensive bio-survey of all insect species present in the new agro-ecosystem. This can be achieved by manually scouting the orchards, the placement of traps (ground, tree, and light), fogging of trees etc. There are numerous ways in which to conduct such a bio-survey and it is therefore essential that the methods used should be relevant and cater for the situation in question. From the bio-surveys conducted a few years prior to the present study it was evident that *A. raptoria* is a primary pest of this nut crop, showing high population numbers and severe damage indices.

In studies of this nature, bio-surveys of the natural vegetation surrounding the agro-ecosystem should also be done. This could provide information on additional potential damaging species and provide information on natural host-ranges of target species. Such a venture could also provide information on the off-season refugia of target pest species.

Just conducting a bio-survey, however, will be insufficient when aiming towards sustainable management of a particular harmful species. In this regard continued monitoring is of prime importance while management practices are being implemented. Thus, good scouting techniques should be implemented as early as possible. This should be an ongoing practice running on a set calendar which will ensure that the necessary data on relevant insect populations are obtained on a timely basis during critical periods. Ongoing monitoring should therefore be done throughout the warmer seasons, including the total growing season of the plant. In more tropical regions monitoring should be conducted all year-round. During the present study, ongoing monitoring was done throughout the growing period of the trees and only terminated during the cold, winter months. Great emphasis should be placed on implementing a well established, high accuracy level scouting program.

After initial bio-surveys have been completed, and the insects identified, a reference collection and spread sheet of potential pests and beneficial insects can be compiled. Insects can further be sub-divided into detailed trophic groups for further reference purposes. All this information will ultimately contribute towards the proactive identification of different categories of pests on the crop.

4. Prioritization of possible insect pests

Many factors contribute to the pest status of a phytophagous species found on a crop. Most important is to evaluate the damage caused by the particular species. The level of damage attributed to the species in question will in all probability be influenced by the type of feeding. Generally, polyphagous foliage feeders will cause less damage to the crop as a whole, than would species directly feeding on the crop product. In the case of *A. raptoria* it was found that this stinkbug feeds on young stems, leaf petioles and individual nuts in the clusters. Sap sucking by these bugs on the nuts at a young age causes tissue necrosis, possible secondary fungal infection and the resultant

premature dropping of nuts. Due to this *A. raptoria* was considered a primary pest, since feeding damage by this species directly influences yield and has a direct economic consequence.

Population dynamics also play a major role in prioritizing pest-status of plant-feeding species. Even though a pest may be damaging, but population numbers are low, crop loss will be insignificant. Thus the fecundity and survival potential of a particular species in an agro-ecosystem should also be taken into account. Also, many species, especially localized species common in an area, may already be kept below damage thresholds by natural enemies also present in the area. *A. raptoria* showed very high population densities in the pistachio orchards, classifying them as a primary pest of pistachio in the Prieska district.

5. Knowledge of pest species

As soon as potential pest species is identified, knowledge of the species becomes an essential requirement. Should the species in question be translocated and introduced together with the host plant, the possibility exists that the species has already been described as pest elsewhere. If this is the case, relevant, existing management practices from the literature can then be adapted to fit the current circumstances. Also, the species may be a cosmopolitan pest, such as *Nezara viridula*, the southern green stinkbug. Such species are usually well researched, and a great deal of relevant information on management practices and species bio-ecology can be found in the literature. From this information, conventional, well researched management practices can then be implemented to suit the specific requirements of the situation.

However, when the pest species is endemic to the area and has not previously been described as a pest on the relevant host plant, far less research data can usually be found. As *A. raptoria* is endemic to South Africa

and pistachio a new crop to South Africa, this was the case during the present study. Very little research had previously been done on this species.

6. Bio-ecology of *Atelocera raptoria*

Due to the above-mentioned reasons, a complete study on the bio-ecology of *A. raptoria* had to be conducted. Understanding the bio-ecology of a potential pest species aids in its management. Studies on the bio-ecology of a target species should include investigation into the life-cycle, number of generations and generation duration, reproductive biology and general ecological pre-requisites. Information on the life-cycle is needed to ensure that accurate scouting data is accumulated, should immature stages be encountered. The generational time of the species is of great importance when considering the timing of management strategies. The upper- and lower developmental threshold of the insect should also be determined experimentally as this will be of use when implementing temperature related management practices.

7. Natural enemies

The contribution of natural enemies in the overall management strategy imposed on a particular pest species should also be taken into consideration, since a positive outcome in this regard could have vital implications with regard to the final implementation and outcome of the management strategy. Natural enemies can be insect pathogens, predators or parasitoids. During the present study, *Trissolcus basalis* (Hymenoptera: Scelionidae) was found parasitizing the eggs of *A. raptoria.* Consequently, the efficacy of natural, existing parasitoid populations within the pistachio orchards at GVN were also examined. Such a parasitoid-host interaction gives rise to options regarding the feasibility of mass-rearing and releasing the parasitoid, thereby augmenting the existing, natural population and implementing a biological control tactic. When considering mass rearing and release, it is, however, important that the parasitoid is fairly host specific, since augmenting the natural population may have an adverse effect non-target species.

In the case of exotic, imported pests, natural enemies can usually be found existing in the area of origin of the target species. Importing these species do, however, require a great deal of research on the possible effects on local species related to the target pest species. During the present study, it was determined that *T. basalis* was released in South Africa for the control of the related cosmopolitan pest species, *N. viridula*. The parasitoid most probably migrated to the Prieska district over time and acquired *A. raptoria* as part of its host range.

8. Pesticides

In many cases, the use of pesticides cannot be avoided. This is necessary for the satisfactory management of most primary pest species. Many factors have to be taken into account when considering the application of pesticides. Ideally, the pesticides selected should be target specific and exhibit a low impact on natural enemies and other non-target species. Systemic pesticides are highly recommended in this regard. These pesticides are absorbed into the plant tissue with the result that species not feeding on the crop are not affected. During the present study, all the pesticides selected exposed satisfactory systemic activity.

In new crop development there are usually no pesticides locally registered for use on the crop. This was the case during the present study, where no specific pesticides were registered for use on *A. raptoria* on pistachio. However, this provides a myriad of choices when selecting pesticides to be used. Besides pesticides preferably having a low impact on natural enemies of the target species, as well as non-target species, the efficacy of the pesticides in managing pest populations is the main factor to consider. For this reason, efficacy studies or bioassays should be conducted on all selected pesticides. These should include contact efficacy, secondary contact efficacy, as well as residual efficacy. There exist a great deal of bio-assay techniques and methodologies. The method used should thus be catered to the specific

requirements of the situation and the relevant conditions on location. In the case of the present study, bioassays were conducted diurnally, as well as nocturnally, simulating natural pesticide application conditions on site at GVN. Also, residual and contact efficacy studies were conducted on all selected pesticides. All these actions contributed towards the selection of a satisfactory pesticide to be used in the management of *A. raptoria*. Based on this approach, Sevin® and Endosulfan® were selected for use in the present study. These two pesticides were selected from a total of seven different pesticides that were tested and will prohibit the use of pesticides that are ineffective.

9. Degree-day models as a management tool

Degree-day models have been used to great effect in the timing of management strategies. If correctly utilized, this tool will assist in the timing of pesticide spraying programs, thereby maximizing efficacy, minimizing excessive, wasteful application and lowering the number of necessary applications. Economically, this is of great significance. Efficacy can be maximized by timing pesticide application to the stages in the life-cycle of the pest when the highest mortality rates will be achieved. During the present study, pesticides were applied after initial biofix, which was the first sampling of egg-packages, at the time when approximately all eggs were hatched, exposing the young, vulnerable nymphs. The immature stages of *A. raptoria* are incapable of flight, maximizing the possibility that all insects will come into contact with the pesticides applied.

Degree-days models are highly reliant on good scouting data, reiterating the point made earlier on the importance of well established orchard monitoring practices being in place. Without reliable scouting data, accurate biofix dates, which sets the degree-day model in motion, will not be possible.

There are many methods in calculating the accumulation of degree-days, and the method chosen should be catered to the needs of the program in mind. Degree-days calculation can be a very exact science, requiring complex

mathematical formulae and computers to calculate. However, simpler methods for the calculation of degree-days do exist, and still provide a very good guideline for the timing of management practices. Basically, the accuracy needed will be determined by the relevant situation, type of pest and type of host-plant. During the present study, a simple rectangle method of calculation was used, delivering very good results.

10. Results of degree-day driven pest management

With the conclusion of this study, it has been comprehensively shown that excellent results can be achieved by the implementation of several management strategies towards the control of *A. raptoria.* It has also been shown that, these strategies do, however, require a fair amount of research to be implemented correctly. The negative impact of *A. raptoria* on pistachio at GVN has virtually been eradicated and recent damage indices attributed to this pest is insignificant. Continued monitoring practices are well in place and degree-day driven management is currently a fixed practice at GVN.

11. Future possibilities

Even though *A. raptoria* is presently well managed at GVN, continued scouting will still be done to monitor the possibly of re-emergence due to the development of resistance across different scales. Should this occur, several strategies can followed. As mentioned earlier, the mass-rearing and release of *T. basalis* can be reconsidered, should this option be economically viable. This should assist in the management of *A. raptoria* should the present management program become less effective. Also, new pesticides are registered on a frequent basis. Continued efficacy studies can be done on viable pesticides, possibly indicating more effective pesticides for use in the management program.

12. Compatibility to other pests on crops

The degree-day driven management strategy described in the present study should be compatible to nearly all similar situations, regardless of pest species or crop host. The basic principles described should apply in most other cases as well. Especially on perennial crops, where continued monitoring and the compilation of complete data sets are possible, good results should be obtained. Also, on crops newly introduced to an area, this study will be of great relevance. However, the present study was targeted towards a conventional pest, feeding on the exterior of the tree host. This basically means the pest is exposed, rendering management more simplified. More un-conventional pests, such as borer and soil-living species, will be more difficult to control using the present management strategy. In most other cases, however, the findings of the present study should aid in the development of a sustainable management program towards the control of a pest species.

I, De Villiers Fourie, hereby declares that the dissertation completed and submitted for the degree Magister Scientiae at the University of the Free State, consists of my own work and has never before been submitted for the purposes of another degree at any other university or faculty. Furthermore, I hereby distance myself of author rights on the dissertation in favor of the University of the Free State.

Ek, De Villiers Fourie, verklaar dat die verhandeling wat hierby vir die graad Magister Scientiae aan die Universitieit van die Vrystaat deur my ingedien word, my selfstandige werk is en nie voorheen deur my vir ’n graad aan ’n ander universiteit of fakulteit ingedien is nie. Voorts doen ek afstand van die outeursreg in die verhandeling ten gunste van die Universiteit van die Vrystaat.