Diversity and ecology of phytophagous weevils in the deciduous fruit industry, South Africa.

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: [December 2018]

Summary

The banded fruit weevil, *Phlyctinus callosus*, Boheman, 1834 (Coleoptera: Curculionidae), is a serious and economically significant pest of apple orchards and vineyards in the Western Cape Province of South Africa. Adults can be accidentally packed with export fruit causing quarantine problems and is regarded as an international phytosanitary pest.

A nine month population survey (during the fruiting season of September 2017 to May 2018) was conducted in three different fruit growing areas, namely; Stellenbosch, Ceres and Grabouw, Western Cape Province, South Africa, to determine the assemblage structure and an identification key was compiled for all weevils found in vineyards and apple orchards during this survey. Fortnightly monitoring using 15 cm cardboard bands (tied around the base of the trunk), has indicated that there were nine weevil species found to occur in apple orchards and vineyards, namely, *Eremnus atratus* (Sparrmann, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Eremnus occatus* Boheman, 1843, *Eremnus setifer* Boheman, 1843, *Naupactus leucoloma* Boheman, 1840, *Pantomorus cervinus* (Boheman, 1840), *Phlyctinus callosus* Boheman, 1834, *Sciobius tottus* (Sparrmann, 1785) and *Tanyrhynchus carinatus* Boheman, 1836. Vineyards recorded the highest diversity with eight species recorded: *T. carinatus*, *P. callosus*, *E. setifer*, *E. atratus*, *E. chevrolati*, *E. occatus, P. cervinus and N. leucoloma,* whereas apple orchards recorded the lowest diversity with only four weevil species found: *S. tottus*, *P. cervinus, E. atratus* and *P. callosus. E. occatus* was collected for the first time in vineyards. All nine species belong to the sub-family Entiminae (root weevils*). T. carinatus* was found in low abundance and it is the only weevil species with a long rostrum and easier to distinguish from the rest of the weevils collected during the study. The rest of the weevils differ only slightly in morphology, especially the *Eremnus*. Based on collected species during the survey, an illustrated morphological key was produced with species identification information. DNA barcoding was supplied to provide a complementary diagnosis tool.

Seasonal monitoring was undertaken to establish population peaks during the fruiting season, in relation to *P. callosus,* which was regarded as a key pest in apple orchards and vineyards. Furthermore, damage assessments were conducted by assessing fruit clusters or bunches and recording the percentage damage per block, first in early December (pre-thinning assessment) and later in early April (pre-harvest assessments). Weevils emerged in mid-October in vineyards and around late-October in apple orchards. A peak in adult populations were reached between November and December, after which the population dropped drastically from January up to May. *P. callosus* was the most abundant weevil species and it accounted for 82 % of all the weevils collected during the survey. Other weevils were found in lower abundance: *E. occatus* (5.9 %), *E. setifer* (5.8 *%), E. chevrolati* (2.1 %), *P. cervinus* (1.9 %), *N. leucoloma* (1.1 %), *T. carinatus* (0.4 %), *S. tottus* (0.3 %), and *E .atratus* (0.3 %). Most damage took place during the pre-thinning assessment and to a lesser extent in the pre-harvest assessment. Most of the damage in apple orchards and vineyards was attributed to *P. callosus*, based on Spearman's rank correlations, with the remaining weevils contributing to some damage.

As it is possible that certain soil physical parameters such as soil texture, soil chemistry and soil bulk density along with the presence of ground cover impact the population of weevils, soil

samples were collected in early September, and analyzed for soil chemistry and soil texture. Groundcover surveys and identification took place every two weeks. The results indicated that there was no significant (P = 0.05) relationship between soil chemistry, soil bulk density, soil texture or ground cover percentage and weevil population. The association between adult *P. callosus* and groundcover was very weak. It is possible that other factors such as soil moisture, cultural practice within the ground cover, relative humidity and soil penetrability impact the weevil population in apple orchards and vineyards.

This study identified and recognized nine weevil species causing damage in vineyards and apple orchards in the Western Cape Province, South Africa, of which one was recorded for the first time in vineyards, as well as assessing the effect of abiotic and biotic attributes towards the population of weevils on these crops. The findings of this study aimed towards supporting the development of ecologically-based control strategies; which enable further research into sustainable and integrated approaches to the management of these weevils.

This thesis is dedicated to

My late mother, Emma Tobhi Nkosi, who taught me to forever soldier on in life but could not see this work finished. This work is also dedicated to my son, Azania Mxolisi Junior.

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Preface

The thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the journal of *African Entomology*.

Chapter 1 **General introduction and project aim**

Chapter 2 **Research results**
Diversity and morphological review of common weevils (Coleoptera: Curculionidae) in vineyards and apple orchards in the Western Cape Province, South Africa.

Chapter 3 **Research results**
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General introduction and project aim

Economic significance of weevils in orchards and grapevines

Apples and table grapes are two of South Africa's commercially produced crops. The South African apple industry is valued at around R4.8 billion annually with a total of nearly 22 923 hectares in production, 16, 250 of which are in the Western Cape Province (DAFF, 2015). The South African table grape industry is valued at around R4 billion annually with a total of nearly 18 212 hectares in production, 11 629 of which are in the Western Cape Province (DAFF, 2016). There are several insect pests threatening both apple and table grape production in South Africa (Pringle, *et al*, 2015). *Phlyctinus callosus* Boheman, 1834 (Coleoptera: Curculionidae), the banded fruit weevil (BFW) (previously known as vine snout beetle), is native to South Africa, where it was first recorded as a pest before the turn of the century (Barnes & Pringle 1989). *P. callosus* is the only curculionid (weevil), regarded as a serious pests in orchards and vineyards (Marais 2003; Marais & Barnes 2004). Since its first recognition as a pest in South Africa in 1896 (Lounsbury, 1896), the weevil has spread to New Zealand and Australia, where it is known as the garden weevil (Kuschel 1972; Fisher 2004). *P. callosus* (Fig. 1.1) has spread from its original find in South Africa to New Zealand, then settled in Tasmania, then dispersed to various Australian states. Although it has been detected in the USA, it has failed to establish itself in the Northern Hemisphere (Myburgh & Kriegler, 1967). A recent discovery has also found that BFW is a major pest of blueberries (Brendhand *et al.* 2010). *P. callosus* is a serious pest of apples, nectarines and grapevines in South Africa and Australia, while in New Zealand it is a key pest of vegetables (Barnes & Pringle 1989; Butcher 1984; Fisher 2004; Walker, 1981). Adult *P. callosus* also attack a variety of ornamental plants in home gardens (Barnes & Pringle, 1989). In South Africa, Western Cape, damage to fruit occurs from December to February (Marais & Barnes, 2003; Fisher & Learmonth, 2004). In Australia the larvae (Fig. 1.2) of *P. callosus* feed on the roots of immature grapevine, resulting in stunted growth and it may appear water-stressed (www.agric.wa.gov.au, 2018) (Fig. 1.3). In Australia both the larvae and adult attack nectarines, olives, parsnips, lucerne, potatoes, apples, plums, walnuts, asparagus, carrots, strawberries and various ornamentals (Pringle, *et al*, 2015). Adult *P. callosus* feed on the skin of the fruit and the inner part of the fruit and in some cultivars they debark the fruit stalk resulting in premature fruit dropping (Barnes and Swart, 1977) (Fig. 1.4). The typical symptoms of the damage caused by adult *P. callosus* on the fruits and leaves, includes fruit scaring, leaf and stem notching, blossom and shoot-tip feeding and shot holing of the leaves (Pringle, *et al*, 2015). Fruit damage resulting from scaring is of economic significance to commercial fruit, demanding weevil management (Pringle *et al.* 2015; Marais & Barnes 2004). Adult feeding results in unmarketable fruits, dropping of fruits and quarantine issue with export fruit (Marais & Barnes 2004). *P. callosus* is regarded as an international phytosatinary pest (Pringle *et al*. 2015; Barnes & Marais 2004).

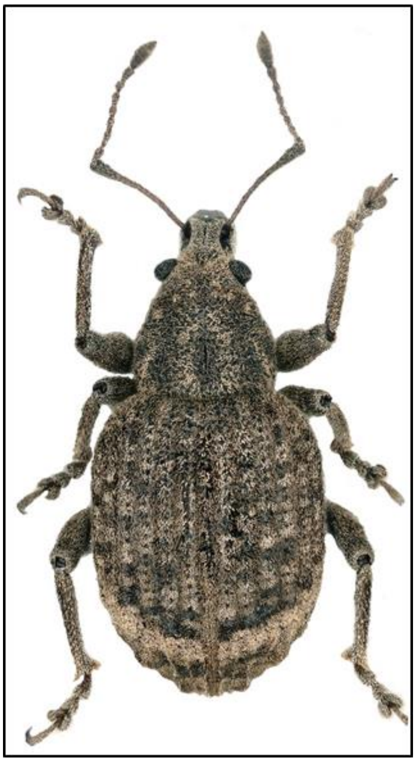

Figure 1.1 *Phlyctinus callosus* adult. Photo by Julien Haran.

Figure 1.2 *Phlyctinus callosus* larva.

Figure 1.3 *Phlyctinus callosus* larval damage to roots. (Source: www.agric.wa.gov.au)

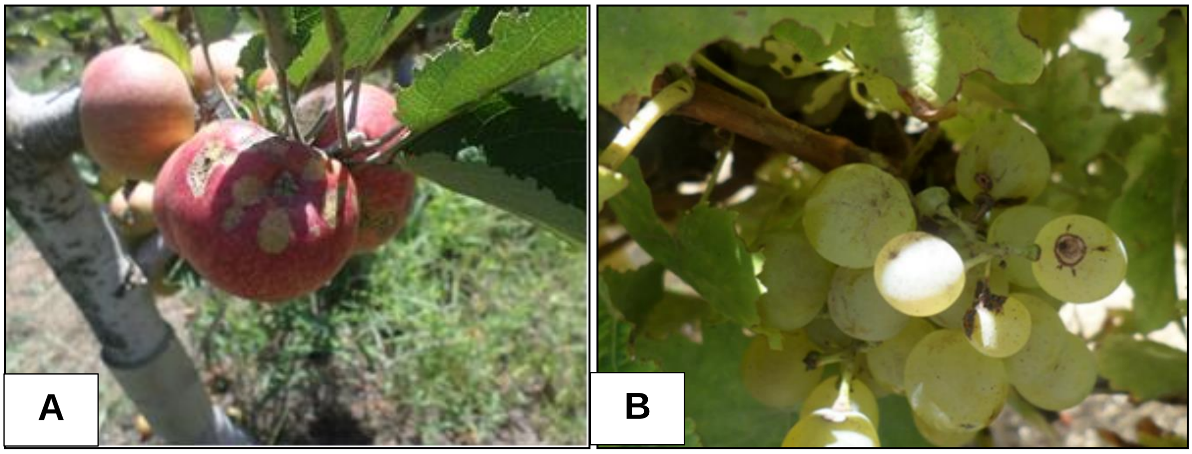

Figure 1.4 Adult *Phylictinus callosus* feeding damage to both apple (A) and grape bunch (B).

Curculionoidea

This superfamily is generally known to represent the most highly evolved of all coleopterans (Scholtz & Holm 1985). Although some species within the superfamily are regarded as specialist feeders, most of its members are polyphagous (Scholtz & Holm 1985). Some species have parasitic or predacious feeding habits, are gall forming, seed eating or even coprophagous (Scholtz & Holm 1985). The adults of most curculinoids are recognized in the adult stage by the following characteristics: the head is nearly always produced into a rostrum which can reach great lengths (excepts in groups such as Platypodinae and Scolytinae) and where the mouth part are located which are used to construct holes for oviposition and feed on plant tissue sometimes resulting in deep cavities (Scholtz & Holm 1985). Antennae are borne on the rostrum and are strongly geniculate with a short terminal club (Scholtz & Holm 1985). The prothorax is rounded and lateral margins absent (Scholtz & Holm 1985). Five visible abdominal sternites occur, some of which are fused; and the body integument is hard and sclerotized often covered with scales and bristles (Scholtz & Holm 1985). This super-family comprise the largest portion of all species of the order Coleoptera and constitutes, as presently understood, the largest family animals, the Curculionidae (Zarazaga & Christopher 1999).

Curculionidae

The order Coleoptera constitutes the largest family of insects in the world (Scholtz & Holm 1985). Within this order, the family Curculionidae (weevils, snout beetles) contains the largest number of species in the animal kingdom, with a total 60 000 species occurring worldwide (Oberprieler *et al.* 2007), 2500 of which occur in Southern Africa (http://www.biodiversityexplorer.org). Curculionidae contains the largest family of animals (Scholtz & Holm 1985). Weevils display enormous diversity in appearance and habits (Scholtz & Holm 1985). Approximately 25 percent of the weevils found in sub-Saharan Africa occur in Southern Africa (Scholtz & Holm 1985). Most weevils are polyphagous with both the adult and larvae feeding all possible parts of the plants; however a few species are saprophagous (Diaz 2005). Flowering plants (Angiospermae) have been exploited by weevils,with not a single member being free from attack of one or other species of weevil species (Scholtz & Holm 1985). Most weevils also cause severe economic damage to plants and crops cultivated by man (Scholtz & Holm 1985). Most weevils are nocturnal feeders, while others are known to be diurnal (Scholtz & Holm 1985). They often fly or move to the other side of the twig when endangered (Metcalf and Metcalf 1993). However, when disturbed most weevils often remain motionless or feign death (O' Brian & Kovarik 2000). Most weevil species stridulate by scratching an elytral file against their abdominal segments (Scholtz & Holm 1985). Most weevils are solitary (Anderson 2002). However, some weevils like *Brachycerus,* congregate during winter under shelter on the ground (Scholtz & Holm 1985).

Weevils are characterized by their elongate rostrum and geniculate antennae (Diaz 2005). The rostrum differs depending on species; shorter on some, while longer and narrow on others (Diaz 2005). In some species the eyes can be well developed, but normally they are very small, or even rudimentary or absent in soil dwelling weevils (Scholtz & Holm 1985). Body shape and size are widely variable. The mouthparts are located at the anterior of the rostrum and these characteristics separate this family into subfamilies (Diaz 2005). Some weevils are fully winged and fly well, others possess fused elytra and no wings (Scholtz & Holm 1985). Weevils are most commonly about 5 mm long in size, but others range from 1 to 60 mm (Scholtz & Holm 1985). Most weevils are cryptically colored and aposematic; metallic colours are rare (Scholtz & Holm 1985). The weevil larvae have a semi-circular shape and are legless (Metcalf and Metcalf 1993). White larvae feeds on plant roots, fruits, nuts, stems and buds (Metcalf and Metcalf 1993). The feeding habit of larvae and adults differ and this variation is useful for classification of weevils into subfamilies (Anderson 2002). The successful classification of weevils into subfamilies and tribes is one of the major constraints in the higher classification of Coleoptera (Scholtz & Holm 1985). Traditionally, weevils are divided into the long nosed Phanerognata where the mouthparts are visible from below and Adelognatha short-nosed weevils where the mouthparts are hidden from below (Scholtz & Holm 1985). However, it is not easy to distinguish between the Phanerognata and Adelognatha (Scholtz & Holm 1985).

Entiminae (Root weevils)

The sub-family Entiminae derived its name from the polyphagous feeding behavior of its adults and larvae (Anderson 2002). They are commonly referred to as broad nosed weevils and according to O' Brian and Kovarik (2000), there are *ca*. 14 000 species described worldwide (Marvaldi 2005). These weevils were previously known as Adelognatha, and consist of approximately 1150 genera and 12 220 species, dispersed in all biogeographical regions of the globe (Morrone 1999). Most authors treated Entiminae as a distinct subfamily (Thompson 1192, May 1994, Zimmerman 1994, Morris 1995, Morrone 1996, 1997, 1998, Marvaldi 1997, 1998), although Kuschel (1995) demoted Entiminae to a tribe of Brachycerinae (Morrone 1999). A large number of entiminae share adelognathous mouthparts and deciduous mandibular processes (Thompson 1992), but these features have proven to be homoplastic as they are also present in other weevil taxa (Marvaldi 1997). They constitute the majority of agriculturally important species in South Africa (Scholtz & Holm 1985). According to the latest detachable analysis based on the features of larvae (Marvaldi 1997), they are classified into five tribes, Alophini, Sitonini, Entimini, Pachyrhynchini and Ectemnorhinini (Morrone, 1999). One of the distinct character for almost all broad nosed weevils is Entiminae is that adults bear a pair of mandibles which are shed immediately after emergence from the soil, retaining a scar at the point of attachment (Anderson 2002; Marvaldi & Lanteri 2005; Gosik & Sprick 2013). Entiminae are mainly apterous beetles, often covered with brilliant scales (Scholtz & Holm 1985). The larvae and adults are generalist feeders, adults feed on foliage and larvae feeds on roots (Diaz 2005). Some weevils within the subfamily Entiminae are parthenogenetic, with no record of any males (Gosik & Spring 2013; Diaz 2005). Little information is available on the diversity of this sub-family in Southern Africa (Scholtz & Holm 1985). Entiminae are almost similar to one another in their habits and life cycle (Anderson 2002). Depending on the species, adults deposit their eggs on foliage of host plants or the soil surface (Gosik & Spring 2013; Diaz 2005). The eggs hatch, then burrow into the soil, where the larvae begin feeding on plant roots (Anderson 2002). Most larvae commence feeding on small fibrous roots and as they mature they feed on much larger roots (Anderson 2002). The pupae develop within an earthen chamber within the soil (Anderson 2002). Adults feeds on younger leaf flushes, leaving semi-secular notches (Diaz 2005). As soon as adults emerge from the soil they begin feeding, mating and laying eggs (Marvaldi & Lanteri 2005).

Genera orchards and vineyards

There are five commonly known genera of the subfamily Entiminae that are reported to occur in apple orchards and vineyards in Western Cape Province South Africa, namely; *Eremnus*, *Naupactus* (only recorded in vineyards), *Pantomorus, Tanyrhynchus*, *Phlyctinus* and *Sciobius* (only recorded in apple orchards) (Marais 2003; Marais & Barnes 2004; Pringle *et al.* 2015; Allsopp *et al.* 2015).

Tanyrhynchus Schönherr, 1826

The type genus of the group, *Tanyrhynchus* not only have a large prementum that covers the maxillae (the adelognathaus condition), but their mandibles also display deciduous cusps or at least cusps where such scars had been attached (Oberprieler 1995). Furthermore, the rostrum is only strongly elongated and correspondingly modified to resemble the phanerognathan rostrum in certain species, whereas in other species it is slightly longer and otherwise no different from that of typical otiorhynchine Adelognatha (Oberprieler 1995). Although it possess an elongate rostrum, it still retains the typically otiorhynchine features of the dorsally inserted antennae and free, long scape that surpass the front eye in repose and is never confined in its entirety to a narrow scrobe, a feature that Kraatz (1864) previously regarded as much of a greater value in defining the sub-family Entiminae than the covered maxillae (Oberprieler 1995). Furthermore, *Tanyrhynchus* is a truly adelognathous weevil irrespective of the importance of any of these characters (Oberprieler 1995). *Tanyrhynchus* is closely related to the *Eremnus* genus (Oberprieler 1995). Presently, there are 50 species of the genus *Tanyrhynchus* and they are only found in the Southwestern Cape Province of South Africa (Oberprieler 1995). Some species are associated with Fabaceae, but a large number are associated with Proteaceae. They feed on leaves and do not occur in flower heads, but the extremely elongate rostrum of many species suggest more specialized feeding habits in such cases (Oberprieler 1995). There is limited information on the larval biology of *Tanyrhynchus* (Oberprieler 1995).

Eremnus Schönherr, 1823

This is an agriculturally important genus and is very large, with about 120 or more species ranging from the southern tip of Africa along higher regions of the eastern side of the continent to plateaus in East Africa (Oberprieler 1995). *Eremnus* must be regarded as a paraphyletic group, since the species included in this genus are currently united by symplesiomorphies (Oberprieler 1995).

Naupactini (*Pantomorus* Schönherr, 1840 and *Naupactus* Deejan 1821).

The genera *Naupactus* and *Pantomorus* fall under the Naupactini tribe, and are naturally distributed in the Neotropical region, having their largest diversity in subtropical and tropical areas of South America (Lanteri & O'Brien 1990; Lanteri & Marrone 1995). *Naupactus* is usually associated with trees and shrubs, where the adults feed on leaves and other green parts of the plant, whereas *Pantomorus* are widely distributed in prairies and steppes, feeding on grasses and other plant groups (Lanteri *et al.* 2002a, b). This tribe is native to South America and have been introduced to other continents, becoming serious pests of agriculture. Some of the latter of this tribe are apomictic parthenogenetic (Lanteri & Normark 1995; Hardwick *et al.* 1997; Normark & Lanteri 1998; Mander *et al.* 2003). The majority of the species classified in *Naupactus* possess metathoracic wings and well-developed elytral humeri, whereas in *Pantomorus* the humeri are absent or reduced, with vestigial membranous wings (Scataglini 2005). Scataglini (2005), in regards to the *Pantomorus-Naupactus* complex (*P-N* complex), specified that "until species can be critically studied the humeri and wings features must be used for dividing these two vaguely defined genera. The taxonomic status of these genera have been transformed through time (Scataglini 2005). They are treated as an independent taxa by other authors and others as synonyms of *Naupactus* or *Pantomorus* (Wibmer & O'Brian 1990; Lanteri & Marvaldi 1995). A large number of nomenclatural changes that have taken place within the *Pantomorus-Naupactus* complex show the challenges in their delimitation and in the exact placement of their species (Scataglini 2005).

Sciobius Schönherr, 1823

There is very limited information on the origin and distribution of the *Sciobius*.

Phlyctinus Schönherr, 1823

There is very limited information on the origin and distribution of the *Phlyctinus*.

Weevils in vineyards and apple orchards distribution, hosts plants and population parameters

A number of native Entiminae species been previously recorded to occur in apple orchards, namely: *P. callosus, S. tottus* (Sparrmann, 1785), *P. cervinus* (Boheman, 1840) and *E. atratus* (Sparrmann, 1785) (Marais & Barnes 2004). In vineyards, several weevil species have been previously recorded to cause serious to minor injuries on grape berries, namely: *Tanyrhynchus carinatus* Boheman, 1836, *P. callosus*, *Eremnus setifer* Boheman, 1843, *Eremnus atratus*

(Sparrmann, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Pantomorus cervinus* (Boheman, 1840), *Eremnus setulosus* Boheman, 1834, *Eremnus cerealis*, Marshall, 1921 and *Naupactus leucoloma* Boheman, 1840 (Marais 2004). Of these species, *P. callosus* is regarded as the primary weevil causing damage in apple orchards and vineyards in the Western Cape (Marais 2003; Marais & Barnes 2004). The distribution, host plants and population parameters of all this species is shown in Table 1.

Table 1. Comparative host plant list and population parameters of weevil (Curculionidae) recorded in apple orchards and vineyards, in Western Cape Province South Africa.

Species	Distribution	Host plants	Population parameters				References
			Oviposition sites	Nr. instars (larval stage)	Duration of pupal stage	Nr. generations	
Eremnus atratus	South Africa	Suspected to be similar to *P. callosus*.	Soil, weeds, leaf litter or host fruit tree	5 to 8	1 – 3 weeks	1 or 2	Bloomfield *et al.* 2015; Allsopp *et al.* 2015; Annecke & Moran, 1982.
Eremnus cerealis	South Africa	Suspected to be similar to *P. callosus*.	Soil, weeds, leaf litter or host fruit tree	5 to 8	1 – 3 weeks	1 or 2	Van Den Berg 1970; Allsopp *et al.* 2015; Annecke & Moran, 1982.
Eremnus chevrolati	South Africa	Suspected to be similar to *P. callosus*.	Soil, weeds, leaf litter or host fruit tree	5 to 8	1 – 3 weeks	1 or 2	Allsopp *et al.* 2015; Annecke & Moran, 1982.
Eremnus setulosus	South Africa	Suspected to be similar to *P. callosus*.	Soil, weeds, leaf litter or host fruit tree	5 to 8	1 – 3 weeks	1 or 2	Allsopp *et al.* 2015; Annecke & Moran, 1982.
Eremnus setifer	South Africa	Suspected to be similar to *P. callosus*.	Soil, weeds, leaf litter or host fruit tree	5 to 8 instars	1 to 3 weeks	1	Giliomee 1961.
Naupactus leucoloma	Chile, Uruguay, Argentina, Peru, Southern United States,	Plants with large broad leaves, Lucerne, clover, potato, tomato, bean, pea and pomegranate.	Soil surface or host plants	7 to 11 instars	4 months	1	Young *et al.* 1950; Prinsloo & Uys, 2015; Johnson & Tappan, 1988.

Table 1. Continued. Comparative host plant list and population parameters of weevil (Curculionidae) recorded in apple orchards and vineyards, in Western Cape Province South Africa.

	Australia and South Africa.						
Pantomorus cervinus	South Africa, New Zealand, USA, Japan and many more.	Broad leaved plants (orchard trees, ornamentals, annual plants and weeds).	Host plants or soil surface in a sticky substance	3	1 -2 months	1	Chadwick 1965; Morse *et al*. 1988; Madge *et al*. 1992; Abbot 2001; Masaki *et al*. 1996; Morse *et al*. 1987. Wee *et al*. 2008; Smith *et al*. 1997; Suck-ling *et al*. 1996.
Phlyctinus callosus	South Africa, Australia and New Zealand	Mono- and dicotyledonous weeds, grasses, herbs and woody plants.	Substrate, soil surface, hollow stacks (dead or alive) or leaf sheaths of host plants	5 to 8, but up to 11 can occur.	1 to 3 weeks.	1 or 2	Barnes 1989; Barnes 1987; Pringle *et al*. 2015; Annecke & Moran 1982; Giliomee 1961.
Sciobius tottus	South Africa, St. Helena.	Apple, pear, plum, maritime pine, *Pinus pinaster* (Pinaceae); Cape beech, *Mysine melanophloes* (Myrsinaceae); Silver birch, *Betula pendula* (Betulaceae), & sage bush, *Buddleja salviifolia* (Scrophulariaceae).	Curled dead leaves, soil surface, inside calyx lobes of fruits, leaves or bark crevices.	5 to 8	1 ½ to 2 months	1 or 2	Pringle *et al*. 2015; Marais & Barnes 2004.
Tanyrhychus carinatus	South Africa	Suspected to be similar to *P. callosus*.	Suspected to be similar to *P. callosus*.	5 to 8	1 to 3 weeks	1 or 2	Allsopp *et al*. 2015; Annecke & Moran, 1982.

Taxonomy and nomenclature of common weevils found in vineyards and apple orchards

Phlyctinus callosus Boheman, 1834

P. callosus was originally described by Schönherr in 1826 in his description of the subgenus *Peritelus* (*Phlyctinus*), although the authority for the description of *P. callosus* commonly appears in the literature as Boheman 1834 (CABI 2018). Since Schönherr was the first author to describe *P. callosus* he remains as the valid author with his name appearing in parentheses because the species was originally described within *Peritelus* (Barnes 1989). When *P. callosus* was first recorded in New Zealand in 1893, Broun (1893) regarded it as a new species and described it, naming it, *Rhyncogonus germanus*, which remains a junior synonym (CABI 2018). The weevil appears as "*Philyctinus" callosus*; this is a misprint of the correct generic name (CABI 2018). In South Africa, *P. callosus* is commonly known as banded fruit weevil (Barnes, 1989), in Australia it is commonly known as garden weevil (Learmonth 2018) and in New Zealand the accepted common name is vine calandra (CABI 2018).

Pantomorus cervinus (Boheman, 1840)

P. cervinus has a complicated taxonomic history with many junior synonyms including, *Pantomorus cervinus* (Boheman, 1840), Kuschel 1949, *Asynonychus cervinus* (Boheman), Hustache 1947, *Pantomorus olindae* Perkins 1900, *Naupactus simplex* Pascoe 1881, *Aramigus fulleri* Horn 1876, *Asynonychus godmanni* Crotch 1867, *Pantomorus cervinus* Boheman 1840 and *Naupactus cervinus* Boheman 1840 (Logan *et al*. 2008). The species *Asynonychus godmani, Asynonychus fulleri*, *Pantomorus olindae* were synonymized with *Asynonychus cervinus* by Hustache in 1947 and 1955, and *Naupactus simplex* was added to the list by Kuschel (Chadwick 1965). The accepted common names includes Fuller's rose weevil in South Africa (Pringle *et al*. 2015), and in Australia and Zealand, it is known as Fuller's rose weevil (Penman 1984; Carne 1987) and in the USA it is known as Fuller's rose beetle (Bosik 1997).

Effect of abiotic and biotic factors on weevil populations

Soil and ground cover

Soil plays a pivotal part in the study of many biological processes (Flint 2012). Its significance has been recognized also by the agronomists, chemists and botanists Hayes & Mccolloch (1922). However, the zoologist and in particular the entomologist, have paid little attention to many fundamental animal activities (Hayes & Mccolloch 1922). The soil is home to a large number of insects during their entire or part of their life cycle; it offers protection and shelter to so many insects; it is a medium for plant growth and a major factor in the food supply of insects (Hayes & Mccolloch 1922). The relative abundance, distribution and seasonal activities of insects can be related to different soil conditions (Flint 2012). The important factors of soil are texture, structure, evaporation, moisture, natural enemies, organic matter and carbon dioxide (Hayes & Mccolloch 1922). Furthermore, organic matter, soil texture and exchange capacity are also regarded amongst the significant attributes for the soil-dwelling insects on cultivated crops (Flint 2012). Out of these three, texture is the most important attribute for soil dwelling insects, especially on agriculturally grown crops (Flint 2012). In most cases, insects use soil for protection, material for shelter, and for some insects that live above the ground, utilize the soil to evade their natural enemies or undergo some developmental growth stages (Hayes & Mccolloch 1922). One of these insects groups is the curculionids, where the adults deposit their eggs in the soil, while larvae and pupae develop within the soil and the adult spend most of their time above ground feeding and sheltering on certain ground covers (Anderson 2002). Diverse groundcover in orchards and vineyards maintain diverse natural enemy assemblages that are significant in regulating pest populations (Flint 2012). Barnes & Pringle (1989), in their study of *P. callosus* in apple orchards, concluded that relative humidity in the cover crop microclimate, soil moisture and cultural practices are considered to be the significant attributes that influence on the number of generations per year. The same author further stated that cover crop and the irrigation system and dry summers are important attributes facilitating the number of adult *P. callosus* in fruit orchards. The larvae easily penetrate soil with adequate moisture gradient (Barnes & Pringle 1989).

Aim and objectives

The aim of this study was to improve weevil management through a better understanding of the effects of both soil texture and cover crop variability, as well as prioritizing the species that should be targeted for management purposes. It is proposed that an analysis be undertaken to determine which abiotic and biotic factors play a role in causing damaging population levels in commercial orchards/vineyards. The objectives of this study were, therefore, to:

1. establish the relative abundance of economically important weevil species and compile user-friendly keys for the identification of the species involved,
2. determine the influence of orchard floor management practices (cover crops), and
3. determine the influence of various soil parameters on weevil distribution.

Each of these objectives are addressed in separate chapters, compiled as individual publications, therefore some repetition may occur.

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Chapter 2: Diversity and morphological review of common weevils (Coleoptera: Curculionidae) in vineyards and apple orchards in the Western Cape Province, South Africa.

Abstract

Agriculturally important components of the Western Cape economy, fruit and wine are important for export markets. The vast majority of the weevils within the subfamily Entiminae (Curculionidae) are pests of cultivated apples and grapevines due to their feeding behaviour, being mainly polyphagous. Weevils are characterized by having elongated mouthparts form a rostrum (snout). Among them, *Phlyctinus callosus* Boheman, 1834 is the most common weevil in both apple orchards and vineyards. However, is it not known whether this weevil is the only fruit-damaging Entiminae on apples and vineyards. Furthermore, the weevil diversity is not well known in deciduous fruit and weevil species are often not easy to distinguish from each other. Surveys were conducted over nine months (September 2017 to May 2018) in three major fruit growing areas, all located in the Western Cape Province, South Africa. Nine weevil species of the sub-family Entiminae were found to occur in apple orchards and grapevines, namely; *Tanyrhynchus carinatus* Boheman, 1836, *Phlyctinus callosus* Boheman, 1834, *Eremnus setifer* Boheman, 1843, *Eremnus atratus* (Sparrmann, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Eremnus occatus* Boheman,1843, *Sciobius tottus* (Sparrmann, 1785), *Pantomorus cervinus* (Boheman, 1840) and *Naupactus leucoloma* Boheman, 1840. *Tanyrhynchus carinatus* is the only weevil species with a long snout within the subfamily Entiminae and easier to distinguish from the rest of the weevils collected during the study, but this species not very abundant. The rest of the weevils differ in morphology by relatively minor differences, especially within the genus *Eremnus*. Based on the examination of the species obtained, an illustrated morphological key was produced. DNA barcoding was applied to provide a complementary means of identification.

Introduction

The order Coleoptera constitutes the largest family of insects in the world (Scholtz & Holm 1985). Within this order, the family Curculionidae (weevils, snout beetles) contains the largest number of species in the animal kingdom, with a total 60 000 species occurring worldwide (Oberprieler *et al*. 2007), 2500 of which occur in Southern Africa (http://www.biodiversityexplorer.org). Southern Africa harbors approximately 150 subfamilies, but many of them are of uncertain status and difficult to distinguish from others (Scholtz & Holm 1985). One of the most species rich subfamilies within the Curculionidae is the Entiminae (Marvaldi 1997). The subfamily Entiminae are all polyphagous in the larval and adult stage (Anderson 2002). They are commonly known as broad-nosed weevils (O'Brian & Kovarik 2000) and there are *ca*. 14 000 species described worldwide (Marvaldi 1997).

Morphologically, their primary distinguishing feature is their short snout compared to the other weevils, with a few exceptions having a long rostrum (Scholtz & Holm 1985). One distinguishable characteristic of almost all the weevils within the subfamily Entiminae is that adults bear a pair of mandibles which are shed immediately after emergence from the soil, retaining a scar at the point of attachment (Anderson 2002; Marvaldi & Lanteri 2005; Gosik & Sprick 2013). Entiminae have variety in body size, ability to utilize plants and flight ability (Gosik & Sprick 2013). Most Entiminae are adapted to both hot and dry grasslands and steppe biotopes, different forest habitats, coastal cliffs and mountainous habitats (Gosik & Sprick 2013). A limited number of species can be found in anthropogenic habitats such as cultivated plants, gardens and nurseries and some are recognized as economic pests of cultivated apple orchards and vineyards in South Africa (Scholtz & Holm 1985).

Broad nose weevils are relatively similar in their feeding habits and biology (Diaz 2005). Depending on species, adults deposit their eggs on the soil surface, organic litter and various host plants (Scholtz & Holm 1985; Diaz 2005). After hatching the larvae drop to the soil surface, burrow into the soil and begin feeding on fibrous plant roots, causing damage as they mature (Diaz 2005). Newly emerged adults feed on immature leaf flushes, and resulting in semi-circular notches on leaf margins (Scholtz & Holm 1985). Some broad nosed weevils reproduce parthenogenetically, with no known males (Scholtz & Holm 1985). A number of species within this subfamily are flightless (Anderson 2002; Emenegger & Berry 1978), with a few having wings (Diaz 2005).

The biology of most Entiminae species is not known in Southern Africa (Scholtz & Holm 1985). Many of the species that have been studied are polyphagous, with only a limited number of oligophagous species (but more frequent in Sitonini) and monophagy being an exception (Gosik & Spick 2013). *Phlyctinus callosus* Boheman, 1834 is the most common Entiminae weevil species damaging fruits in apple orchards and vineyards (Marais 2003; Marais & Barnes 2004) in South Africa; it has been accidentally introduced into Australia & New Zealand and is a quarantine threat (Pringle *et al.* 2015). The diversity of weevils in cultivated apple orchards and vineyards is, however, not well known and it could be possible that other species also cause economic damage to fruits in the area. A survey was undertaken to document which weevil species occur in deciduous fruits in the Western Cape to contribute knowledge of their pest status. The aim of this study was, therefore, to identify the weevil assemblage in apple orchards and vineyards, which have reported the most damage, and compile a taxonomic identification key based on morphological characteristics to make species identification accessible.

Materials and Methods

Description of study sites

Apple orchards and vineyards with a record of weevil infestation, were selected in three regions in the Western Cape Province: Ceres, Stellenbosch and Grabouw (Table 2.1). Table 2.2 list the details of each site, including the size, irrigation type, cultivar, annual rain fall, the location of the block, slope and crop type. The annual rainfall data were derived from 90 meter resolution long term (± 30 years) climate layers developed by Van Niekerk & Joubert (2011).

Table 2.1. Location, coordinates and elevation of apple orchards and vineyard farms selected for the study.

Farm location	Coordinates	Elevation in meters
Stellenbosch (Vineyard block A & vineyard block F)	**S** 33° 57’ 03.46” **E** 18° 52’ 09.14”	171 m
Grabouw (Apple orchard block E3a & vineyard block 6b)	**S** 34° 08’ 23.80” **E** 19° 01’ 16.86”	178 m
Ceres (Apple orchard block 11D and apple orchard block G8)	**S** 33° 13’ 25.28” **E** 19° 13’ 40.96”	914 m

Table 2.2. Size , slope, age, cultivar, irrigation type and annual rain fall of all the blocks located in three fruit growing areas, namely; Stellenbosch, Grabouw and Ceres, which were sampled for abiotic and biotic factors.

Block name	Size (ha)	Age (years)	Crop	Cultivar	Slope	Irrigation type	Annual rainfall (mm)
Stellenbosch:							
Vineyard block A	0.6	7	Vines	Pinotage	7.7	Micro-sprinkler	830.20
Vineyard block F	1.03	15	Vines	Shiraz	2.1	Micro-sprinkler	830.20
Grabouw:							
Apple block E3a	1.02	8	Apple	Granny smith	4.1	Micro-sprinkler	952.13
Vineyard block 6b	1.02	12	Vines	Shiraz	1.3	Micro-sprinkler	952.13
Ceres:							
Apple block 11D	1.02	8	Apple	Golden delicious	9.5	Micro-sprinkler	1015.90
Apple block G8	1.02	10	Apple	Granny Smith	7.0	Micro-sprinkler	1015.90

Adult weevil monitoring

The monitoring technique for adult weevils was adapted from De Villiers & Pringle (2008), as described below, and was carried out from September 2017 to May 2018. The selected blocks were monitored every two weeks for adult weevils, using marked weevil bands (single-sided cardboard bands about 15 cm wide), tied around the stem of the apple trees and vines with corrugated side against the stem (Figure 2.1). Adult weevils crawled into the bands during the day to shelter. In the grapevine blocks 20 vines, evenly spaced throughout the block, were selected, marked and sampled every two weeks (De Villiers & Pringle 2008) (Figure 2.2) (1). In the apple blocks 25 trees, evenly spaced throughout the block, were selected, marked and sampled every two weeks (De Villiers & Pringle 2008) (Figure 2.2) (2). Single-sided cardboard bands were removed from the stems, then a white cloth was tied and placed at the base of the stem (Figure 2.1). This proved to be useful for collecting the weevils, since most adult weevils feign death and fall to the ground when disturbed. Weevils were then collected and stored in marked containers during transportation back to the laboratory.

Figure 2.1. A grapevine stem tied with single-sided cardboard band (15 cm wide) with a thin piece of wire used to tie the cardboard band to the stem for adult weevil monitoring (left). A white cloth placed at the base of the stem of an apple tree to ensure all weevils were captured (right).

Figure 2.2. A 0.8 ha vineyard block, outlined in red (1) and a 2 ha apple block, outlined in green (2) used during the study illustrating how the cardboard bands were evenly distributed in the apple and vineyard blocks. The red and green dots represent the selected trees in both apple and vineyards (marked and tied with single-sided corrugated bands).

Species identification

The collected adult weevils were transferred into marked zip lock bags, then placed into the freezer for a period of approximately 24 hours to ensure no weevils survived. After removal from the freezer, the dead adult weevils were sorted, counted and identified into morphospecies. After counting, the adult weevils were then transferred into marked containers filled with ethanol (96 % ethanol) for preservation. The weevils were pinned and stored. As no synoptic work exists for weevil identification in South Africa, the determination of species identity of a reference collection of morphospecies was made by Dr Julien Haran (CIRAD, France), with the use of reference collections housed at Iziko museum (Cape Town) and relevant literature (Oberprieler 1995). Subsequent identifications were made based on this reference collection. Images of five weevil species, namely: *Eremnus cerealis* Marshall, 1921, *Naupactus leucoloma* Boheman, 1840, *Pantomorus cervinus* Boheman, 1840, and *Tanyrhynchus carinatus* Boheman, 1836 were taken using a Leica MZ 16A auto montage microscope with a Leica DFC 290 fixed digital camera and the other five (*Eremnus atratus* (Sparrmann, 1785), *Eremnus chevrolati* (Oberprieler, 1988), *Eremnus occatus* Boheman, 1843, *Eremnus setifer* Boheman, 1843 and *Phlyctinus callosus* Boheman, 1834) were photographed at CBGD (Montpellier, France) using a Keyence® VHX5000 imaging system. Extended depth-of-field images were obtained using the software of the imaging station. Measurements were made with an optical micrometer. Body length refers to the distance from the apical margin of the head (excluding the rostrum) to the apex of the elytra. Length of the rostrum refers to the distance between the apical margins of the eyes and the apex of the rostrum. The ratio of width to length (w/l) was calculated based on the width being the widest point of the elytra. The ratio of width to length (w/l) was measured at the widest point of the prothorax and the elytra. The length of the elytra was measured between the anterior part of the scutellum and the apex of elytra. Scales are important features to distinguish species, but they are fragile and may be partly lacking on some individuals. Diagnoses provided refer to fresh specimens with entire vestiture. To ensure the body parts were symmetrical and the background of the images balanced, GIMP version 2.10.0 was used to edit the images. A dichotomous key was compiled using relevant distinguishing morphological features such as the rostrum shape and size, the general body shape and vestiture, the pronotum shape and the presence of tooth on femora.

Rank abundance calculation

Rank abundance was calculated by obtaining the total count of each four weevil species collected during the entire seasonal population survey in apple orchards. In order to obtain a log abundance value which designated each species a ranking of 1 to 4 according to their total abundance in apple orchards, these values were logged. Similarly, in vineyards, rank abundance was calculated by obtaining the total count of each of the eight weevil species collected during the entire seasonal population survey in vineyards. In order to obtain a log abundance value which designated each species a ranking of 1 to 8 according to their total abundance in vineyards, these values were logged.

DNA extraction

Adult specimens collected from apple orchards and vineyards were placed in 96 % ethanol and sent to CIRAD (UMR CBGP, Molecular biology platform, Montpellier, France) for DNA barcoding. DNA was extracted from a leg with using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), as per manufactures instructions. PCR amplification was conducted using the standard primers for barcoding (mitochondrial cytochrome c oxidase subunit I) of invertebrates: LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). The method for PCR reactions were adapted from Brévault *et al.* (2018). PCR reactions were conducted on a Mastercycler® nexus (Eppendorf, Hamburg, Germany) in a volume of 10 µl of Multiplex Master Mix (Qiagen, Hilden, Germany), 0.8 µl primers (Forward and Reverse at µM) and 2 µl of DNA. The PCR conditions were as follows: initial DNA denaturation at 94 °C for 15 minutes, followed by 10 cycles of 30 s at 70 °C, followed by 30 cycles of 30 s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C with a final extension of 20 min at 72 °C. Sequencing of the PCR products were performed by Eurofins Genomics (http://www.eurofinsgenomics.eu/). All vouchers specimens placed in 96% ethanol and were deposited in the general collection at CBGP. Barcode sequences were aligned according to Haran *et al.* (2015). The tool BLAST (https//www.ncbi.nlm.nhih.gov/) was used to confirm the amplification of the target group (absence of non-target amplifications) based on the existing references in the NCBI database. Based on the aligned sequences a preliminary phylogenetic tree was constructed to show the genetic relationships among the pool of samples. Phylogenetic reconstruction included four congeneric species (*E. chevrolati*, *T. carinatus, P. callosu*s, *E. setifer*, *E. atratus*, *E. chevrolati*, *E. occatus*, *N. leucoloma* and *P. cervinus*) (Figure S1) in order to make a preliminary exploration of the relatedness of pest species with neighbor wild species, which were obtained from a separate study conducted by Dr Julien Haran. Phylogenetic reconstruction were performed with 1000 bootstrap replicates using the software RAxML v.8.2.4 (Stamatakis 2014). *N. leucoloma* and *P. cervinus* were used as an outgroup.

Results

Adult weevil monitoring and species identification

Nine weevil species of the subfamily Entiminae were found to occur in the selected vineyards and apple orchards in Ceres, Stellenbosch and Grabouw, namely: *E. atratus*, *E. chevrolati*, *E. occatus, E. setifer, N. leucoloma,* P. *cervinus*, *P. callosu*s and *T. carinatus.* A brief review of their comparative host plants and biological parameters is provided (Table 1). Nine of the species found were already reported as pests, and one species, namely: *Eremnus occatus* was reported for the first time in vineyards. *Eremnus cerealis* Marshall, 1921 and *Eremnus setulosus* Boheman, 1834 and, which are regarded as a widespread pest, were not found in the current study. Rank of each species in terms of abundance in apple orchards is illustrated in Figure 2.3 and in grapevines is illustrated in Figure 2.4. The log abundance in Figure 2.3 indicates that *P. callosus* was the most abundant species in apple orchards in a period of nine months (September 2017 to May 2018), followed by *P. cervinus*, *S. tottus* and *E. atratus*. The log abundance in grapevine blocks illustrated in figure 2.4, indicates that *P. callosus* was the most abundant weevil species, followed by *E. occatus*, *E. setifer*, *E. chevrolati, P. cervinus*, *N. leucoloma*, *T. carinatus*, and *E. atratus*. Diagnoses for each species are provided in the following sections, which were compiled with the assistance of Dr. Julien Haran.

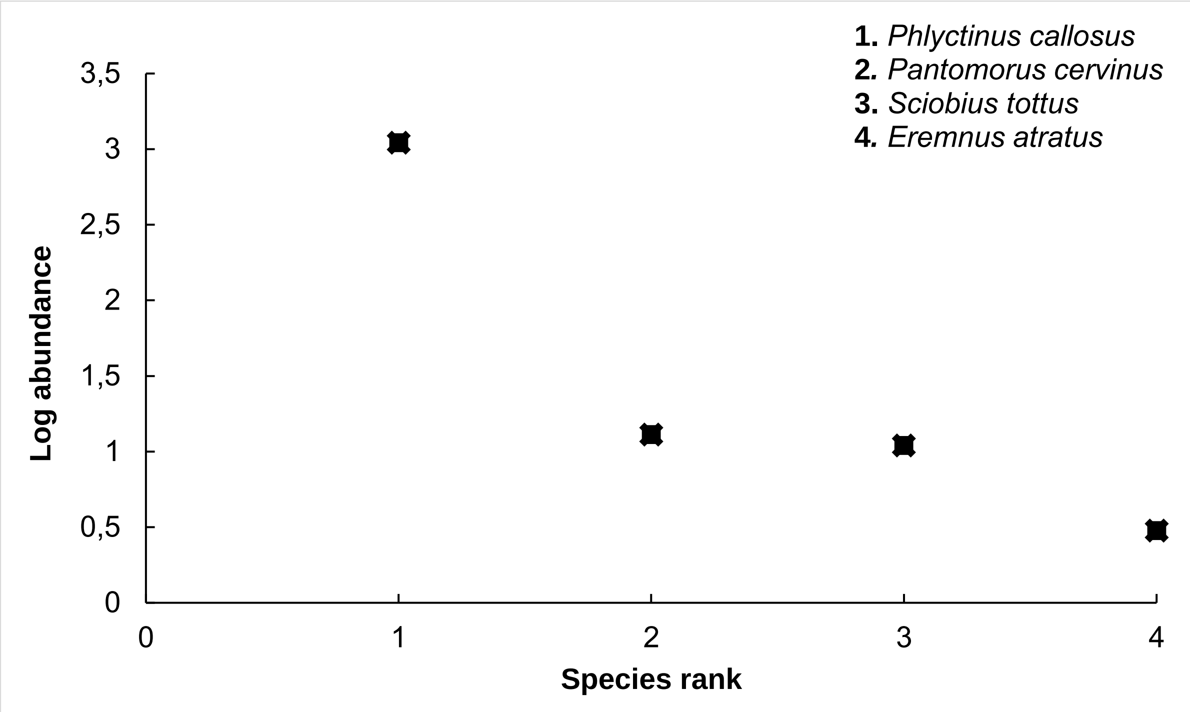

Figure 2.3 Rank abundance curve of the four most abundant Curculionidae species found in the Western Cape (apple orchards), based on the population density survey carried out from September 2017 to May 2018 in three study sites.

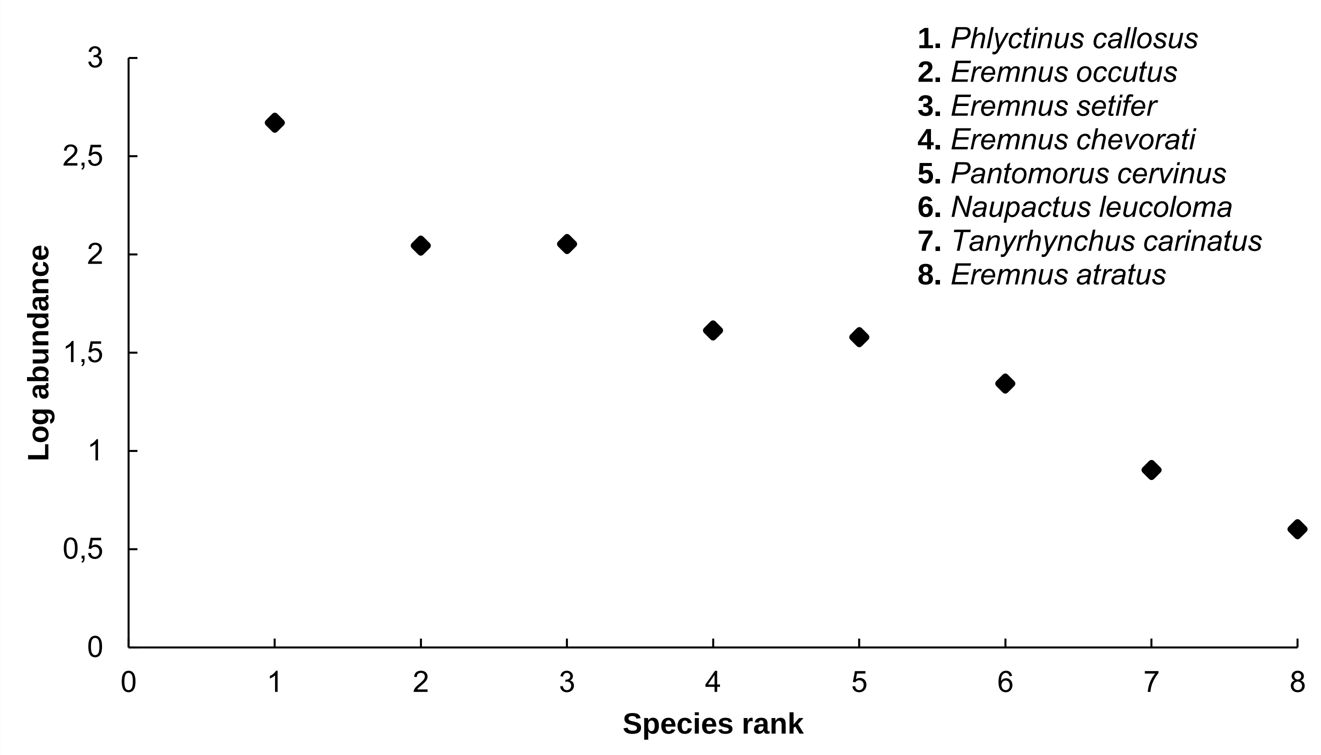

Figure 2.4 Rank abundance curve of the nine most abundant Curculionidae species found in the Western Cape (grapevines), based on the population density survey carried out from September 2017 to May 2018 in three study sites.

***Phlyctinus callosus* Boheman, 1834**

Identification

P. callosus are grey-brown, body length up to 7 mm; the *P. callosus* adults are flightless (Annecke & Moran 1982) (Figure 2.5). In appearance, the adult *P. callosus* is similar to other apple weevils, such as vegetable weevil (*Listroderes obliquus*) and Fuller's rose weevil (*Asynonychus cervinus*) (Fisher & Learmonth 2003). The BFW is characterized by a black and shiny tip on the rostrum and its abdomen is distinctly globular (CABI 2018). Beyond the transversal white band, towards the posterior of the abdomen, the elytra are markedly 'bumpy' (CABI 2015). Each bump is covered by numerous setae (Annecke & Moran 1982).

Diagnosis. Body length 6 to 7 mm. **Colour.** Body integument brown; vestiture of elytra comprising recumbent, slightly overlapping scales, distinctly longer than wide, not concealing integument, and series of semi-erect scales on all interstriae; scales of elytra pale brown, usually forming a pale transversal band at apical ¼, surrounded by darker transversal bands. **Head.** Rostrum longer than wide (w/l ratio: 1.08), narrower at antennal insertion than at base, covered with pale brown scales; eyes protruding, almost conical, non-regularly convex.

Prothorax. Slightly wider than long (w/l ratio: 1.4), sides moderately curved, constricted apically, with a longitudinal median carina, mostly visible in basal half. **Elytra.** Sides subparallel in basal two thirds (w/l ratio: 0.8), rounded toward apex in apical third; interstriae raised; apical visible on interstria 3-5. **Legs.** Femora clavate, unarmed, tibiae straight.

Damage

Adults cause extensive damage on the leaves, fruits and stems (Pringle *et al.* 2015). Short holes with serrated edges are the typical symptoms on leaves (Learmonth 2018) (Figure 2.6). In South Africa, grapevines damage usually occurs around November and December when emerging grapes are attacked following flowering (Barnes 1987). In Australia severe adult infestations lead to fruit scarring on grapes (Learmonth 2018). Adults can also injure berries and attack young grape bunches, chewing off the entire bunches (Mayburgh *et al*. 1973; Annecke and Moran 1982; Fisher & Learmonth 2004).

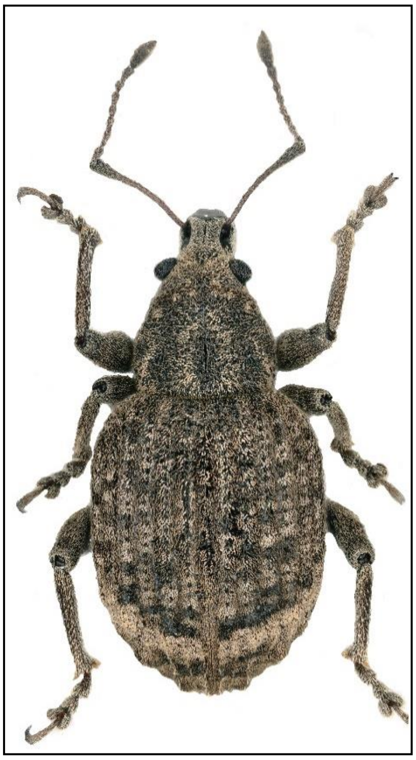

Figure 2.5 *Phlyctinus callosus* adult. (Photo by Julien Haran).


Figure 2.6 Typical weevil adult leaf feeding damage.

Pantomorus cervinus **(Boheman, 1840)**

Identification

P. cervinus adults are dull brown (with intermixed white scales) with the length of about 6 – 8.5 mm (Woodruff & Bullock 1979) (Figure 2.7). Sides of the elytra bearing a short oblique strip of white scales near the hind of the femora (Grout 2012). In dorsal view the head shape is trapezoidal with the back of the head slightly longer than the width of the front head (Grout 2012).

Diagnosis. Body length 6 to 8.5 mm. **Colour.** Body integument brown; vestiture of elytra comprising recumbent, slightly overlapping scales, distinctly longer than wide, not concealing integument, and series of semi-erect scales on all interstriae; scales of elytra brown, usually forming a short oblique strip of scales, near the hind of femora. **Head.** Rostrum shorter than wide (w/l ratio: 0.5), covered with white scales; bearing a longitudinal furrow, eyes protruding, very convex. **Prothorax**. Slightly wider than long (w/l ratio: 1.4), sides moderately curved, constricted apically. **Elytra.** Sides convex (w/l ratio: 0.79), widest near middle of length, rounded toward apex in apical third. **Legs.** Femora clavate, unarmed.

Damage

Both adults and larvae cause damage (Grout & Moore 2015). Adults are active at night, feed on the buds and leaves while the larvae feed on the roots and may lead to stunted growth (Prinsloo & Uys 2015). In the USA, adults feed at night in citrus, damage young shoots or new foliage and remain inactive during the day (McCoy *et al.* 2006). The symptoms on the leaves include serrations on the edge producing a ragged appearance to foliage (McCoy *et al.* 2006). Adults can consume the entire leaf under severe infestation, leaving only the midrib (Prinsloo & Uys 2015).

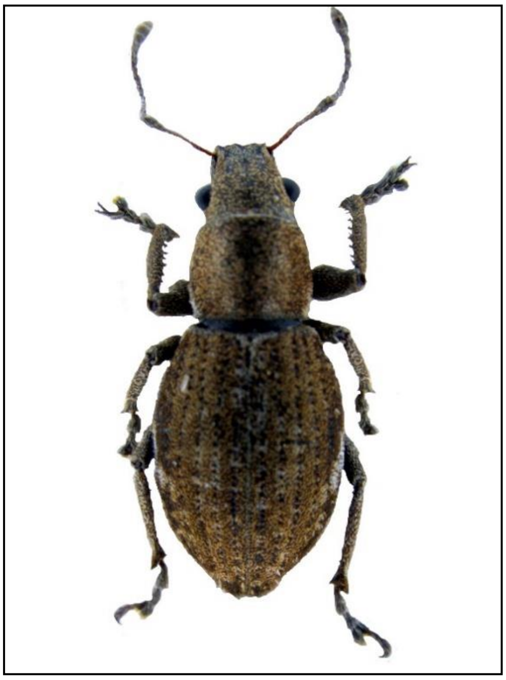

Figure 2.7 *Pantomorus cervinus* adult.

***Naupactus leucoloma* Boheman, 1840**

Identification

The flightless (with fused elytra), adult weevils are oval shaped, greyish bearing longitudinal white bands on each side of the elytra; scutellum bearing a contrasting spot of white scales (Figure 2.8). They are 12 – 15 mm in length with a short, stout snout (Lanteri & Marvaldi 1995). Males are scarce and have only been recorded in South America (Lanteri & Marvaldi 1995).

Diagnosis. Body length 12 to 16 mm. **Colour.** Body integument grey; vestiture of elytra comprising recumbent, slightly overlapping scales, distinctly longer than wide, not concealing integument, and series of semi-erect scales on all interstriae; scales of elytra pale grey, scutellum bearing a contrasted spot of white scales . **Head.** Rostrum longer than wide (w/l ratio: 1.7), bearing a fovea; covered with pale grey scales; eyes almost protruding, convex. **Prothorax**. Slightly wider than long (w/l ratio: 1.2), sides moderately curved, constricted apically. **Elytra.** Sides subparallel in basal two thirds (w/l ratio: 0.7), rounded toward apex in apical third; bearing longitudinal white bands on each side. **Legs.** Femora clavate, unarmed.

Damage

Adult weevils feed on leaves and cause little damage unless there's a high population (Prinsloo & Uys 2015). In South Africa the larvae have been reported to attack the tap roots of lucerne, which leads to subsequent death of the plant (Prinsloo & Uys 2015). They often burrow next to plant roots causing minor damage; older plants withstand the effect and survive, while the newly emerged plants die as a consequence and the damage leads to yield reduction (Johnson & Tappan 1988). Higher population densities lead to complete loss of pasture stands (Johnson & Tappan 1988).

Although present only sporadically in vineyards, this weevil is regarded as a significant pest of lucerne in South Africa (Prinsloo & Uys 2015). It can lead to severe economic injury even at low infestation rates (Prinsloo & Uys 2015). In America, they are considered a serious pest of agricultural crops such as peas, okra, velvet beans, cowpeas, soybeans, beans, sweet potato and cotton (Young *et al.* 1950; Johnson & Tappan 1988). The larvae can also injure roots of peanuts and lower the yield (Boutwell & Watson 1978).

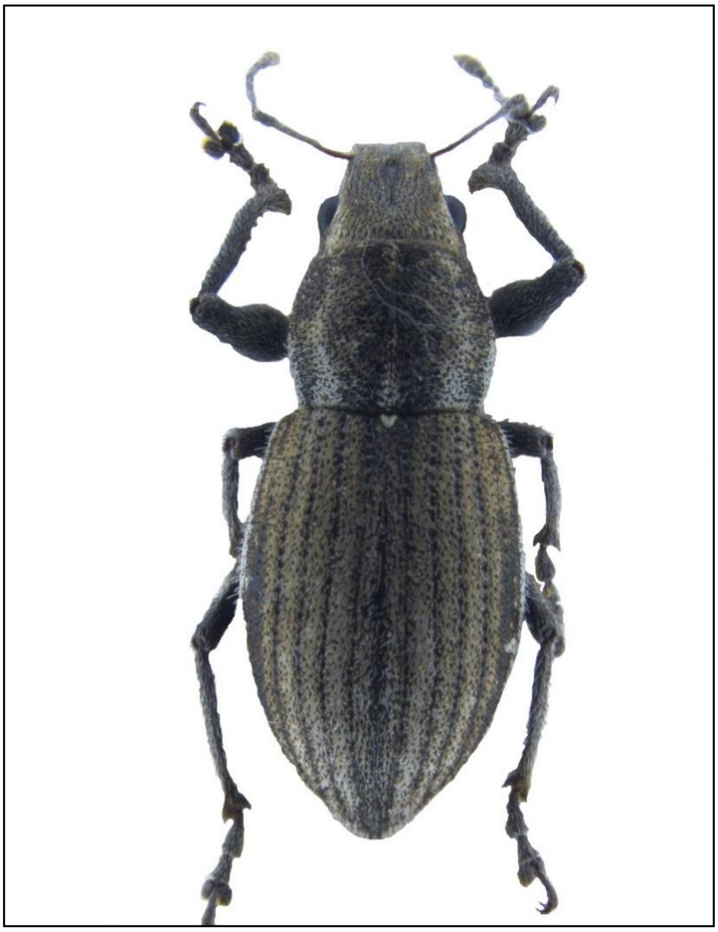

Figure 2.8 *Naupactus leucoloma* adult.

***Sciobius tottus* (Sparrmann, 1785)**

Identification

The adult is flightless, about 8 – 12 mm in length and the overall body is shiny brown to dark brown (Pringle *et al.* 2015) (Figure 2.9). The antennae is very long and slender, their length being half that of the body (Pringle *et al.* 2015). Elytra bear longitudinal rows of tiny, closely spaced tubercles and short colored hairs (Pringle *et al.* 2015).

Diagnosis. Body length 8 to 12 mm. **Colour.** Body integument reddish brown, mostly bare scales. **Head.** Rostrum shorter than wide (w/l ratio: 1.2), narrower at antennal insertion than at base, bearing three distinct longitudinal carinae, separated from eyes and frons by a transversal furrow, antennae elongated, segment of funicle longer than wide; eyes almost convex. **Prothorax**. Slightly wider than long (w/l ratio: 1.5), sides moderately curved, constricted apically, bearing distinct granules. **Elytra.** Sides convex (w/l ratio: 0.7), widest slightly before middle of length, rounded toward apex in apical third; interstriae slightly raised. **Legs.** Long and slender legs, femora clavate, unarmed, last tarsal segment arched and long.

Damage

The weevil causes damage to blossoms, leaves and buds of apples, pears and plums (Pringle *et al.* 2015). High population expansion lead to severe decline in the numbers of buds, young leaves and blossoms, which subsequently lead to crop loss in orchards (Pringle *et al*. 2015). Previous reports by Marais & Barnes 2003, suggest that the weevils do not inflict direct injury to fruit.

Figure 2.9 *Sciobius tottus* adult.

Tanyrhynchus carinatus **Boheman, 1836**

Identification

T. carinatus adults are about 4.5 – 6.3 mm long (Allsopp *et al*. 2015) (Figure 2.10). Elytra covered with tiny whitish to greyish scales; in an immature adult specimen the scales make a pale, longitudinal line down the median of the elytra; unlike *P. callous*, *T. carinatus* has a long rostrum; *T. carinatus* is the first weevil to emerge in grapevines (Allsopp *et al*. 2015).

Diagnosis. Body length 6 to 7 mm. **Colour.** Body integument brown; vestiture of elytra comprising recumbent, slightly overlapping scales, distinctly longer than wide, not concealing integument, and series of semi-erect scales on all interstriae; scales of elytra white. **Head.** Rostrum longer than wide (w/l ratio: 0.5), covered with white semi-erect scales, eyes flat.

Prothorax. Slightly wider than long (w/l ratio: 1.4), sides moderately curved, constricted apically. **Elytra.** Sides slightly convex (w/l ratio: 0.7), widest near base and regularly converging from there to apex; interstriae not raised. **Legs.** Femora clavate, armed with a distinct ventral tooth.

Damage

Damage is thought to be similar to that of *E. cerealis*.

Figure 2.10 *Tanyrhynchus carinatus* adult.

***Eremnus atratus* (Sparrmann, 1785)**

Identification

Adult black snout weevil are 7 to 10 mm long, body integument black, shiny, bare scales (Bloomfield *et al.* 2015) (Figure 2.11). The pronotum is smooth and lacks pits (Blomefield *et al*. 2015). Elytra with longitudinal rows of tiny pits (Allsopp *et al*. 2015).

Diagnosis. Body length 8 to 11 mm. **Colour.** Body integument black, shiny; bare of scales. **Head.** Rostrum slightly wider than long (w/l ratio: 1.6), bearing a fovea; bare scales; convex eyes. **Prothorax**. Slightly wider than long (w/l ratio: 1.4), sides moderately curved, constricted apically. **Elytra.** Sides subparallel in basal two thirds (w/l ratio: 0.7), rounded toward apex in apical third; interstriae raised; declivity with tubercles on interstriae 1, 3 and 5. **Legs.** Femora clavate, unarmed.

Damage

The adults feed on leaves and fruits of host plants which leads to notching along the margins of newly emerging leaves and stems and shoot tips (Blomefield *et al*. 2015). Damage on the fruit include fruit scarring and fruit stem notching (Blomefield *et al.* 2015). The weevils begin feeding immediately after adult emergence (Annecke & Moran 1982; Blomefield *et al.* 2015). The distribution of the weevil population within apple orchards is patchy and occurs sporadically (Blomefield *et al.* 2015). Adults feed on young nectarine fruits in early spring and summer, which can lead to economic injury (Annecke & Moran 1982). Adults attack the lower part of the tree, close to the trunk (Annecke & Moran 1982). They consume the leaf of apple tree from inwards and sometimes injure the bark of the tree to such an extent that the tree wilts and drop the fruit (Annecke & Moran 1982). If specifically targeted insecticides are not applied early on in the season, the weevil can reach pest status in apple orchards if the population increase (Blomefield *et al.* 2015).

Figure 2.11 *Eremnus atratus* adult. (Photo by Julien Haran).

Eremnus chevrolati **Oberprieler, 1988**

Identification

The adult weevil is 6 to 8 mm long and the body is dark to reddish-brown with no discernable scales; the elytra consists of longitudinal rows of pits and pale setae (Allsopp *et al*. 2015) (Figure 2.12).

Diagnosis. Body length 6 to 9 mm. **Colour.** Body integument reddish brown, covered with erected setae; bare of scales. **Head.** Rostrum longer than wide (w/l ratio: 0.8) bearing a distinct longitudinal furrow. **Prothorax**. Slightly wider than long (w/l ratio: 1.3), sides moderately curved, constricted apically, with erected setae on the sides **Elytra.** Sides subparallel in basal two thirds (w/l ratio: 0.8), rounded toward apex in apical third; interstriae flat. **Legs.** Femora clavate, unarmed.

Damage

Damage is thought to be similar to that of *E. cerealis*.

Figure 2.12 *Eremnus chevrolati* adult. (Photo by Julien Haran).

***Eremnus setifer* Boheman, 1843**

Identification

The weevil is 5 to 8 mm in length and the body is shiny metallic green in color (Allsopp *et al*. 2015) (Figure 2.13).

Diagnosis. Body length 6 to 9 mm. **Colour.** Body integument brown; almost entirely covered with recumbent, shiny metallic scales, distinctly wider than long, concealing body integument and erected setae. **Head.** Rostrum longer than wide (w/l ratio: 0.8), covered with shiny metallic scales. **Prothorax**. Slightly wider than long (w/l ratio: 1.2), sides moderately curved, constricted apically **Elytra.** Sides subparallel in basal two thirds (w/l ratio: 0.6), rounded toward apex in apical third **Legs.** Femora clavate, unarmed.

Damage

No previous record of damage from literature for this species

Figure 2.13 *Eremnus setifer* adult (Photos by Julien Haran).

***Eremnus occatus* Boheman, 1843**

Identification

No information available from literature on this species

Diagnosis. Body length 6 to 9 mm. **Colour.** Body integument brown; vestiture of elytra comprising recumbent, slightly overlapping scales, distinctly longer than wide, not concealing integument, and series of semi-erect scales on all interstriae; scales of elytra pale brown. **Head.** Rostrum longer than wide (w/l ratio: 0.1), covered with pale brown scales. **Prothorax**. Slightly wider than long (w/l ratio: 1.8), sides moderately curved, constricted apically **Elytra.**

Sides subparallel in basal two thirds (w/l ratio: 0.7), rounded toward apex in apical third. **Legs.** Femora clavate, armed with distinct ventral tooth.

Damage

No previous record of damage from literature for this species

Figure 2.14 *Eremnus occatus* adult (Photos by Julien Haran).

Molecular identification

Sequences were obtained and specimen codes available in Appendix A. A phylogenetic tree was compiled with other Entiminae of the genus *Eremnus* and *Phlyctinus*, found in and around orchard habits (Appendix B). The barcode sequences do not allow conclusions to be made about deep relationships between Tanyrhynchini and Oosomini, but it helps to verify if morphologically-recognized species form genetically distinct clusters. All species clustered separately between wild-caught (in natural habitats) and those in orchards. Based on this preliminary result, it appears further that *P. callosus* is a complex of several closely-related species. All barcodes obtained were new, except for those of *Naupactus leucoloma* and *Panthomorus cervinus*, which were already available on Genbank.

Identification keys (refer to appendix C)

The following characters were found to be useful for compiling a taxonomic key: length of rostrum, general body shape, texture of pronotum and elytra, colour of elytra, presence of teeth on femora, presence of scales and general colouration of body.

Discussion

Diversity of weevils in agricultural vineyards was higher compared to apple orchards, with nine species found in total, but not all of them having been previously recorded. *Eremnus occatus* was recorded for the first time on grapevines and as shown if figure 2.4 it was the second most dominant weevil. The higher diversity in grapevines could be attributed to different management practices employed by the growers of deciduous fruits (Barnes 1989), or various biotic or abiotic factors that the two crops are typically planted under. The impact of these factors on weevil occurrence is investigated further in Chapter 4. The findings in apple orchards during the course of the study were aligned with those of Marais & Barnes (2004), who reported four indigenous weevils in cultivated apple and pear orchards, namely: *E. atratus, P. cervinus, P. callosus* and *S. tottus.* The current population survey has shown that *P. callosus* is the dominant curculionid in both apple orchards and vineyards in the Western Cape. The complex life cycle (above and below ground, cryptic nature) of *P. callosus* in fruit orchards make it one of the most challenging pests to manage (Barnes & Giliomee 1992). The adult of *P. callossus* is the only curculionid currently regarded as a primary pest of deciduous fruits in the Western Cape (Barnes & Giliomee 1992). It is easily distinguished having a shiny black tip on the rostrum, a v-shaped white band at the rear of the abdomen and a shiny and the abdomen is distinctly globular (CABI 2018).

Continual monitoring is important to assess future pest status of weevil pests, due to the general nature of their damage symptoms. Stem banding is therefore currently the only method recommended for monitoring weevils (De Villiers & Pringle 2008; Brown & Pringle 2006). All *Eremnus* species collected can easily be recognized by their short rostrum (snout) and the elytra having a homogenous colour. The morphological difference among the *Eremnus* species collected during the study were minor (size of the rostrum, presence of scales in the body integument and scutellum, coloration and luster of the body integument, shape of the eyes (convex or conical), presence of the longitudinal carina in the rostrum, size of the rostrum, presence of a transversal furrow at the apex of the rostrum, presence of a fovea between the eyes, presence of distinct granules in the pronotum, presence of erected or semi-erected setae on the side of the pronotum, presence of distinct ventral tooth in the femora, presence of tubercles on elytral declivity). Of all the weevils collected in the present study, *N. leucoloma* was the largest, measuring up to 15 mm in length and further easy to distinguish by the white fringes on either side of the elytra. *N. leucoloma* was first recorded in Cape Town, South Africa in 1941 and it is assumed to have entered through imported fodder; since its establishment it has spread to various parts of the country, occurring in southwestern and central parts of South Africa (Prinsloo & Uys 2015). *T. carinatus* is the only species having a long, slender rostrum (longer than the head plus prothorax) compared to the *Eremnus* species; and it's reported to be the first species to emerge in grapevines (Allsopp *et. al.* 2015; Annecke & Moran 1982). *S. tottus* can be easily distinguished from the rest of the weevils by the length of the antennae, which are almost half that of the body; and it is reported to be the first *Eremnus* species to emerge in apple orchards (Marais & Barnes 2004; Pringle *et. al.* 2015). Being able to distinguish between these weevils during routine monitoring procedures, can therefore aid in assessing their future pest status. Barcoding to clarify the unique genetic sequence for most of the species found in this study had not previously been undertaken. The next step would be for the sequences to be made available on Genbank, and to further investigate the species complex of *P. callosus*, which may have management implications.

The other eight species, namely, *E. occutus*, *E. setifer, E. chevrolati, P. cervinus, N. leucoloma, T. carinatus, S. tottus and E. atratus* were found at lower abundance, but shouldn't be neglected as a potential threat to cause damage if population densities increase in future. For example, Marais & Barnes 2004, reported that when the population of *P. cervinus* are high in certain apple orchards in the Western Cape it leads to considerable problems with micro irrigation system, when the adult females deposit eggs in micro-jets, causing malfunction or failure; in Australia it is regarded as a minor defoliating pest of grapevines (Fisher & Learmonth 2007); in New Zealand *P. cervinus* is regarded as an important quarantine pest of kiwi fruit exported to Asian markets (Logan *et. al.* 2008); the larvae *P. cervinus* are regarded as a minor pest of citrus in Australia, Brazil, and Florida because of root feeding activities (Hely 1948; Tarrant & McCoy 1989; Lanteri et. al. 2002). *S. tottus* has been recorded to damage developing buds, blossoms and leaves only and do not cause any direct damage to ripening fruit in apple orchards. It can also cause damage when the populations are very high, especially on young trees which can be defoliated (Marais & Barnes 2003). In Australia, Fisher & Learmonth (2007) reported that adult *N. leucoloma* feed on foliage of young grapevines when the population is high; in Tasmania, Allen (2015) reported that when the population of *N. leucoloma* is high, the soil dwelling larvae cause significant economic damage on the roots and tubers of potatoes; in the United States of America, Boutwell & Watson (1978), reported that yield of peanut production was reduced by 15 % (on average) by the attack of *N.*

leucoloma in non-insecticide-treated crops. All of the weevils studied here have a similar life history, except for *S. tottus*, where eggs are laid in masses on leaves cemented together during oviposition. *S. tottus* was first reported as a pest in 1996 in South Africa, after apple growers in the Langkloof (Eastern Cape region) recorded severe damage caused by the weevil to the blossoms, leaves and buds of apple trees (Pringle *et. al.* 2015).

Surprisingly, in this study I did not collect either *E. cerealis* and *E. setulosus,* which are regarded as some of the weevils causing direct damage to grape berries (Allsopp *et al.* 2015). The present study was conducted during a period of drought, which may have impacted on the occurrence of some weevils. Unlike *P. callosus* and *T. carinatus*, there are no reports suggesting that *E. cerealis* along with other *Eremnus* species attack the grape berries (Prinsloo & Uys, 2015). Seven of the weevil species found in the present study, *P. callosus*, *E. setifer*, *E. chevrolati*, *E. atratus*, *E. cerealis*, *S. tottus* and *T. carinatus*, are regarded as international phytosanitary pests (Pringle *et. al.* 2015).

These findings have important implications for management practices, as it gives an indication of which species is the most dominant one and which should be given more priority. This is the first study to provide an illustrated morphological key for accurate species identification of weevil pests in apple orchards and grapevines. The pest status of *P. callosus* was confirmed as still being the dominant weevil in apple orchards and grapevines, but due to the polyphagous and largely indigenous nature of all weevils collected, they do all remain a threat to various crops grown in the Western Cape, and accurate monitoring will assist to prevent quarantine issues of export fruit.

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Chapter 3: Seasonal trends – monitoring, damage assessment and pest status

Abstract

Previous studies on seasonal monitoring of weevils and the extent of damage in both apple orchards and vineyards, were mainly on *Phlyctinus callosus* Boheman, 1834 since it is considered a key pest. However, little attention has been paid to the behavior and extent of damage caused by the population of other weevils in commercial apple orchards and vineyards. Six study sites, three in apple orchards and three in vineyards were selected for monitoring, assessing fruit damage and determining pest status of weevil species in the Western Cape for a period of nine months (during the period from September 2017 to May 2018). Damage assessments were conducted on two occasions, first in mid-December (pre-thinning assessment) and second in mid-March (pre-harvest assessment). Weevils started emerging in mid-October in vineyards and towards the end of October in apple orchards. In vineyards the population reached a peak in late December then decreased until April, while in apple orchards the population peaked from late November to mid-January and then decreased until April. *P. callosus* was found to be the most abundant weevil in both apple orchards and vineyards and accounted for 82 % of the weevils collected during the study. Other weevils were present in relatively low abundance , namely*; Eremnus setifer* Boheman, 1843 (5. 9 %), *Eremnus occatus* Boheman, 1843 (5.8 *%), Eremnus chevrolati* Oberprieler, 1988 (2. 1 %), *Pantomorus cervinus* (Boheman, 1840) (1.9 %), *Naupactus leucoloma* Boheman, 1840 (1.1 %), *Tanyrhynchus carinatus* Boheman, 1836 (0.4 %), *Sciobius tottus* (Sparrmann, 1785) (0.3 %), and *Eremnus atratus* (Sparrman, 1785) (0.3 %). In both apple orchards and vineyards, during the pre-thinning (mid-December) assessment, fruit damage was positively and significantly ($p = 0.05$) correlated with the number of *P. callosus* observed, while correlations were generally weak for the other weevil species. It is therefore suggested of all species recorded, that *P. callosus* is currently the most damaging in vineyards and apple orchards.

Introduction

Banded fruit weevil, *Phlyctinus callosus* Boheman, 1834 is regarded as the only Curculionid inducing primary damage to deciduous fruits (Barnes 1987) and a serious pest in vineyards in the Western Cape Province, South Africa (De Klerk 1981; Barnes 1989). The adults feed on the grape berries and apples leaving shallow scars (Marais & Barnes 2004; Marais 2003) (Fig.

3.1). The feeding can result in severe economic injury on apples by reducing the cosmetic value of fruit for export, but it rarely causes significant economic damage to pears (Marais & Barnes 2004). It is regarded as one of the most difficult pests to control in apple orchards due to its complicated life history, lack of accurate monitoring system and lack of effective insecticides (Barnes & Giliomee 1992).

Despite so much focus on the behavior of adult *P. callosus* in vineyards and apple orchards, little is known about the behavior of other common weevils known to exist in apple orchards and vineyards. Annecke & Moran (1982), reported that in vineyards *T. carinatus* is the first weevil to emerge on vines, with other weevils emerging later in October and November. Contrary to the report by Annecke & Moran (1982), Allsopp *et al.* (2015), reported that in vineyards the time of adult weevil emergence in spring varies from species to species with *Eremnus setuloses* Boheman, 1834 emerges first, in late September, followed by *P. callosus*, *E. cerealis* Marshall, 1921 and *E. atratus* (Sparrman, 1785) in succession. In apple orchards, Pringle *et al.* (2015), reported that *Sciobius tottus* (Sparrmann, 1785) is the first weevil to emerge earlier in October followed by *P. callosus*. In a study conducted to monitor the adult *P. callosus* population, Whitehead (1961), used corrugated cardboard bands tied around the base of vine stems to monitor the emergence of adults and found that adults in vineyards begun emerging from late October to early November and reached a peak from mid-November to mid-December. Employing the same sampling method, Kriegler (1970), reported that weevil emergence in South Western Cape apple orchards started from mid to the end of November. Using the same method, Barnes (1989), concluded that in apple orchards in the Western Cape, adult emergence started in October and peaked in early to mid-November. *P. callosus* is regarded as one of the primary pests of deciduous fruits, but currently there is no reliable and practical monitoring method, with most growers using corrugated cardboard bands tied around trunks to monitor the population as they shelter during the day (Barnes 1989) (Fig. 3.2) (B).

Information on the exact time and extent of the damage caused by weevils in apple orchards and vineyards is very limited. Most literature has focused on *P. callosus* since it is suspected to be the primary weevil pest on grapevines and in apple orchards. Barnes & Giliomee (1992), conducted research on the fruit feeding behavior of *P. callosus* in apple orchards and concluded that fruit damage reached a peak between late November and mid-December and thereafter the damage decreased for the next six to eight weeks with a very small peak in January. Myburgh *et al*. (1975), estimated that the damage caused by fruit feeding of *P. callosus* lead to an average crop loss of about 3.0 % in sprayed commercial orchards, and Sproul *et al.* (1986) reported that in vineyards adult *P. callosus* caused yield loss of up 30 %.

Barnes & Swart (1977), reported that in apple orchards fruit damage appeared to occur mainly during December and between January and February.

Control of *P. callosus* in the Western Cape is hampered by the lack of knowledge on when damage occurs and uncertainty over the species complex involved. Seasonal monitoring yields valuable information on the status of the pests and such knowledge could be used to predict the exact date of damage in apple orchards and vineyards. Knowledge about the seasonal behavior of pests is required for planning the initiation of monitoring and predicting when damage can be expected (De Villiers & Pringle 2006). This study aims 1) to yield valuable information on which weevil species should be targeted for improved management practices in apple orchards and vineyards located in Ceres, Grabouw and Stellenbosch, and 2) to determine the potential impact of various weevil species found to attack apples and grapes. This study will provide new information on the entire weevil complex found in apple orchards and vineyards, to complement what is known about *P. callosus*.

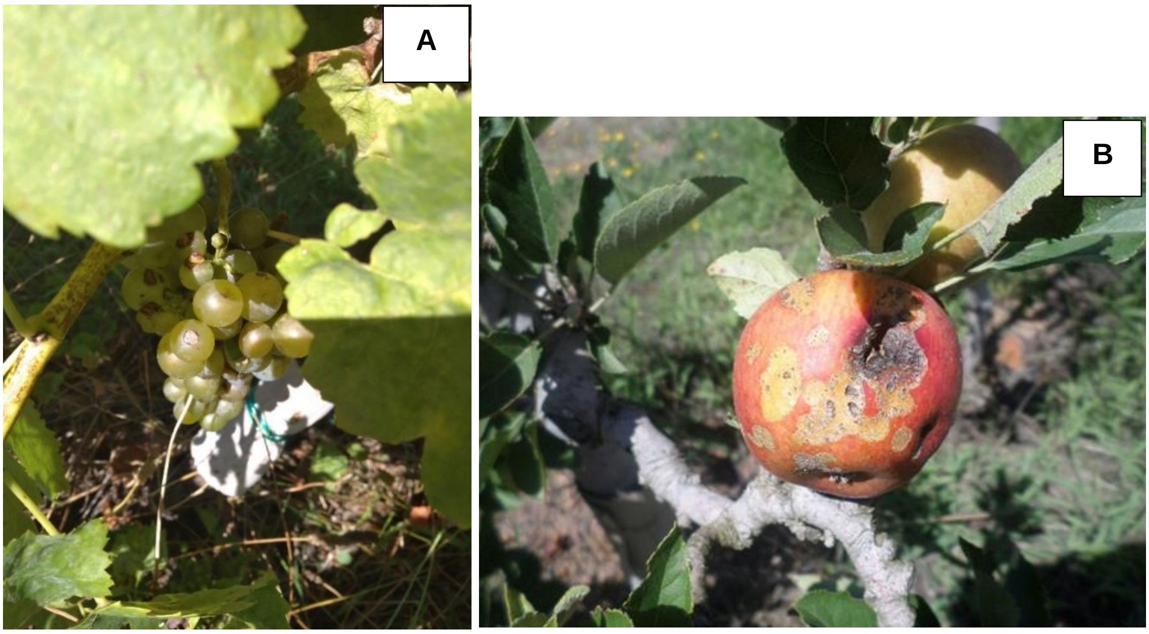

Figure 3.1 Adult *Phlyctinus callosus* feeding damage to grapes (A) and apples (B).

Materials and methods

Seasonal Monitoring

A total of three sites were selected in the Western Cape (Grabouw, Stellenbosch and Ceres) known to be infested with weevils and surveyed for a period of nine months (September 2017 – May 2018). Each site consisted of two blocks, one vineyard and one apple block (Table 3.1).

Blocks were monitored fortnightly for adult weevils using labeled cardboard bands (single sided cardboard bands about 15 cm wide) tied around the stem of the apple trees and vines with the corrugated side against the stem (Refer to the previous chapter). Twenty evenly spaced trees were selected in each vineyard block, marked and surveyed every two weeks (De Villiers & Pringle 2008) (Figure 3.2) (A). Twenty-five evenly spaced trees were selected in each apple block, marked and surveyed every two weeks (Brown & Pringle 2006) (Figure 3.2) (B). A white cloth was placed around the base of the stem, before corrugated cardboard bands were dislodged from the stem; since adult weevils feign death when disturbed and ensured all weevils were captured (Figure 3.3) (B). Collected adult weevils were transported back to the laboratory for counting and identification.

Table 3.1. Location, coordinates and elevation of Apple orchards and vineyard farms selected for the study.

Farm location	Coordinates	Elevation in meters
Stellenbosch (Vineyard block A & vineyard block F)	**S** 33° 57’ 03.46” **E** 18° 52’ 09.14”	171 m
Grabouw (Apple block E3a & Vineyard block 6b)	**S** 34° 08’ 23.80” **E** 19° 01’ 16.86”	178 m
Ceres (Apple block G8 & Apple block11D)	**S** 33° 13’ 25.28” **E** 19° 13’ 40.96”	914 m

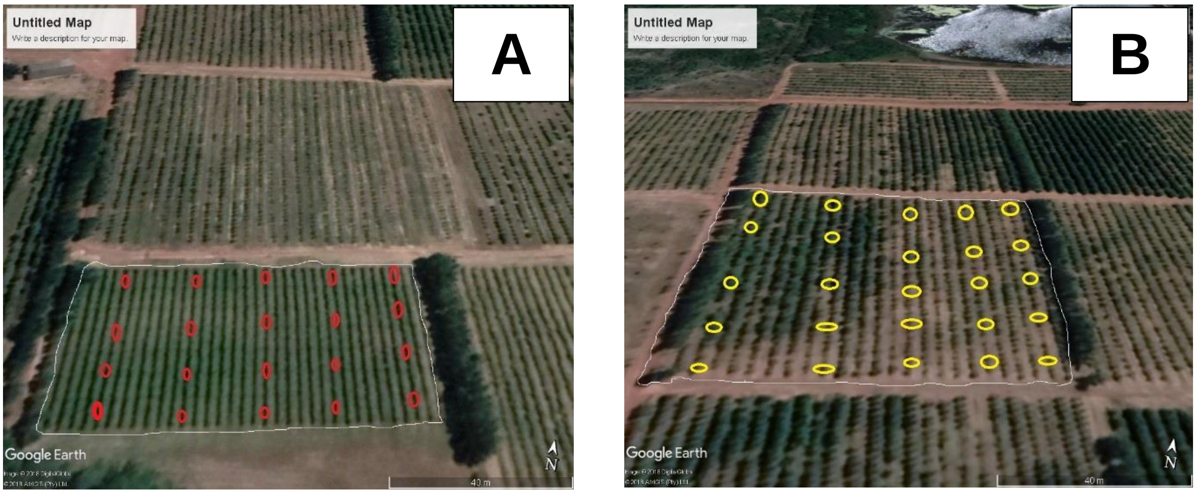

Figure 3.2. A 1.02 ha vineyard block, outlined in white (A) and a 1.2 ha apple block, outlined in white (2), used during the study illustrating the position of the bands (the red and yellow oval shaped circles representing the selected trees) in both apple orchards and vineyards.

Damage Assessments

The fruit damage assessments employed during the course of the study were from De Villiers & Pringle 2006. Damage assessments took place twice during the season: First during mid-December (the pre-thinning assessment) and secondly during mid-April (the pre-harvest assessment). Assessments were carried out on the same trees as those used when monitoring with cardboard bands. Five bunches and fruit clusters on each of the 20 vines in vineyard blocks and 25 trees in apple blocks, respectively, were inspected and all damaged fruits were recorded.

Weather data

The weather data for a period of nine months for all the three study areas were obtained from the Agricultural Research Council (ARC) Institute for Soil, Climate and Water (Agrimet, Stellenbosch). This includes the minimum, maximum, daily average temperatures and average relative humidity.

Statistical analysis

The percentage fruit damage/block and the average number of adult weevils over the whole season/block were analyzed using the non-parametric Spearman (rank-order) correlations, as data were not normally distributed, and the assumptions for normality were violated irrespective of transformation. Two dimensional scatterplots illustrating the correlation between percentage damage versus the number of weevil adults were used graphically present data. Analyses were conducted in Statistica v.13.2 (Stat-Soft Inc., 2013).

Results and Discussion

Weevil population in vineyards

The seasonal cycle of the average adult weevil population in vineyards is illustrated in Fig. 3.3 (A), except for adult *P. callosus* which is illustrated in Fig. 3.3 (B). All weevil species collected belonged to the family Curculionidae and to the sub-family Entiminae. In total, 796 adult weevils were collected. In all three study areas, *P. callosus* was the most dominant weevil through the duration of the study and it accounted for 58 % of the catches, followed by *Eremnus occatus* Boheman, 1843 (14 %), *Eremnus setifer* Boheman, 1843 (13 %), *Eremnus chevrolati* Oberprieler, 1988 (5 %), *Pantomorus cervinus* (Boheman, 1840) (3 %), *Naupactus leucoloma* Boheman, 1840 (2 %), *Tanyrhynchus carinatus* Boheman, 1836 (1 %) and *E. atratus* (0.9 %). Surprisingly, in our findings we did not record any *E. setulosus* and *E. cerealis* species, which are both regarded as one of the most fruit damaging weevils in vineyards and are also regarded as an international phytosanitary pest (Allsopp *et al.* 2015). *T. carinatus* was the first weevil observed in the vines during the second week of October (Fig. 3.3) (A). Later towards the end of October the following adult weevils emerged, namely: *E. setifer*, *E. occatus*, *N. leucoloma*, *E. chevrolati* (Fig. 3.3) (A) and *P. callosus* (Fig. 3.3) (B). Interestingly, our findings were similar to those of Annecke & Moran 1982 who reported that *T. carinatus* was the first weevil to emerge in vines; but contrary to Allsopp *et al* 2015 who reported that *E. setulosus* was the first to emerge in vines followed by *P. callosus*, *E. cerealis* and *E. atratus*. The weevil population increased from mid-October to late November. Populations reached a peak in November (Fig. 3.3) (A), with the exception of *P. callosus* (Fig. 3.3) (B), which continued to increase until it reached a peak in December. In the case of most weevil species, a drop in their abundance appears to be linked to a drop in temperature after December (Fig. 3.4). Similarly, Whitehead (1961) found that *P. callosus* emerged in late October to early November and reached a peak from late November to late December. There does not appear to be any relation to weevil abundance during the growing season and average relative humidity (Fig. 3.5). In late November to early December most of the weeds within the interrows of the two vineyard study sites in Stellenbosch were killed with herbicides (Personal observation). Since most adults feed at night and shelter on the weeds, the weeds (cover crops) served as an alternative host to the weevils during the day and some adults were also observed to feed on plant leaves so this may have affected their abundance (personal observation). From January to mid-April the population decreased to zero and in May there were no weevils collected. During this period most of the grape berries were harvested and leaves dropped to the vineyard floor, providing no incentive for weevils to enter the canopy.

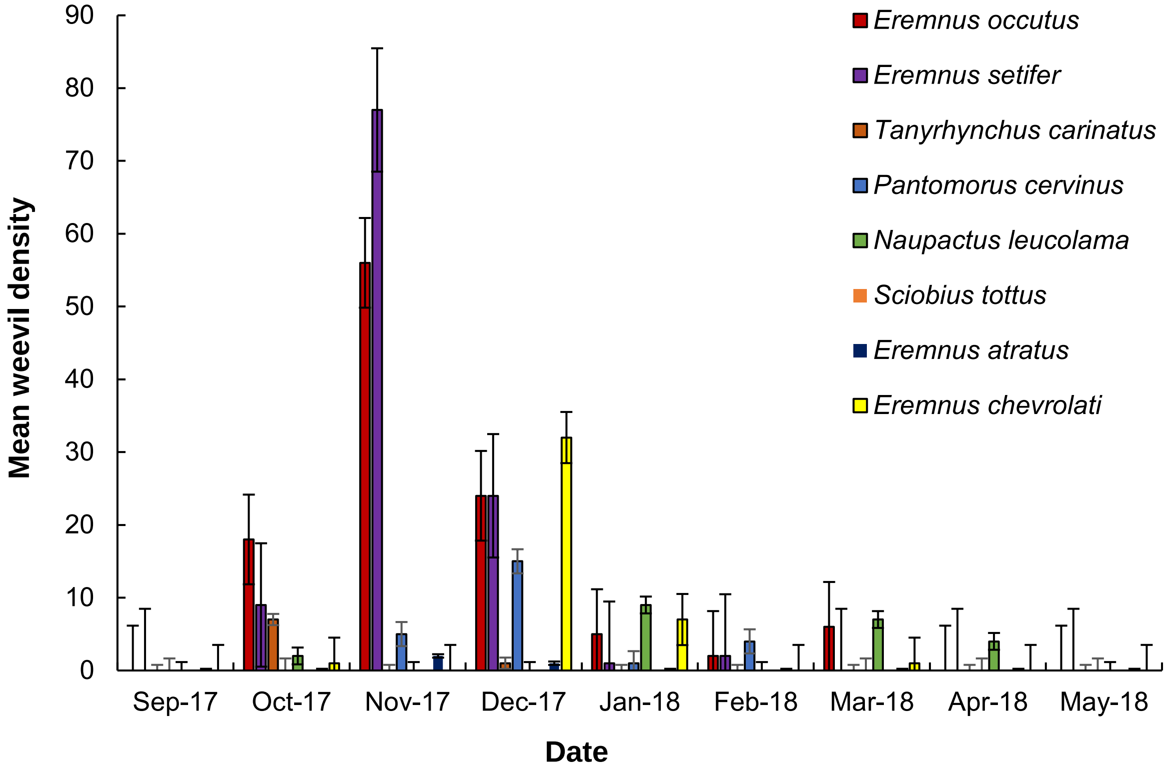

Figure 3.3 (A) Mean weevil density (± S.E.) (*Eremnus occatus*, *Eremnus setifer*, *Tanyrhynchus carinatus*, *Pantomorus cervinus*, *Naupactus leucoloma*, *Eremnus chevrolati* and *Eremnus atratus*) in three vineyard blocks, one located in Grabouw and two in Stellenbosch from September 2017 to May 2018.

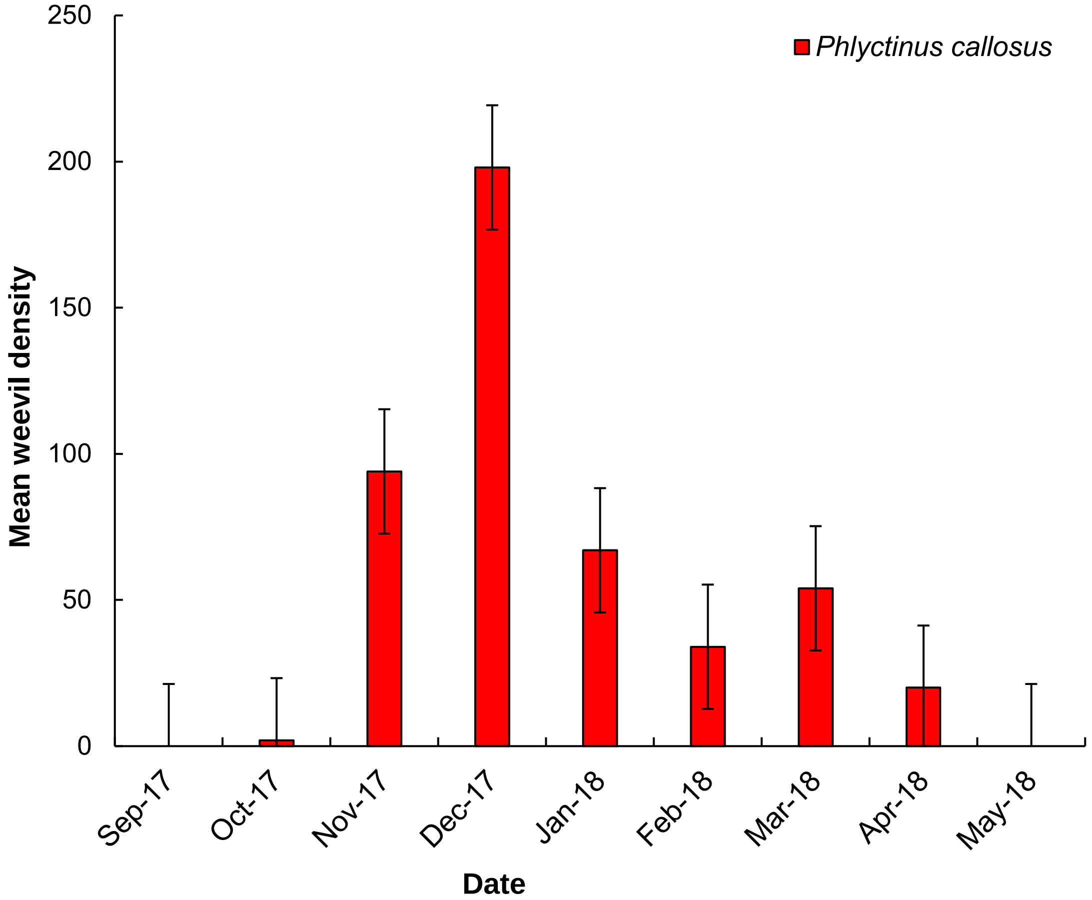

Figure 3.3 (B) Mean weevil (*P. callosus*) density (± S.E.) in three vineyard blocks, one located in Grabouw and two in Stellenbosch from September 2017 to May 2018.

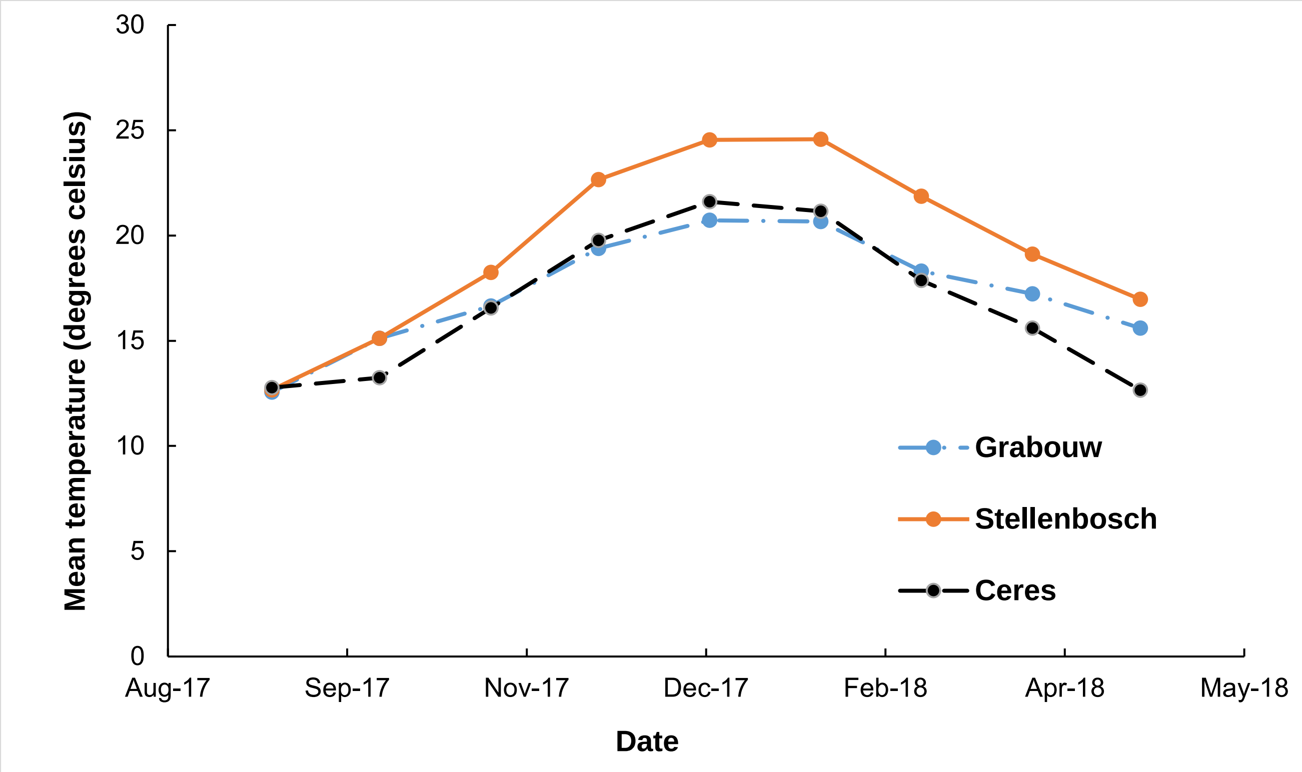

Figure 3.4 Mean monthly temperatures for Grabouw, Stellenbosch and Ceres for the 2017 to 2018 growing season.

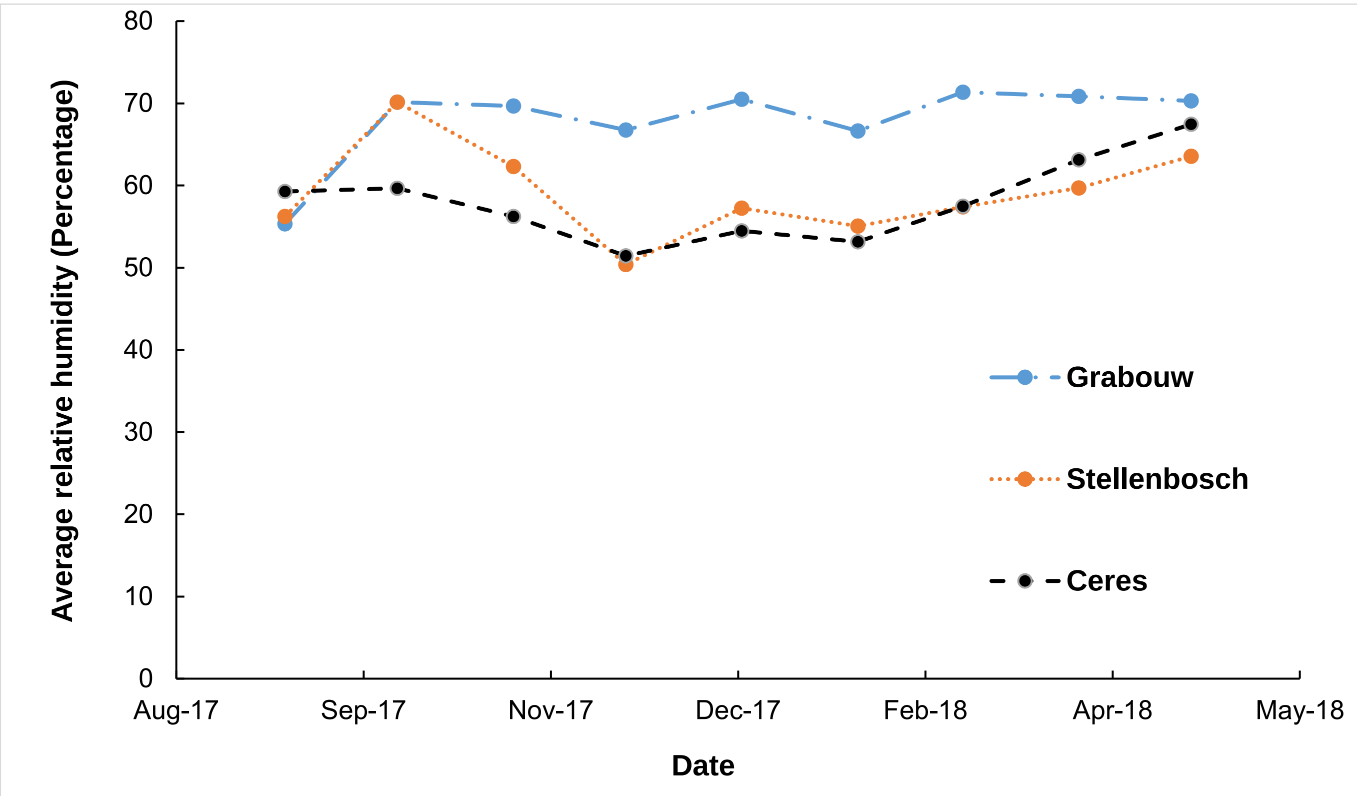

Figure 3.5 Average relative humidity for Grabouw, Stellenbosch and Ceres for 2017 to 2018 growing season.

Weevil population in apple orchards

The seasonal cycle of the weevil population in apple orchards is illustrated in Fig. 3.6 (A), except for adult *P. callosus* which is illustrated Fig. 3.6 (B). All weevil species collected belonged to the family Curculionidae and to the sub-family Entiminae. In total, 1124 adult weevil species were collected in apple orchards. In all three study areas, *P. callosus* were the most active and visible weevils and accounted for 98 % of the trap catches, followed by *P. cervinus (*1 %), *Sciobius tottus* (Sparrmann, 1785) (0.8 %) and *E. atratus* (0.7 %). Adult *P. callosus* begun moving into trees in late-October, which is contrary to the findings by Pringle *et al.* (2015) and Marais & Barnes (2004) where *S. tottus* was reported to be the first weevil to emerge in apple orchards. This may suggest that weevil emergence is influenced by management practices employed from orchard to orchard, or climatic factors. The population of *P. callosus* increased from October to mid-November, again correlated with increasing spring temperatures (Fig. 3.6), and as was also found by Barnes (1987), who monitored the population of *P. callosus* in apple orchards for a period of three years and found that the population increased from November to December. In a study conducted in a laboratory in Australia by Walker (1981) where adult *P. callosus* were reared at constant temperatures, they found that *P. callosus* adults performed better at a temperature of 15 °C with a recorded 15 % mortality in the first 100 days after emergence from the soil; as the temperature increased to 20 °C, the mortality rate increased to 70%. What this seems to indicate is that weevils are more active at a mean temperature between 15 to 20 °C, but beyond that weevils fail to tolerate temperatures of up to 25 °C. By early November, *S. tottus* started moving into the trees, and the population reached a peak in late December. From late December *Eremnus atratus* began moving into the trees. Aside from declining temperatures (Fig. 3.4), the sharp decline in *P. callosus* from the end of January till mid-April, which was also observed in vineyards, could also have been influenced by fruit thinning, which took place during mid-December (Fig. 3.7). Many of the fruit were on the orchard floor and it is assumed that weevils were less likely to climb the trunk since the fruits were available in surplus on the floor, resulting in the number of catches dropping from early January to April. In a study of seasonal cycles of *P. callosus* in apple orchards in the Western Cape, Barnes (1987) used corrugated cardboard bands to collect the adult *P. callosus* population for three consecutive years (1983/84, 1984/85 and 1985/86) and suspected that the decrease in adult *P. callosus* from January could be explained by the fluctuation in sex ratio. In their study they found that most females begun leaving trees in November and January for the purpose of oviposition which occurred off the trees. Only a limited number of *P. cervinus* were recorded in February, which differs from the report by Marais & Barnes (2004), that adult *P. cervinus* population peak around December.

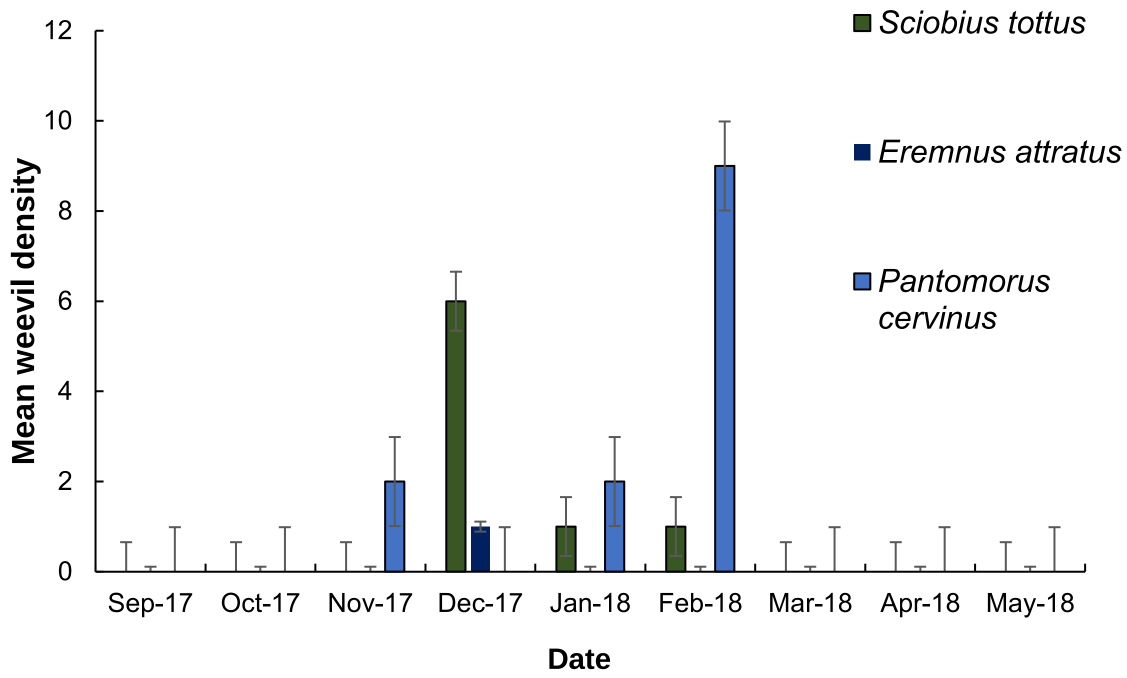

Figure 3.6 (A) Mean number of weevils (± S.E.) (*Pantomorus cervinus, Sciobius tottus and Eremnus atratus*) in apple orchards located in Ceres and Grabouw from September 2017 to May 2018.

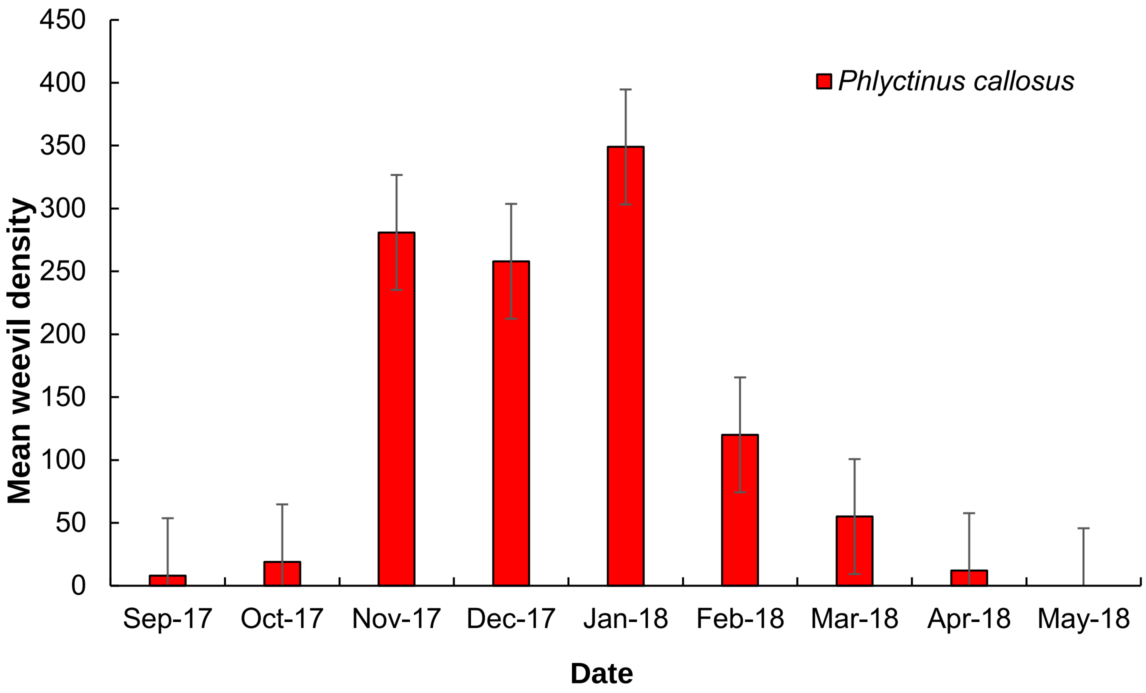

Figure 3.6 (B) Mean number of weevils (± S.E.) (*Phlyctinus callosus*) in apple orchards located in Ceres and Grabouw from September 2017 to May 2018.

Figure 3.7 The apple orchard during fruit thinning in Ceres during mid-December 2017.

Fruit Damage Assessments in Vineyards

Pre-thinning and pre-harvest assessment (December)

During the pre-thinning assessment, fruit damage was positively and significantly correlated with the number of weevils observed, excluding *P. callosus* (Spearman: r = 0.41, P < 0.001113) (Fig. 3.8) (A). Damage to vineyards was positively and significantly correlated with the *P. callosus* population (Spearman: r = 0.75, P < 0.0000001) (Fig. 3.8) (B), with a stronger correlation observed. During the pre-harvest assessment, fruit damage was positively and non-significantly correlated with the number of weevils observed, excluding *P. callosus* (Spearman: r = 0.096357, P < 0.63933) (Figure 3.9) (A). Damage to vineyards was, however, again positively and significantly correlated with the *P. callosus* population (Spearman r = 0.54, P < 0.000009) (Fig. 3.9) (B). This suggests that *P. callosus* is responsible for the majority of damage observed on vineyards in this study (both pre-thinning and pre-harvest). At this period (pre-thinning assessment) the vineyard floor was full of plant cover (weeds), the mean temperature (Fig. 3.4) and relative humidity (Fig. 3.5) were also increasing. In their requirements for the management of *P. callosus* on table grapes destined for Israel in 2006, the Department of Agriculture in the Republic of South Africa suggested that if the vineyard floor is kept clear of plant cover (weeds), *P. callosus* will be better managed (DAFF 2006). This seems to suggest that in vineyards the cover crops (weeds and grasses) seem to play an imperative role as being the alternative source of food for weevils and acting as hosts. The effect of weeds on weevils is investigated further in Chapter 4. It should be noted that most of the grape berries sampled in mid-December (pre-thinning assessment) were again sampled in March (pre-harvest assessment), therefore recording the same damaged grape berries. At this time of the growing season (March), the weevil population was declining (Fig. 3.3 A and Fig. 3.3 B) and therefore too low to cause the significant damage observed.

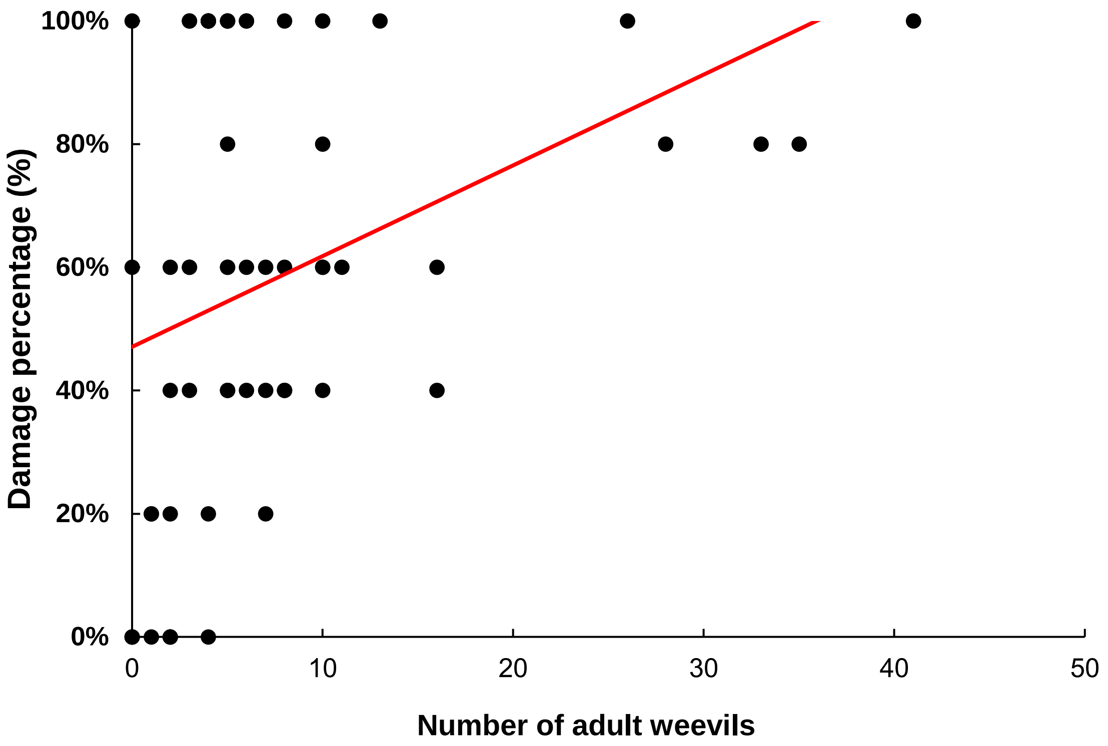

Figure 3.8 (A) Scatterplot illustrating the relationship between the mean number of adult weevils (*Pantomorus cervinus*, *Naupactus leucoloma*, *Eremnus atratus*, *Eremnus chevrolati*, *Eremnus occatus* and *Tanyrhynchus carinatus*) & the percentage fruit damage observed during the pre-thinning assessment in three vineyard blocks located in Stellenbosch and Grabouw, Western Cape, South Africa.

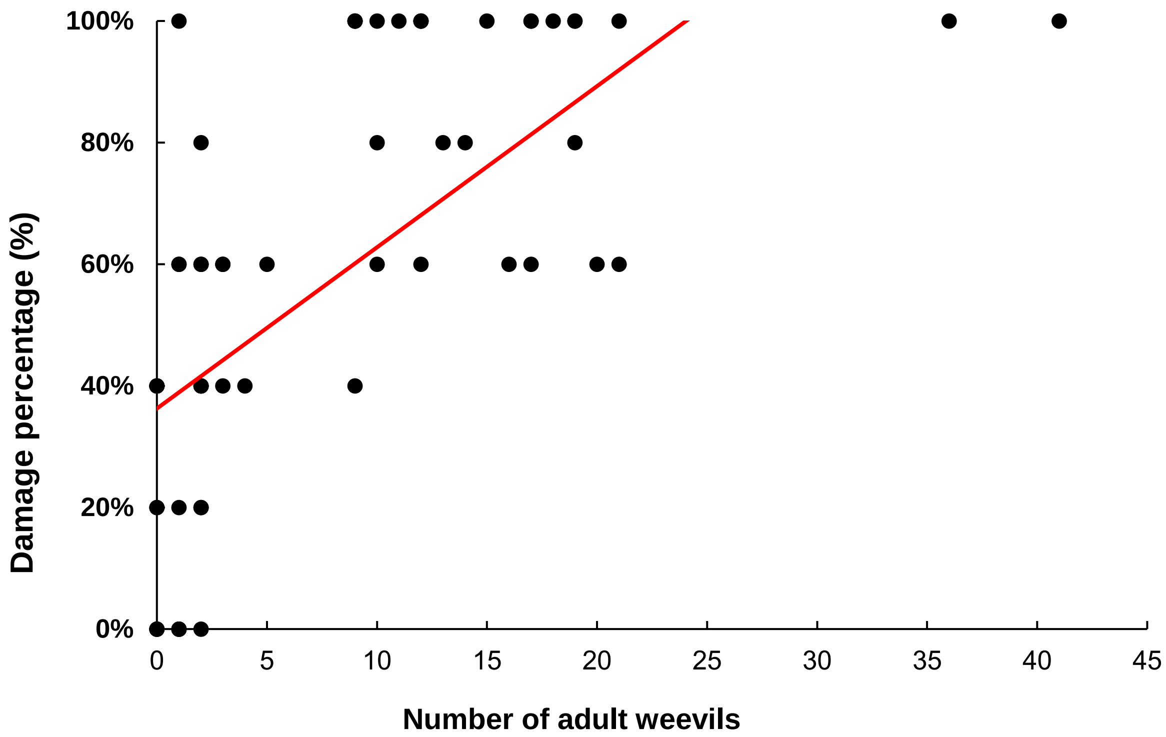

Figure 3.8 (B) Scatterplot illustrating the relationship between the mean number of adult weevils (*Phlyctinus callosus*) & the percentage fruit damage observed during the pre-thinning assessment in three vineyard blocks located in Stellenbosch and Grabouw, Western Cape, South Africa.

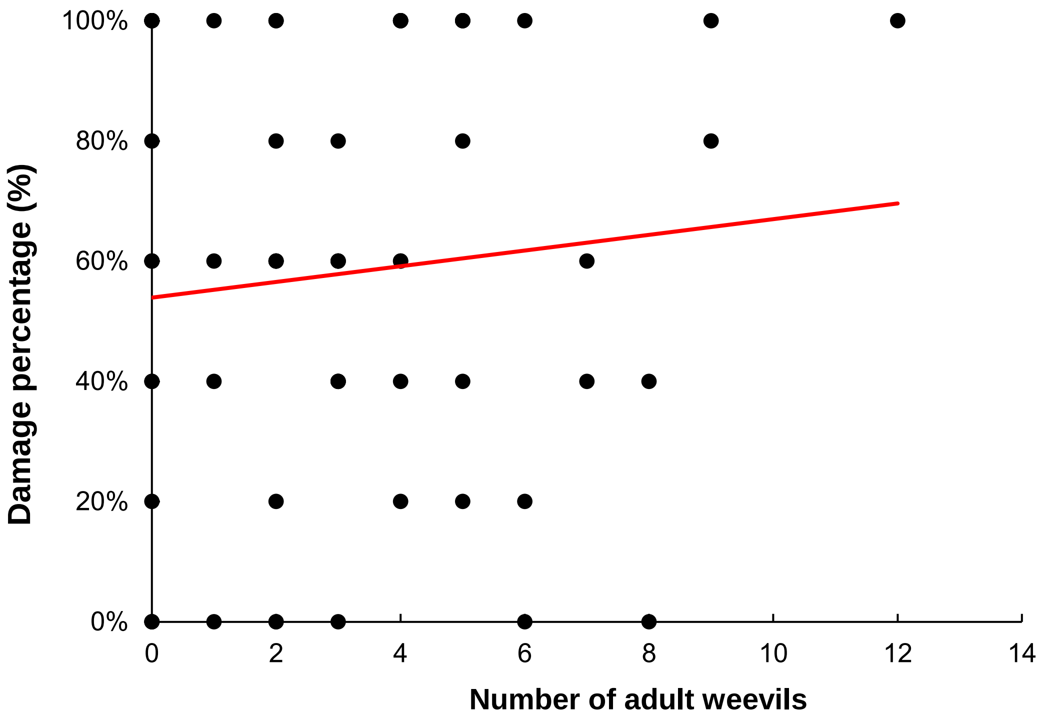

Figure 3.9 (A) Scatterplot illustrating the relationship between the mean number of adult weevils (*Pantomorus cervinus*, *Naupactus leucoloma*, *Eremnus atratus*, *Eremnus chevrolati*, *Eremnus occatus* and *Tanyrhynchus carinatus*) & the percentage fruit damage observed on three vineyard blocks located in Stellenbosch and Grabouw, Western Cape, South Africa. Pre-harvest assessment.

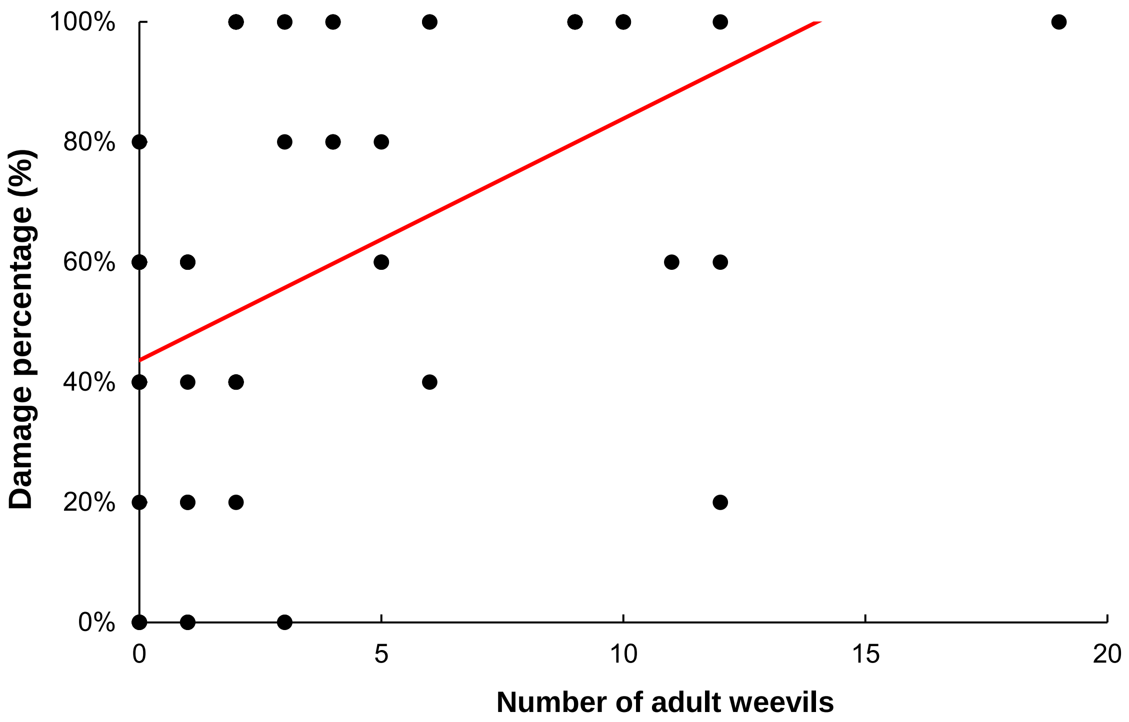

Figure 3.9 (B) Scatterplot illustrating the relationship between the mean number of adult weevils (*P. callosus* & the percentage fruit damage observed during the pre-harvest assessment in three vineyard blocks located in Stellenbosch and Grabouw, Western Cape, South Africa.

Fruit Damage Assessments in Apple Orchards

Pre-thinning assessment (December) and pre-harvest assessment (March)

During the pre-thinning assessment, fruit damage was positively and significantly correlated with the number of weevils observed, excluding *P. callosus* (Spearman r = 0.357035, P < 0.001664) (Fig. 3.10) (A). Damage to orchards was positively and significantly correlated with the *P. callosus* (Spearman r = 0.57, P < 0.0000012) (Fig. 3.10) (B), with a strong correlation observed. During the pre-harvest assessment, fruit damage was positively and non-significantly correlated with the number of weevils observed, excluding *P. callosus* (Spearman r = 0.18, P > 0.12) (Fig. 3.11) (A). Damage to orchards was negatively and non-significantly correlated with the *P. callosus* population (Spearman r = -0.13, P > 0.28) (Fig. 3.11) (B). This suggest that *P. callosus* is responsible for a majority of damage observed in orchards in this studyand that damage could only be significantly correlated during pre-thinning assessments in apple orchards. These findings were aligned with those of Barnes & Giliomee (1992), who found that the most fruit-damaging period associated with adult *P. callosus* occurred from late

November to mid-December, sometimes extending to late January. In their study, Barnes & Giliomee (1992) also assessed the mandibles of newly emerged weevils before feeding begun and later in mid-December. They suggested that the higher weevil damage to apples in December could be attributed to the newly emerged adults which had sharp mandibular cusps which could easily penetrate the skin of the apple compared to older weevils. This meant that at this stage (pre-thinning assessment) the newly emerged adults had much stronger mandibles to penetrate the cuticle of the apple and as a result fruit damage increased with the population of weevils. In their study of the fruit-feeding behavior of *P. callosus* in apple orchards, Barnes & Giliomee (1992), recorded a sharp decrease in fruit feeding from December to March. They contended that the sudden decrease in the fruit-feeding could be associated with reduced strength of the mandibles and the increase in the wax cuticle of the apples from December onwards. Djamin & Pathax (1967) and Raupp (1985), reported that the wearing of insect mandibles adversely affect their feeding efficiency. In a study of fruit feeding behavior of *P. callosus* in the Western Cape, Barnes (1989) found that they may be up to two generations in an orchard with an adult peak population occurring between March and April depending on irrigation practice. The timing of damage to fruit recorded in the current study is more consistent with the occurrence of only one generation per year and a limited number of adults recorded at this stage (pre-harvest assessment), which seems to explain the negative correlation between weevil abundance and little increase in damage at the later time of assessment.

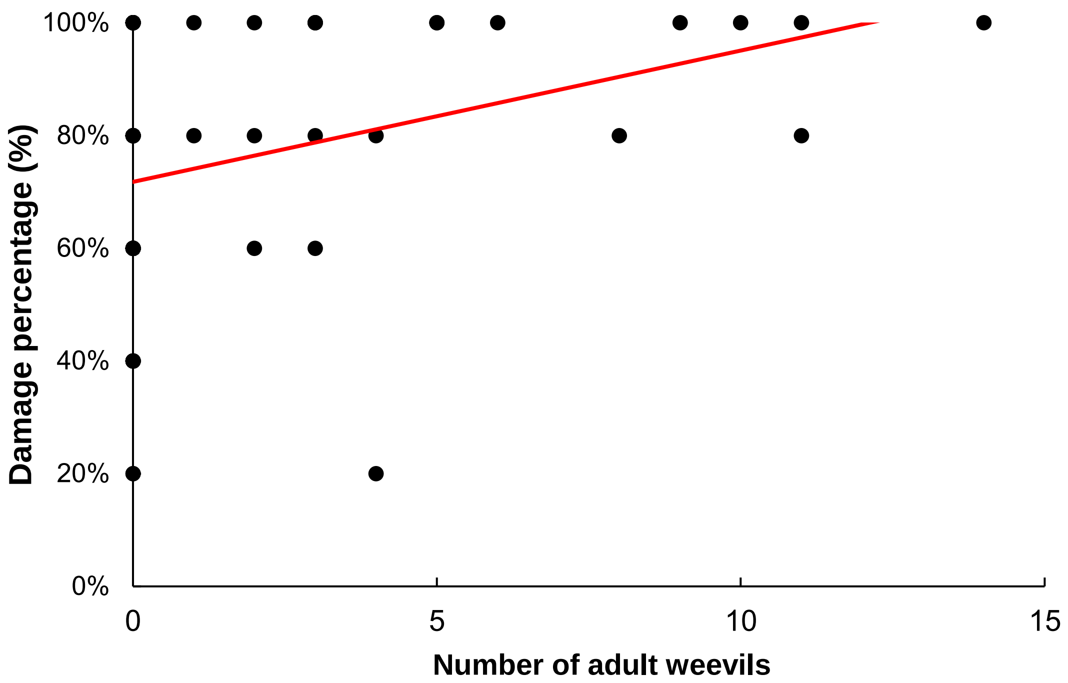

Figure 3.10 (A) Scatterplot illustrating the relationship between the mean number of adult weevils (*Sciobius tottus*, *Pantomorus cervinus* & *Eremnus atratus*) & the percentage fruit damage observed during the pre-thinning assessment in three apple blocks located in Ceres and Grabouw, Western Cape, South Africa.

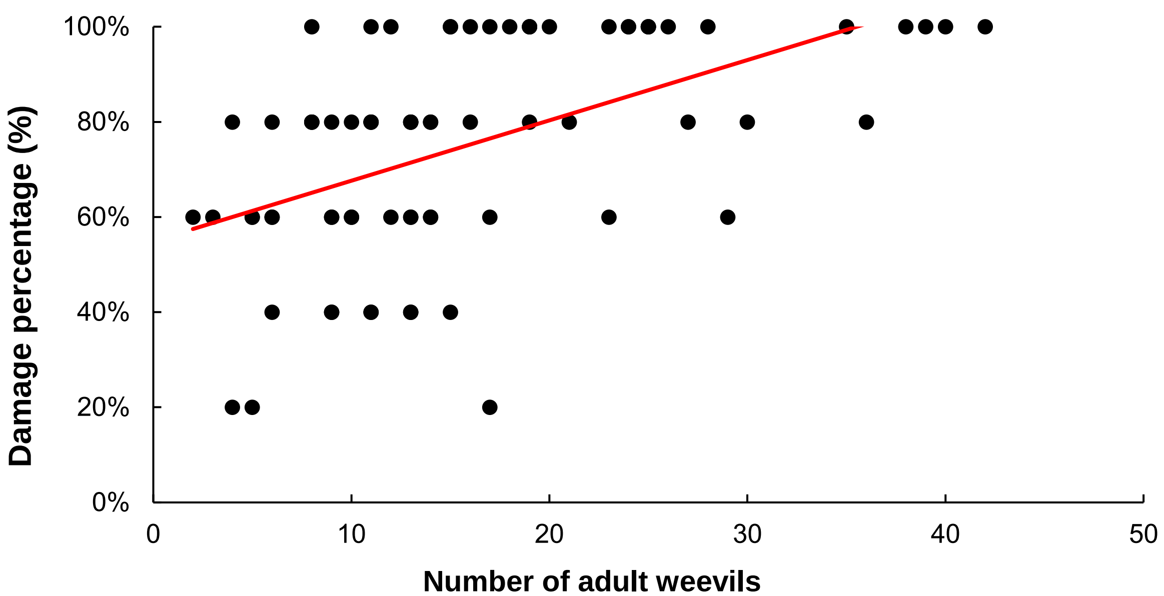

Figure 3.10 (B) Scatterplot illustrating the relationship between the mean number of adult weevils (*Phlyctinus callosus*) & the percentage fruit damage observed during the pre-thinning assessment in three apple blocks located in Ceres and Grabouw, Western Cape, South Africa.

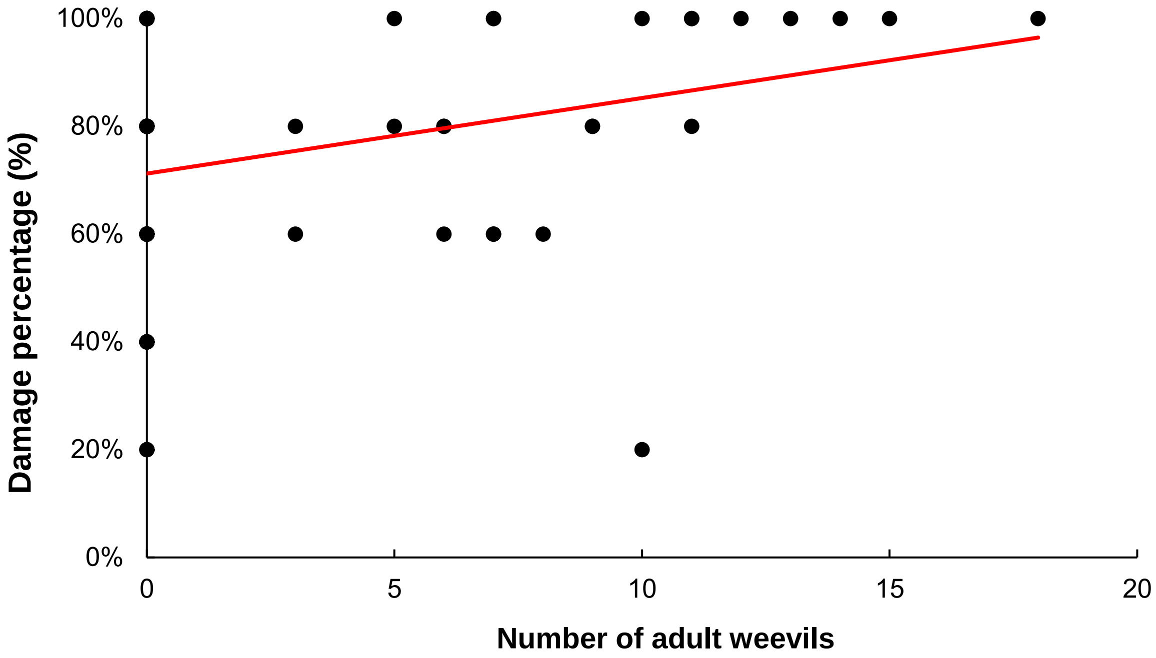

Figure 3.11 (A) Scatterplot illustrating the relationship between the mean number of adult weevils (*Sciobius tottus*, *Pantomorus cervinus* & *Eremnus atratus*) & the percentage fruit damage during the pre-harvest damage assessment, observed in three apple blocks located in Ceres and Grabouw, Western Cape, South Africa.

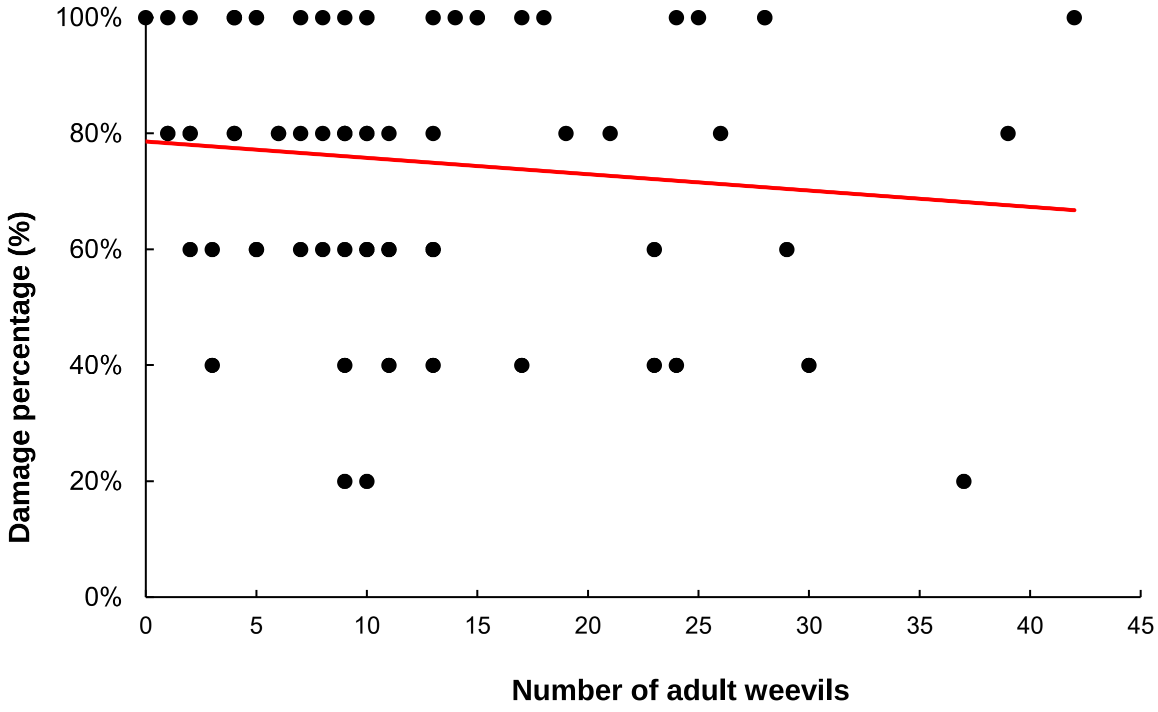

Figure 3.11 (B) Scatterplot illustrating the relationship between the mean number of adult weevils (*Phlyctinus callosus*) & the percentage fruit damage during the pre-harvest damage

assessment, observed in three apple blocks located in Ceres and Grabouw, Western Cape, South Africa.

Conclusion

Seasonal monitoring has shown that *P. callosus* is the dominant weevil species in both apple orchards and vineyards, and it is suggested that *P. callosus* represents a potential threat if the population is left untreated. In terms of the seasonal behavior of adult *P. callosus* in apple orchards and vineyards, our results showed the same trends as previous studies (Barnes & Giliomee 1992; Barnes 1987; Annecke & Moran 1982; Whitehead 1961). The other weevil species: *E. atratus*, *E. setifer*, *E. occatus, E. chevrolati*, *P. cervinus*, *N. leucoloma*, *T. carinatus* and *S. tottus* were present in low numbers and it is expected that these species will not cause any significant economic losses to apple orchards and vineyards unless the populations increase rapidly in the future. This study provided information on the occurrence of fruit damage, indicating the period during the seasonal cycle when crops are most susceptible to attack by weevils. In particular for *P. callosus*, the data indicate that most of the damage occurs prior to pre-thinning, i.e. December. The same trend was observed for weevil damage in both apple orchards and vineyards.

This study is important for understating the behavior of adult weevils in commercial apple orchards and vineyards that may be related to damage. It also documents which weevils should be targeted by growers for monitoring practices. It is strongly recommended that future research focus on temperature and relative humidity as they appear to be amongst the important environmental factors influencing weevil populations in deciduous fruits. Since the presence of weeds on the orchard or vineyard floor may influence weevils, this aspect is further investigated (see Chapter 4).

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Chapter 4: The effect of soil texture, soil bulk density and ground cover on the adult weevil population in apple orchards and vineyards

Abstract

Most weevils of the sub-family Entiminae are regarded as economically significant pests of ornamental, agricultural or forest plants. *Phlyctinus callosus* Boheman, 1834 is a key pest of a number of fruit crops including apples, grape berries and nectarines, where the adult feeds on the fruit leaving shallow scars, which reduce the marketing value of the fruit. Adult weevil populations were monitored every two weeks in three major fruit growing areas, all located in the Western Cape Province of South Africa were investigated for a period of nine months (September 2017 to May 2018). Nine weevil species of the sub-family Entiminae were collected in both apple orchards and vineyards during the investigation, namely: *Eremnus atratus* (Sparrmann, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Eremnus occatus* Boheman, 1843, *Eremnus setifer* Boheman, 1843, *Naupactus leucoloma* Boheman, 1840, *Pantomorus cervinus* (Boheman, 1840), *P. callosus*, *Tanyrhynchus carinatus* Boheman, 1836 and *Sciobius tottus* (Sparrmann, 1785). In addition, the effect of soil texture, soil bulk density and ground cover percentage on seasonal adult weevil populations was investigated. The association of adult weevils with ground cover plant species was investigated also. The results indicated that adult weevil abundance was non-significantly correlated with either soil bulk density or groundcover percentage. Furthermore, weevils were not associated with any particular groundcover plant species. Adult weevil abundance could be influenced by soil moisture in the cover crop micro-climate, soil temperature, soil penetrability, different cultural practices and relative humidity.

Introduction

The majority of curculionids (weevils) are injurious to cultivated crops (Scholtz and Holm 1985). All weevils are primarily phytophagous in the larval and adult stage feeding on all possible parts of the plants (Scholtz and Holms 1985). The feeding habits of adult and larval weevils can vary and this variation is useful in the classification of weevils into different subfamilies (Anderson 2002). In Southern Africa very limited information is available on the life cycle of most weevils (Scholtz & Holm 1985). *Phlyctinus callosus* Bohemian, 1834, is the most common weevil in vineyards and is also a significant pest of apples and pears (Marais 2003). *P. callosus* is the only major pest for which there is no reliable and practical monitoring system within deciduous fruit orchards (Barnes & Capatos 1989). Lack of accurate information on the exact times of emergence, and on fluctuations and population levels, makes it complicated for optimal management of *P. callosus*, resulting in uncertainty on the need for and correct timing of insecticide sprays (Barnes & Capatos, 1989).

The subfamily Entiminae (root weevils) is regarded as one of the most species-rich within the Curculionidae family (Marvaldi 1997). The root weevils are relatively similar to each other in their life cycle and habits (Diaz 2005). Depending on the species, adults deposit their eggs on the soil surface, fruits or foliage of host plants (Diaz 2005). Most root weevils lay their eggs in plant tissue, in holes prepared by the rostrum or in some cases on the host fruit tree (Anderson 2002). After the eggs hatch, the larvae drop to the soil surface and disperse into the soil to find plant roots (Diaz 2005). The larvae of some root weevils have a relatively long development period and feed on roots of grapevines and weeds (Allsopp *et al.* 2015). The pupae develop in an earthen cell in the soil (Allsopp *et al.* 2015). Most adults emerge from the soil and begin feeding, mating and laying their eggs (Anderson 2002; Diaz 2005). Adults of most root weevils are flightless and nocturnal, they climb most fruit trees to feed on buds and fruit trees (Annecke & Moran 1982). During the day most weevils hide in the soil or debris near the base of host plants or some crevices in the rough bark (Annecke & Moran 1982). This close association with cover crops and soil is the basis for which this study was conducted.

No previous studies in the Western Cape have attempted to assess the effect of soil texture, bulk density and cover crop density on weevil abundance in apple orchards and vineyards. Regarding the effect of soil texture, Duncan (2003) in the USA, found that soil texture has been implicated as a significant cause of variation in the control of *Diaprepes abbreviates* (Linnaeus, 1785) larvae by entomopathogenic nematodes. In the USA, Harris and Ring (1980), found that dry hardened clay soil hamper the emergence of *Curculio caryae* (Horn, 1873), but irrigation allowed normal emergence. Furthermore, the same authors, found that by withholding soil moisture, the emergence of *C. caryae* can be delayed, but it cannot be accelerated by the addition of moisture before the normal emergence window. Cresswell and Hamilton (2002), reported that silt and clay soil have a lower bulk density compared to sandy soils which usually have larger, but fewer pore spaces. Cresswell and Hamilton (2002), further reported that bulk density increases with compaction. Stephen *et al*. (1998), reported that soil penetrability acts as a passive barrier to the emergence of *C. caryae* and is a function of a percentage soil moisture. Blanchard (1981), measured soil penetrability, percent soil moisture and *C. caryae* emergence and found that the highest soil penetrability value in the study that permitted weevil emergence was 72.3 kg/cm^2 at a soil moisture of 16.4%. Stephen *et al*. (1998), found that drought conditions dried and hardened the upper layer of the soil which impeded the emergence of *C. caryae* while a portion of *C. caryae* population emerged through the cracks in soil and root channel. DAFF (2006), in the Western Cape, reported that *P. callosus* population was significantly reduced in vineyards were the floor was free of weeds.

Since weevils spend the majority of their life cycle within the soil and feed on weeds and grasses, it is highly probable that soil texture, bulk density and percentage groundcover would influence the abundance of weevils in apple orchards and vineyards. The aim was, therefore, to determine whether or not soil texture, soil bulk density and ground cover percentage influence the dispersal and abundance of weevils in apple orchards and vineyards.

Methods and materials

Description of study sites

Fruit growing areas (apple orchards and vineyards) with a history of weevil infestations were selected in three regions in the Western Cape Province: Stellenbosch, Grabouw & Ceres (Table 2.1, Table 2.2, Chapter 2)). Details of each site, including the location of the block, size, cultivar, irrigation type, annual rainfall and age are all listed in Table 4.2. The annual rain fall weather data was derived from 90 meter resolution long term (± 30 years) climate layers developed by Van Niekerk & Joubert (2011).

Adult weevil monitoring

Sampling to monitor the presence of adult weevils was carried out for a period of nine months (September 2017 to May 2018). The methods described by De Villiers & Pringle (2008) were used as reference to monitor adult weevils. The selected blocks were monitored every two weeks for adult weevils, using marked weevil bands (single-sided cardboard bands about 15 cm wide), tied around the stem of the apple trees and vines with corrugated side against the stem). Adult weevils crawled into the bands during the day to shelter. In the grapevine blocks, 20 vines, evenly spaced throughout the block, were selected. In the apple orchards, 25 trees, evenly spaced throughout the block, were selected (Brown & Pringle 2008). A white cloth was tied and placed at the base of the stem since most adult weevils feign death and fall to the ground when disturbed. Then single-sided cardboard bands were dislodged from the stems. Weevils were then collected and stored in marked containers during transportation back to the laboratory. See Figs. 2.1 and 2.2 (Chapter 2) for images depicting sampling methods.

Soil sampling for soil texture and bulk density analysis:

Soil sampling design

Soil samples (soil texture sampling and soil bulk density) were collected in early November 2017 (at this stage soil was easily penetrated) between the rows of each orchard and vineyard used when scouting for weevils, therefore a total of three orchards and three vineyards. A grid sampling layout, adopted from Pennock & Yates (2008), was employed to establish five parallel transects in three apple orchards and three vineyards (Figure 4.1). In the apple blocks, thirteen trees were selected as shown in Fig. 4.1 (A) and in the vineyard blocks, ten trees were selected as shown in Fig. 4.1 (B). The design was selected to ensure homogeneity. Soil samples were collected adjacent to trees as indicated in Fig 4.2 (B).

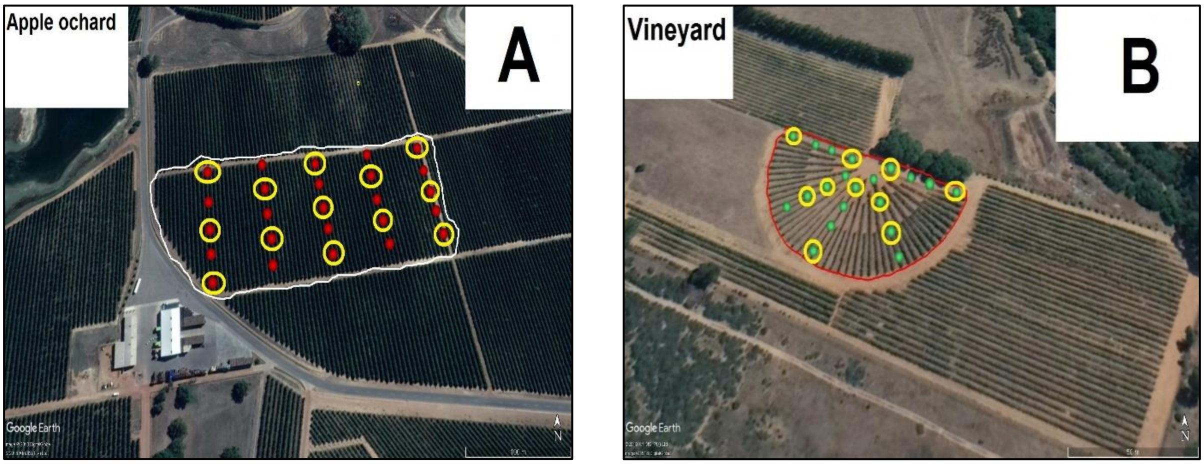

Figure 4.1. A 2 ha Apple block **(A)** and a 0.8 ha vineyard block **(B)** used during the study to indicate sampling design. The yellow oval rings represent the selected trees where soil samples were taken, the green and red dots represent all the trees which were used for weevil monitoring purposes and the red (vineyard) and white (apple) line represents the selected block within the sites.

Soil texture and chemistry sampling

The sampling technique for soil texture analysis were adapted from Pennock & Yates (2008). The following steps were undertaken in sampling soil texture (Fig. 4.2): **(A)** soil samples were collected by first removing the top surface of the soil (1 to 3 cm)**, (B)** an auger (4 cm in diameter) was driven to a depth of 8 cm and soil samples were drawn, **(C)** sample was placed in a 2 liter bucket, **(D)** the sub-samples (13 in apple orchards and 10 vineyards) were thoroughly mixed and foreign materials like roots, stones, pebbles, and gravels were discarded, **(E)** the bulk was reduced to half a kilogram by dividing the soil into four equal squares (quartering), **(F)** two opposite quarters were thoroughly mixed, **(G)** transferred into clean and marked zip lock bags and the remaining two quarters were discarded. The samples were submitted to Bemlab (Somerset West, South Africa) for soil texture and chemical analysis.

Figure 4.2. Steps indicating the steps followed for soil texture analysis.

Soil bulk density analysis

The methods employed for soil bulk density analysis was adapted from Hao *et al.* (2008). The following steps were undertaken in sampling soil bulk density (Fig. 4.3): **(A)** Soil samples were obtained by first driving a metallic rod (4.5 cm in diameter) to a depth of 4 cm through an undisturbed soil surface, **(B)** the soil core was gently removed from the soil and any signs of compression or shattering in the rod were examined, **(C)** a sharp blade was used to level the base of the soil core after soil extraction, **(D)** soil sub-samples (13 in apple orchards and 10 vineyards) were placed in clean and labeled zip lock bags, and taken to the laboratory at Stellenbosch University (Department of Conservation Ecology and Entomology). **(E)** Samples were then transferred to labeled paper containers (10 x 10 cm), **(F)** placed in an oven set to 60°C over a period of 48 hours, **(G)** oven dried samples were then weighed and the mass of the dry soil recorded and then bulk density was obtained using the following formula:

- by first calculating the volume (V) (cm^3) of the rod from the known height or depth (h) (cm), radius squared (r) (cm) and pi (π) (3.14159265) :

$$V = (\pi \times r^2 \times h)$$

- And soil bulk density (D_b) (g/cm^3) was obtained from the ratio of the oven-dried soil in grams (M_s) (g) to the bulk volume of the soil (V):

$$D_b = M_s / V.$$

Figure 4.3. Steps showing all the necessary procedures followed for soil bulk density analysis.

Ground cover estimation and identification

Ground cover estimation was conducted in the inter-rows of the apple orchards and vineyards. Grabandt (1985) was used as a reference for plant identification. The method for ground cover estimation was adapted from Canfield (1941). Ground cover estimation was conducted on the same trees used for weevil monitoring (Figs. 2.2, Chapter 2). A stainless steel rod (2 meter in length) with 10 equally spaced non-adjustable nails (Fig. 4.6) was used to estimate the ground cover percentage. The stainless steel rod was placed at the base of the tree (perpendicular) leading into the row. If the nails touched grass, organic matter, pebbles, weeds or dead plants, a value of one was assigned, which meant ground cover was present. Nails not touching anything were assigned a value of zero (indicating ground cover was absent). If the nail touched ground cover, the ground cover was identified and the number of intercepted ground cover summed in each block. The numbers where the nails touched ground cover were added and divided by 10 (referring to the ten units on the metal rod), then multiplied by 100 to obtain the percentage ground cover. All weeds touching the metal rod were also identified using Grabandt (1985).

Figure 4.4 Stainless steel rod (2 m in length) with 10 equally-spaced non-adjustable nails used to estimate the ground cover percentage.

Data analysis:

Rank abundance

Rank abundance plot was computed by first calculating the total number of all weed species intercepted in each commercial block during ground cover estimation (only weeds). These values were then converted to log abundance value which gave each plant species a ranking from 1 to 9 in apple blocks and 1 to 10 in vineyard blocks according to their total abundance in the three fruit growing areas (Ceres, Stellenbosch & Grabouw).

Correlation of seasonal population (number of adult weevils) with ground cover percentage and soil bulk density.

The ground cover percentage/block, soil bulk density/block and the average number of adult weevils/block were analyzed using the non-parametric Spearman (rank-order) correlations, as data were not normally distributed, and the assumptions for normality were violated irrespective of transformation. Two dimensional scatterplots illustrating the correlation between the ground cover percentages versus the number of adult weevils; and soil bulk density and the number of adult weevils, were used to graphically present data. Analyses were conducted in Statistica v.13.2 (Stat-Soft Inc., 2013).

Correlation with soil chemistry and soil texture

Soil texture, soil chemistry and the number of adult weevils were analyzed using the non-parametric Spearman (rank-order) correlations, as data were not normally distributed, and the assumptions for normality were violated irrespective of transformation. Analyses were conducted in Statistica v.13.2 (Stat-Soft Inc., 2013).

Correspondence analysis

A multiple correspondence analysis (MCA) was used to compare whether adult *P. callosus*, *E. setifer* and *E. occatus* were associated with any weed species in the six apple orchards and vineyard blocks, with weevil species as column variables and weeds as supplementary column variables. This method is suitable for categorical data, as it makes no distributional assumptions (Compton, 1994).Only the four most common weed species were used in the analysis. The analysis was performed in Statistica v.13.2 (Stat-Soft Inc., 2013).

Results

The mean seasonal population density among between different vineyard and apple orchard blocks is illustrated in Fig. 4.5. Nine weevil species of the sub-family Entiminae were collected during the survey, eight of them were collected in vineyards (*Tanyrhynchus carinatus* Boheman, 1836, *Phlyctinus callosus* Boheman, 1834, *Eremnus setifer* Boheman, 1843, *Eremnus atratus* (Spearman, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Eremnus occatus* Boheman, 1843, *Pantomorus cervinus* (Boheman, 1840) and *Naupactus leucoloma* Boheman, 1840 and four were collected in apple orchards (*P. callosus, E. atratus, P. cervinus* and *Sciobius tottus* (Spearman, 1785). In general, apple orchards recorded higher and more even abundance than vineyards.

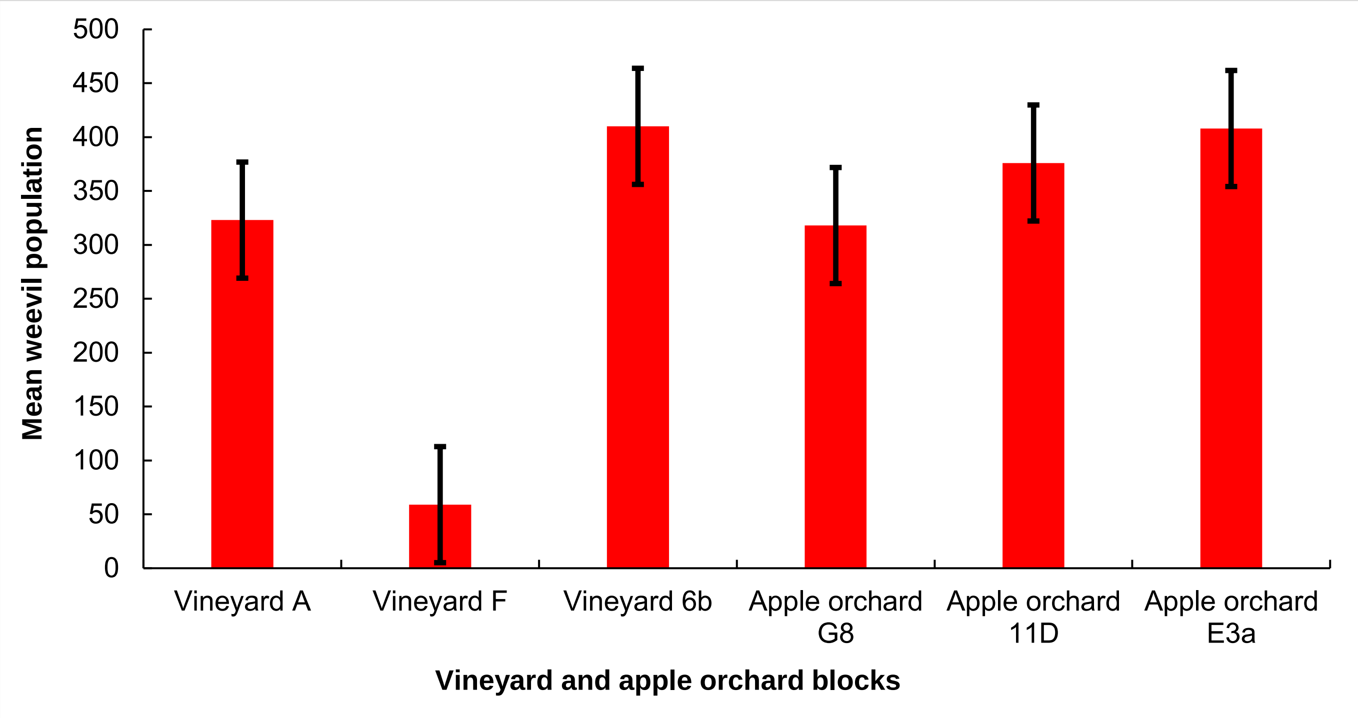

Figure 4.5 Mean weevil density (Seasonal population) (± S. E.) (*Eremnus atratus, Eremnus chevrolati, Eremnus occatus, Eremnus setifer, Naupactus leucoloma, Pantomorus cervinus, Phlyctinus callosus, Sciobius tottus & Tanyrhynchus carinatus)* collected in Ceres (block 11D & G8), Grabouw (block E3a & 6b) and Stellenbosch (block A & F) for a period of nine months (September 2017 to May 2018) in three different apple orchard and vineyard blocks.

Weed survey and abundance

The weed species recorded in both vineyards and apple orchards during the survey are listed in Table 4.3. The rank abundance of each species in vineyards is illustrated in Fig. 4.6 and in apple orchards is in Fig. 4.7. Fig. 4.6 illustrates that *Plantago lanceolate,* was the most dominant species in vineyards And that *Hypochaeris radicata* was the most dominant species in apple orchards (Fig. 4.7).

Table 4.3. List of weed species recorded in three study sites, namely; Stellenbosch, Grabouw and Ceres for a period of 9 months (September 2017 to May 2018).

Weeds	**Stellenbosch**		**Grabouw**		**Ceres**	
	Vineyard A	**Vineyard F**	**Apple orchard E3a**	**Vineyard 6b**	**Apple orchard G8**	**Apple orchard 11D**
• *Amaranthus retroflexus* (Red pigweed)						✓
• *Bidens pilosa* (Common black-jack)			✓	✓		✓
• *Chenopodium album (White goose foot)*		✓		✓	✓	✓
• *Conyza bonariensis* (Flax-leaf fleabane)	✓	✓				
• *Datura stramonium* (Thorn apple)						✓
• *Dimorphotheca sinuate* (Cape marigold)			✓	✓	✓	✓
• *Hypochaeris radicata* (Hairy wild lettuce)	✓		✓	✓	✓	✓
• *Lactuca virosa* (Wild lettuce)			✓	✓	✓	✓
• *Malva parviflora* (Small mallow)					✓	
• *Medicago polymorpha (Bur clover)*		✓		✓	✓	

Table 4.3 Continued. List of weed species recorded in three study sites, namely; Stellenbosch, Grabouw and Ceres for a period of 9 months (September 2017 to May 2018).

• *Oxalis corniculata* (Creeping-sorrel)		✓				✓
• *Plantago lanceolata* (Narrow-leaved ribwort)		✓	✓	✓		✓
• *Polygonum aviculare* (Prostrate knotweed)			✓		✓	✓
• *Portulaca oleracea* (Purslane)					✓	✓
• *Raphanus raphanistrum* (Wild radish)	✓	✓	✓			
• *Rapistrum rugosum (Wild mustard)*	✓			✓		✓
• *Sonchus oleraceus* (Sow thistle)		✓		✓		
• *Taraxacum officianale* (Common dandelion)	✓	✓			✓	
• *Trifolium repens* (White clover)	✓		✓	✓	✓	
• *Vicia benghalensis* L. (Narrow-leaved purple vetch)	✓	✓				

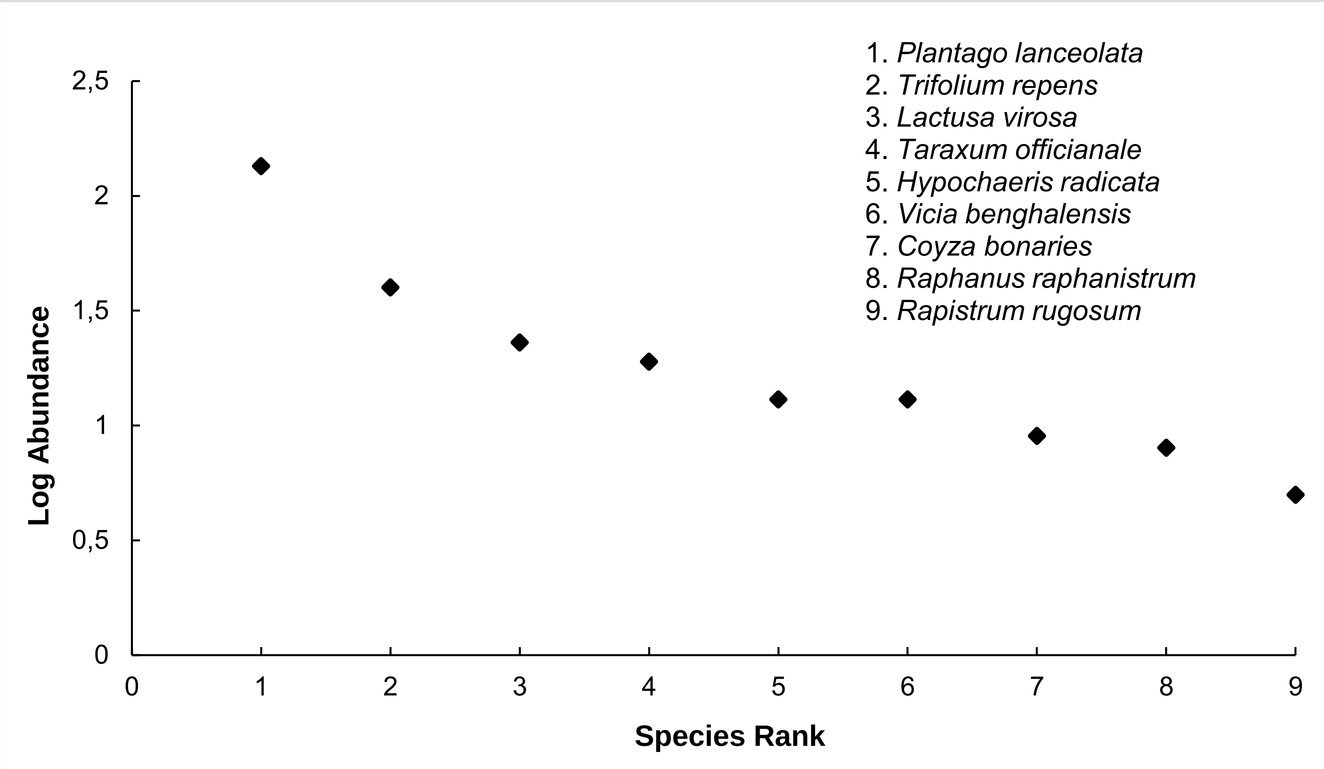

Figure 4.6 Rank abundance plot of the nine most prominent weed species found in three vineyard blocks (two in Stellenbosch and one in Grabouw), based on a nine month survey (September 2017 to May 2018).

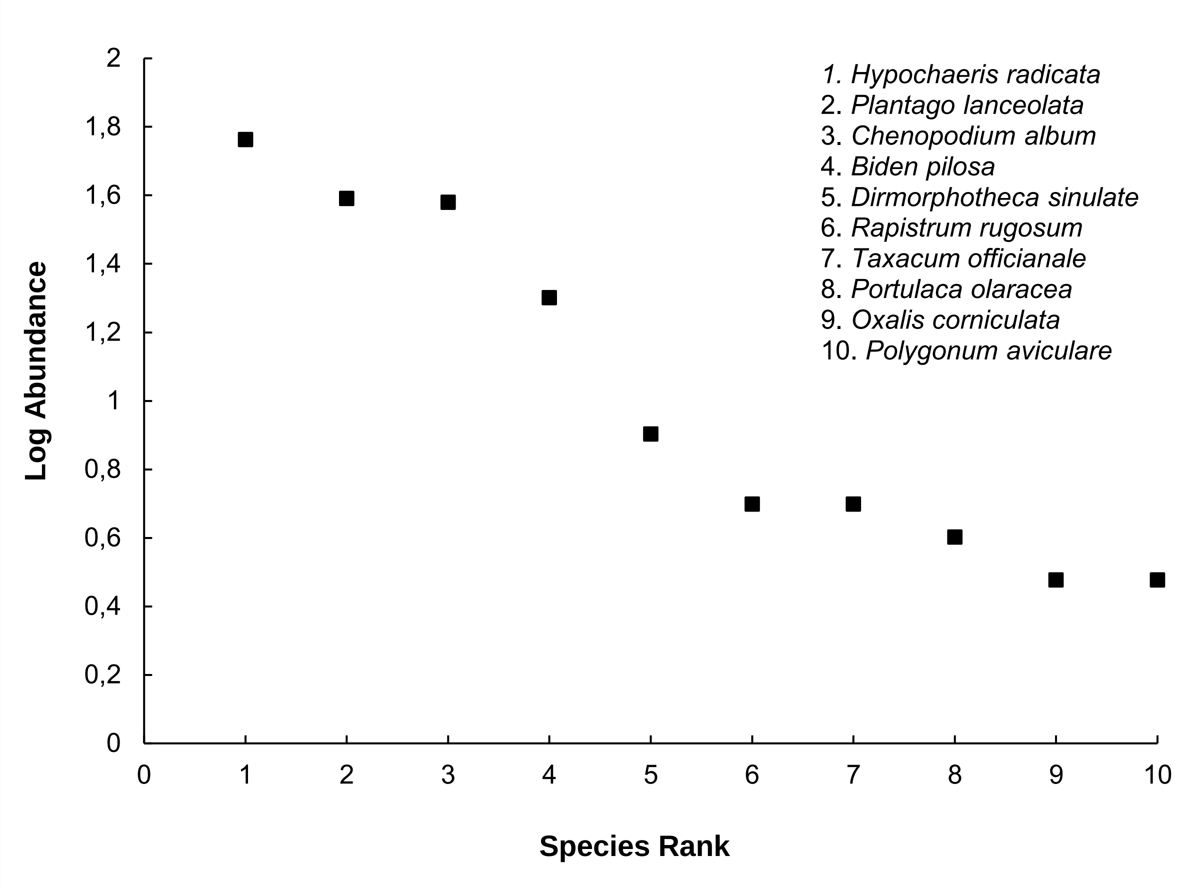

Figure 4.7 Rank abundance plot of the 10 most prominent weed species found in three apple orchards blocks (two in Ceres and one in Grabouw), based on a nine month survey (September 2017 to May 2018).

Correlation of seasonal population (adult weevil population) with ground cover percentage

Seasonal population (adult weevil's abundance) was not correlated ($P < 0.05$) with ground cover percentage in either apple orchards or vineyards (Table 4.4).

Soil texture and soil analysis

Table 4.5. indicates the soil texture of six orchard and vineyard blocks in three different fruit growing regions. Overall, soil texture varied from sandy, sandy loam to sandy clay loam. Both the highest and lowest weevil abundance was found in sandy loam soils, indicating that soil texture was likely not a factor influencing weevil occurrence during this study. As indicated in Table 4.6 (A), there was no significant correlation ($P < 0.05$) between soil texture parameters [clay (%), silt (%), sand (%), fine sand (%), medium sand (%), course sand (%) and stone (%)]

and mean weevil population. Table 4.6 (B) indicates soil chemistry analysis showed no significant correlation ($P < 0.05$) between soil chemistry parameters [ph, resistance, sodium (Na), stone, phosphorus (P), Zinc (Zn), Manganese (Mn), iron (Fe), soluble salt (Soluble S) and carbon] and mean weevil population.

Seasonal population (adult weevil abundance) showed a negative trend with soil bulk density, but was non-significantly correlated ($P < 0.05$) with soil bulk density in both apple orchards and vineyard blocks, as shown in Table 4.7.

Table 4.4. Non-parametric (Spearman rank order) correlation in apple orchards and vineyards for seasonal population of weevils versus ground cover percentage.

Block	**r**	**P**
Vineyard 6b	0.2703	0.2491
Vineyard A	-0.4096	0.0729
Vineyard F	-0.1873	0.4291
Apple orchard 11D	0.1716	0.4120
Apple orchard G8	0.3582	0.0787
Apple orchard E3a	-0.0180	0.9334

Table 4.5. Soil texture in the three fruit growing areas of the apple and vineyards production in the Western Cape (Stellenbosch, Ceres & Grabouw).

Site	Block	Clay (%)	Silt (%)	Sand (%)	Fine sand (%)	Medium sand (%)	Course sand (%)	Stone (%)	Classification	Mean weevil population
Stellenbosch	Vineyard A	33	12	55	38.4	8.4	8.2	0.0	Sandy clay loam	323
	Vineyard F	19	14	67	44.3	17.5	5.2	0.0	Sandy loam	59
Grabouw	Apple orchard E3a	17	18	65	42.4	18.2	4.4	0.0	Sandy loam	408
	Vineyard 6b	11	10	79	43.4	24.2	11.6	1.0	Sandy loam	410
Ceres	Apple orchard G8	5	6	89	55.4	24.4	9.2	0.9	Sandy	318
	Apple orchard 11D	23	26	51	35.0	5.0	11.0	5.3	Sandy clay loam	376

Table 4.6 (A) Spearman rank order correlations for soil texture analysis and mean weevil population in the three fruit growing areas of the apple and vineyard production regions studied in the Western Cape (Stellenbosch, Ceres & Grabouw).

	Mean weevil population	Clay (%)	Silt (%)	Sand (%)	Fine sand (%)	Medium sand (%)	Course sand (%)	Stone (%)
Mean weevil population	1.000000	-0.600000	-0.428571	0.428571	0.142857	0.657143	0.257143	0.151794

Table 4.6 (B) Spearman rank order correlations for soil chemistry analysis and mean weevil population in the three fruit growing areas of the apple and vineyards production regions studied in the Western Cape (Stellenbosch, Ceres & Grabouw).

	Mean weevil population	pH	Resistance	Stone	P Bray II	Na	Potassium	Ca	Mg	Cu	Zn	Mn	Beryllium	Fe	Soluble S	Carbon
Mean weevil population	1,000000	-0,666737	0,753702	0,092582	-0,542857	-0,371429	-0,657143	-0,428571	0,085714	-0,771429	-0,428571	-0,600000	-0,289886	0,028571	-0,371429	0,085714

Table 4.7. Spearman rank order non-parametric correlations in both apple orchards and vineyards for seasonal weevil population versus soil bulk density.

Block	**Spearman**	
	r	**P**
Vineyard 6b	-0.20	0.57
Vineyard A	-0.42	0.22
Vineyard F	-0.31	0.4863
Apple orchard 11D	0.07	0.82
Apple orchard G8	0.08	0.50
Apple orchard E3a	-0.22	0.46

Correspondence analysis

Dimension 1 accounted for 59.09% of the variation in the graph and dimension 2 accounted for 32.87 % (Fig. 4.8). *Eremnus setulosus* was strongly associated with *E. occatus* at a high order of magnitude on dimension 1, but neither of these weevils were associated with any of the major weeds. In fact, their absence was associated with the weeds, but around the origin of the graph, indicating a low order of magnitude.

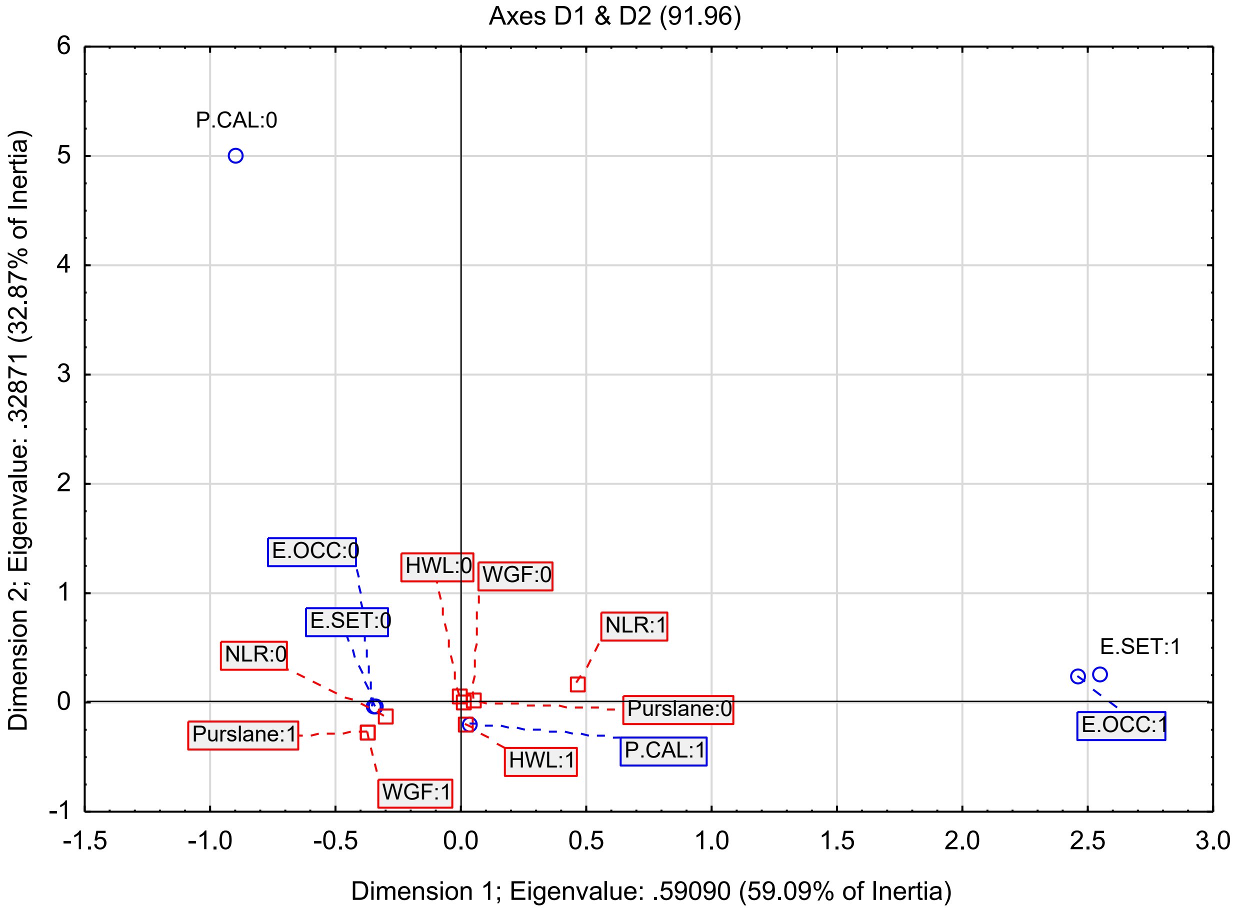

Figure 4.8 Correspondence analysis showing the association between adult *Phlyctinus callosus* (P.CAL), *Eremnus setifer* (E. SET) and *Eremnus occatus* (E. OCC) in relation to ground cover (Hairy wild lettuce (HWL), Purslane, Wild goose feet (WGF) and Narrow leaf ribwort (NLR)) located in three fruit growing areas, namely, Stellenbosch, Grabouw and Ceres sampled over a period of 9 months (September 2017 to May 2018). One (1) behind each abbreviation refers to presence, while zero (0) refers to absence.

Discussion

Overall, *P. lanceolata* was the dominant groundcover species in vineyards and *H. radicata* was the most abundant species recorded in apple orchards. This may explain why *P. callosus* was weakly associated with these species in the correspondence analysis, but may indeed point to these weeds also being good hosts. Other weeds in both apple orchards and vineyards were recorded in low densities, such as *Oxalis corniculata*, *Chenopodium album*, *Biden pilosa*, *Dirmorphotheca sinulate*, *Rapistrum rugosum*, *Taxacum officianale*, *Portulaca olaracea, Polygonum aviculare, Lactusa virosa, Vicia benghalensis, Coyza bonaris* and *Trifolium repens*. Similarly, in deciduous fruit orchards in Grabouw, Western Cape, Barnes & Pringle (1989) found that most adult *P. callosus* could be collected at any time of the year on *P. lanceolata* while experienced difficulty in collecting it on any other ground cover species. In their study for oviposition by *P. callosus* in deciduous fruit orchards, Barnes & Pringle (1989) found that adult *P. callosus* laid more eggs on *Lolium multiflorum* than on either *Paspalum dilatatum* or *P. lanceolata* and more eggs were also laid on *H. radicata* than on *P. dilatatum*. Barnes (1987), asserted that the larvae of *P. callosus* don't disperse much in the soil, which suggests that in vineyards dominant weeds such as *P. lanceolata* were the preferred host for the larvae of *P. callosus*; also in apple orchards it is also suspected that dominant weeds such as *H. radicata* and *P. lanceolata* were preferred hosts for adults *P. callosus* either for sheltering or oviposition. Barnes & Pringle (1989) working in deciduous fruits, suggested that *P. lanceolata* was more favored than *H. radicata* either for sheltering or oviposition. In vineyards, *P. lanceolata* was the most dominant weed species of all the groundcover composition, which suggest that weevils were also more likely to prefer one of the two species as they were found in abundance. Similarly in New Zealand kiwi fruit orchards, Logan *et al.* (2008) in their study for larval survival of *Pantomorus cervinus* (Boheman, 1840), found that *T. repens* were preferred host compared to *Lolium perenne.* Johnson *et al*. (2004), in their laboratory study for host plant preference by the root feeding clover weevil, *Sitona lepidus,* found that the newly hatched larvae preferred the roots of *T. repens* more compared to other groundcover species. The non-significant correlations of weevil abundance with cover crop density or percentage ground cover in the present study indicates that the weevil population is not driven by the attributes of groundcover.

The soil texture in all study areas were almost similar with sandy loam to sandy clay loam with a slight difference in soil texture, with no apparent relevant relationship with weevil abundance. Likewise the correlation of soil bulk density with weevil abundance was non-significant.

Barnes (1989) suggested that in autumn, temperature plays a significant role for the larvae to pupate as soon as they reach maturity. Barnes (1989) further suggested that relative humidity and soil moisture in the cover crop micro-climate, as driven by climate and different cultural practices are considered to be the key factors influencing the number of generations per year of *P. callosus* in fruit orchards. Barnes (1989) found that soil with adequate moisture gradient enable larval penetration into the soil, and well drained and warmer soil provides a favorable habitat for the development of the immature stages, highlighting the importance of soil texture. Cover crops influence soil moisture soil temperature, soil penetrability, and relative humidity, which could be factors influencing weevil abundance in both apple orchards and vineyards, but further detailed studies of the micro-climate are required to test these hypotheses.

In conclusion, it would therefore be advisable to assess the impact of soil parameters and micro-climate in more detail, using a larger sample size in apple and vineyards blocks with weevil infestations. A larger sample size would impact on the amount of data obtained from increased sampling efforts, such as obtaining data on seasonal abundance and hence pest status.

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Chapter 5: General discussion and recommendations

Phlyctinus callosus Boheman, 1834 is regarded as the only curculionid (weevil) causing serious damage on apples and in vineyards (Marais 2003; Marais & Barnes 2004), however, the impact and extent of damage caused by other species of weevils known to occur in apple orchards and vineyards is not well understood. At the beginning of this study limited information was available on the diversity and of the weevils known to occur on vineyards and apple orchards in the Western Cape, South Africa. Moreover, population surveys of weevils in apple orchards and vineyards here focused mainly on *P. callosus* as it is considered a key weevil pest in apple and nectarine, with very little information is available on the seasonal ecology of other weevils. As well as clarifying the identity and prevalence of other species of weevils, this study also aimed at assessing the effects soil texture, soil physical parameters and ground cover occurrence on the weevil population in apple orchards and vineyards. The information gathered from this study could be useful in developing long-term control methods for suppressing these pests.

Chapter 2: Diversity and morphological review of common weevils (Coleoptera: Curculionidae) in vineyards and apple orchards in the Western Cape Province, South Africa.

The diversity of weevils in cultivated apple orchards and vineyards is, however, not well known and it could be possible that other species also cause economic damage to fruits in the Western Cape. At the beginning of this study it was not clear whether *P. callosus* is still the most dominant/common weevil in apple orchards and vineyards. It was shown that the biodiversity and abundance of weevils substantially differed between the selected study areas. The study shows the first detailed account of the biodiversity of weevils occurring in both vineyards and apple orchards, therefore providing the first identification key of weevil species present in both cultivated apple orchards and vineyards. Nine weevil species were collected in three apple orchard blocks and three vineyard blocks namely: *Tanyrhynchus carinatus* Boheman, 1836, *P. callosus*, *Eremnus setifer* Boheman, 1843, *Eremnus atratus* (Sparrmann, 1785), *Eremnus chevrolati* Oberprieler, 1988, *Eremnus occatus* Boheman, 1843, *Sciobius tottus* (Sparrmann, 1785), *Pantomorus cervinus* (Boheman, 1840) and *Naupactus leucoloma* Boheman, 1840. Furthermore, the study has indicated that *P. callosus* was still the most dominant weevil species recorded in both apple orchards and vineyards which suggests that it is still the weevil causing significant damage, while the other species were found in lower abundance, but shouldn't be neglected as a potential threat to cause damage if population densities increase in future. However, the extent of damage inflicted by each weevil species it not well known and this was therefore investigated further in Chapter 3. *Eremnus setulosus* Boheman, 1834, regarded as one of the weevils causing direct damage to grape berries (Marais 2003), was not collected during the study. Furthermore, *Eremnus cerealis* Marshall 1921, has been previously recorded to occur in vineyards in the Western Cape (Allsopp *et al*. 2015; Annecke & Moran 1982), but was not recorded in this study. The study was conducted during a period of drought, which may have impacted on the occurrence of some weevils. *Eremnus occatus* Boheman, 1843 was recorded for the first time in vineyards in the Western Cape. This study also shows that diversity was much higher in vineyards compared to apple orchards.

Wrongly identified pests can lead to unsuccessful pest control measures (Flint 2012). Many insect pests look similar and some could be easily confused with beneficial organisms (Flint 2012). Morphologically some weevils are also similar and difficult to distinguish, thus a taxonomic key was compiled using relevant distinguishing morphological features such as the rostrum shape and size, the general body shape and vestiture, the pronotum shape and the presence of a tooth on femora. This dichotomous key could be useful for future researchers exploring the impacts of weevils in cultivated vineyards and apple orchards.

This is the first study to provide an illustrated morphological key for accurate species identification of weevil pests in apple orchards and grapevines. The pest status of *P. callosus* was confirmed as still being the dominant weevil pest in apple orchards and vineyards, but due to the polyphagous and largely indigenous nature of all weevils collected, they do all remain a threat to various crops grown in the Western Cape, and accurate monitoring will assist to prevent quarantine issues in export fruit.

Chapter 3: Seasonal trends – monitoring, damage assessment and pest status.

Information of what is known about the biology and monitoring of important pests is necessary for choosing the best strategy for making informed decisions about pest status (Flint 2012). Lack of accurate information on the exact times of emergence, and on fluctuations of population levels, of weevil complexes complicate efficient pest management, resulting in uncertainty on the need for and correct timing of insecticide spray (Barnes & Capatos, 1989). Seasonal cycles of adult *P. callosus* in cultivated apple orchards and vineyards observed during the study were similar to those reported by Barnes & Giliomee (1992), Barnes (1987), Annecke & Moran (1982) and Whitehead (1961).

Results indicated that *P. callosus*, accounted for more than 78% of the weevil population collected in apple orchard and vineyards. Weevils emerged in late October and in vineyards, reached a peak in late November, while in apple orchards weevil populations peaked in late December. These two peaks observed during the study showed two possible generations to target for the application of control measures. During the survey in vineyard blocks sprayed with herbicides in late October it was observed that adult weevil populations were drastically reduced in late December. This suggests that by spraying herbicides earlier, or even prior to adult weevil emergence can significantly reduce the weevil population.

Abiotic factors impact the growth, development, and ability of an organism to thrive in an ecosystem (Flint 2012). Furthermore, when temperatures are warmer, more generations of invertebrates can be expected per year (Flint 2012). The average maximum temperature recorded for all the three study sites from August 2017 to May 2018 was 20 to 25 °C and the average maximum relative humidity between 55 to 70 % was recorded from August 2017 to May 2018 (Agrimet, Stellenbosch). The results from the weather data has shown that the maximum average temperature was recorded from November 2017 to December 2017. The peak in weevil population during the month of November 2017 and December 2017 could be influenced by the maximum mean temperature recorded at this stage in both apple orchards and vineyards.

The study has shown that significant fruit damage in both apple orchards and vineyards took place during the pre-thinning assessment (early December) and to a lesser extent the pre-harvest assessment (mid-March). During the pre-thinning assessment in both apple orchard and vineyards, fruit damage was positively and significantly correlated (Spearman $r = 0.57$, $P < 0.0000012$) with the number of *P. callosus* observed and was also positively and signicantly correlated (Spearman $r = 0.357035$, $P < 0.001664$) with the rest of weevil complex combined, but their correlation were not as strong as that of *P. callosus* with the fruit damage. Literature has highlighted that at this stage (pre-thinning assessment) of the year the mandibular cusps of adult *P. callosus* were still sharp enough to penetrate the skin of apple fruit and as weevils mature monthly the mandibles wear out (Barnes & Giliomee 1992). Furthermore, the *P. callosus* population was too low during the pre-harvest assessment to cause any significant damage. This suggests that *P. callosus* is still the most important curculionid causing economic damage to cultivated apple orchards and vineyards. Previous literature has shown that the presence of other weevils occurring in vineyards do not cause damage to grape berries, except *P. callosus*, *E .cerealis* and *E. setulosus* (Allsopp *et al.* 2015). The present study indicated further that in the absence of *P. callosus* during the Spearman correlation (Spearman: $r = 0.41$, $P < 0.001113$) of weevil abundance with fruit damage was positive and significantly correlated, *E. occutus* being the most dominant weevil, so its presence (for the first time) might suggest that it also contributes to the damage recorded in vineyards along with *P. callosus*.

This study provided information on the occurrence of fruit damage, indicating the period during the seasonal cycle when crops are most susceptible to attack by weevils. As the symptoms of fruit damage are very non-specific to any one species, this study was therefore able to provide better insights into the identity of those species of weevil causing damage in apple orchards and vineyards.

It is strongly recommended that future research focus on temperature and relative humidity as they appear to be important environmental factors influencing weevil populations in deciduous fruits. Since the presence of weeds either on the orchard or vineyard floor may act as hosts for sheltering weevils, this aspect is further investigated (see Chapter 4).

Chapter 4: Abiotic and biotic factors – effect of soil texture, soil bulk density and ground cover on the adult weevil population in apple orchards and vineyards.

The abiotic components that influence most organisms in the managed crops include water, soil, light, mineral, gases, temperature and climate (Flint 2012). DAFF (2006), reported that the absence of biotic factors such as ground cover in vineyards, significantly reduced the population of *P. callosus.* The action of one species in an ecosystem can impact the population growth and well-being of many other species (Flint 2012). According to the log abundance results, weed dominance varied between apple orchards and vineyards. This may explain why *P. callosus* was weakly associated with these species in the correspondence analysis, but may indeed point to these weeds also being hosts. The results also showed that ground cover density was too low to cause any significant effect to the population of adult *P. callosus.* The influence of soil texture and soil chemistry was not significant relation to weevil populations. This results indicated that ground cover percentage was non-significantly correlated with adult weevil population. These results suggest that the presence of ground cover, irrespective of density, has little impact on the population of weevils. Furthermore, literature has emphasized that a vineyard floor kept free of weeds, significantly reduces the number of weevils (DAFF 2006). Since weevil larval stages occur in the soil, it was thought that soil bulk density might influence the larval abundance in the soil. The results have shown that soil bulk density was non-significantly correlated with adult weevil abundance. The study was unable to pinpoint the main abiotic and biotic factors affecting weevil population in apple orchard and vineyards within the nine month period.

This study may be useful for future researchers to expand on the limited information on the effects of abiotic and biotic factors on the population of weevils in commercial apple orchards and vineyards and may also improve our understanding for effective control and management of weevils.

Research recommendations

One of the key limitations to this study was the challenge in attaining and locating study sites with a confirmed history of previous weevil infestations and, more importantly, sites with different soil characteristics, such as soil texture. These would have enabled an in-depth field survey for assessing and comparing weevil populations from different soil textures, soil chemistry and soil bulk density. Increasing sample sizes should be considered for future long term studies. The drought in the Western Cape during field surveys might have further been another constraint in limiting the weevil diversity in vineyards and apple orchards, as two of the most common weevils previously reported in vineyards were not recorded in our field studies, namely; *E. cerealis* and *E. setulosus*. The presence of these species would have played a major role in compiling a detailed morphological key of all the weevils commonly known to occur in commercial orchards and vineyards for future researchers.

Confirmation of the occurrence of *E. occatus* for the first time in vineyards and most importantly to rank as the second most dominant weevil in vineyards would require a detailed future study to clarify its importance and possible management practices as no previous literature on its biology and ecology exist.

Future research must also focus on surveying adjacent natural habitats and surrounding apple orchards and vineyards for the biodiversity of weevils, as this might help determine a trends and identify possible alternative host plants.

In both cultivated apple orchards and vineyards the population build up in late in mid-October to mid-November is very important as more damage took place around this time. The fluctuation in weevil populations recorded during the present study is relevant for the control measures for all the study sites. Fruit growers should initiate monitoring prior to mid-October. Any spray program aimed at suppressing weevil population should be applied much earlier or prior to weevil build up. Furthermore, any spray program aimed at suppressing or killing groundcover should be applied earlier than mid-October. Although, most fruit growers use sticky bands to prevent weevil from climbing the three trunks to access the fruit, much longer weeds could provide a path-way for weevils to access the fruits. Groundcover must be constantly trimmed, as this might reduce the costs of sticky bands as they are more labor intensive. Furthermore, trimming the weeds rather than completely destroying them has a potential for increasing natural enemies that have the capability of suppressing other pests known to cause damage in apple orchards and vineyards. Strategies for monitoring should begin earlier in October. Inspection should be conducted on a weekly basis and apply control as soon as the first weevil emerges (Marais & Barnes 2004). Furthermore, inspect the water shoots, as they provide the first sign of the feeding damage. Remove any ground cover preferred as a host by weevils for feeding or shelter (Marais & Barnes 2004). Instead, establish ground cover such as rye, oats or triticale in the inter-row and apply herbicides to keep ridges weed free, especially during autumn and winter (Marais & Barnes 2004).

The close association of *E. occutus* and *E. setifer* creates a need for a further research on these two species. Considering the fact that *E. occutus* was recorded for the first time in vineyards and it was the second most dominant weevil particular attention to this species is recommended. The impact of drought may well have influenced the species diversity and abundance found during the study period, and therefore should be repeated in seasons with more abundant water. Further molecular investigations are required to establish the nature of the species complex of *P. callosus*, which could have management implications.

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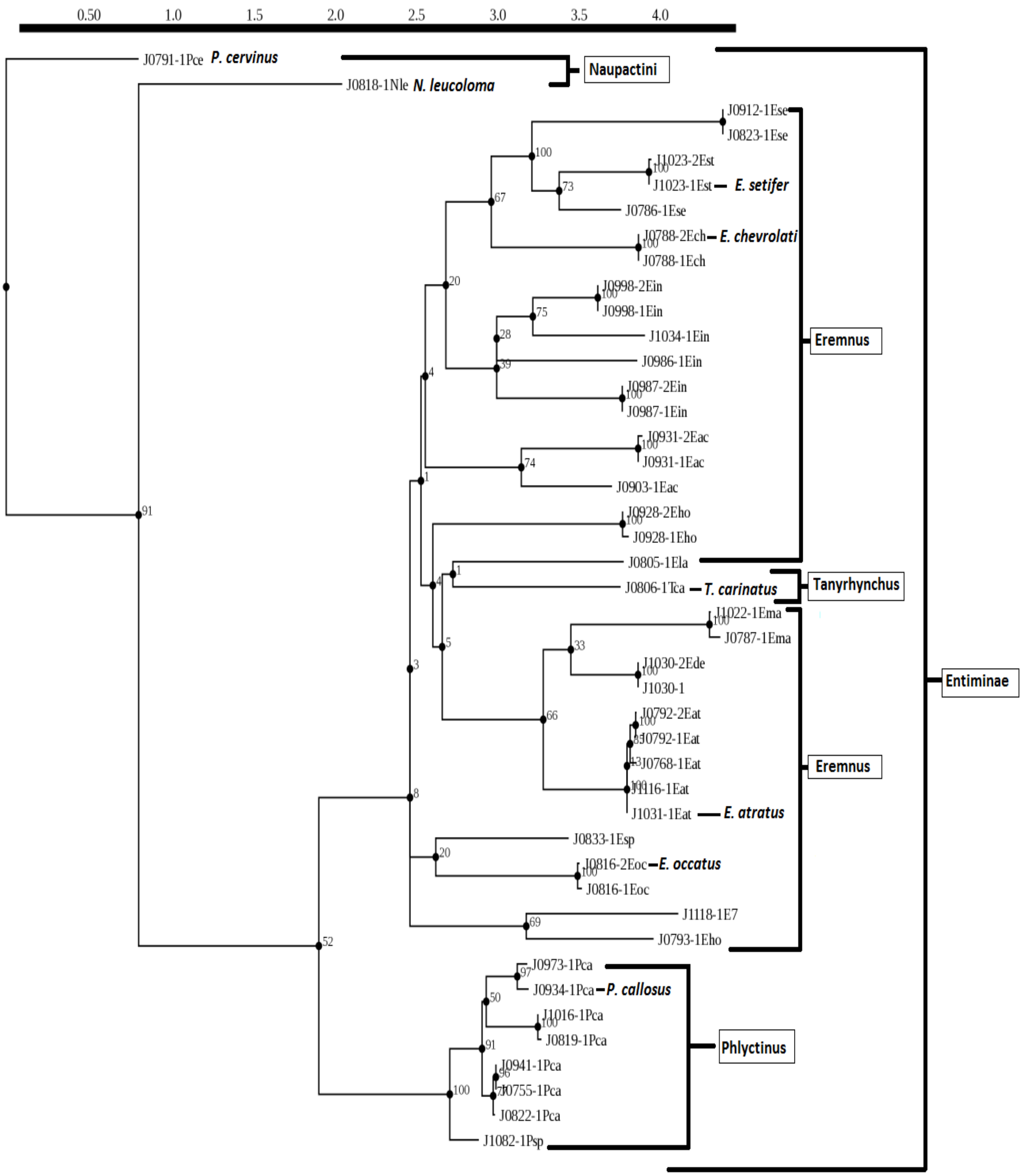
Appendix A

Table S1. List of adult weevil samples collected in Stellenbosch, Western Cape (September 2017 –May 2018).

Specimen code	Tribe	Author & date of description	Host	Genus	Species
JHAR01031_0101	Entiminae	(Sparrmann, 1785)	Grapevine	*Eremnus*	*atratus*
JHAR00788_0102	Entiminae	Oberprieler, 1988	Grapevine	*Eremnus*	*chevrolati*
JHAR01023_0101	Entiminae	Boheman, 1843	Grapevine	*Eremnus*	*setifer*
JHAR00816_0102	Entiminae	Boheman, 1843	Grapevine	*Eremnus*	*occatus*
JHAR00791_0101	Entiminae	(Boheman, 1840)	Grapevine	*Naupactini*	*cervinus*
JHAR00818_0101	Entiminae	Boheman 1840	Grapevine	*Naupactini*	*leucoloma*
JHAR00806_0101	Entiminae	Boheman, 1836	Grapevine	*Tanyrhynchus*	*carinatus*
JHAR00755_0101	Entiminae	Boheman, 1834	Grapevine	*Phlyctinus*	*callosus*

Appendix B

Figure S1. Phylogenetic tree (RAxML) inferred from 658 bp mitochondrial cytochrome oxidase I (COI) sequence. Bootstrap support values were obtained for a number of 1000 replications. The species indicated in bold were collected in both apple orchards and vineyards from September 2017 to May 2018. The species indicated in bold were collected in apple orchards and vineyards from September 2017 to May 2018.



APPENDIX C

Identification key of common weevils (Curculionidae) in vineyards and apple orchards of the Western Cape Province, South Africa. (Bold capital letters indicate the key features for weevil's identification as shown by the images below)

1 - Long rostrum (rostrum longer than the head plus prothorax); femora bearing a small tooth **(A)**……………………….……***Tanyrhnchus carinatus***

1' - Short rostrum (rostrum shorter than head plus prothorax); femora bearing a small tooth or not………………………..…………………….………..2

2 - Pronotum with a longitudinal carina; elytra generally with transversal white bands in apical 2/3; elytra usually have tubercles at the apex; declivity at the apex of the elytra vertical in lateral view **(B)**……………………………………………………………………………………… ***Phlyctinus callosus***

2' - Pronotum lacking longitudinal carina; elytra lacking transversal white-bands in apical 2/3 of the elytra; elytra with tubercles at the apex or not; declivity at the apex of the elytra oblique or vertical in lateral view ……………………………………………………………………………………..3

3 - Elytra with long setae, longer than width of interstriae……………..4

3' – elytra without long setae, always shorter than width of interstriae…………………………………………………………………………………….6

4 – Elytra with a longitudinal white band on each side of the elytra; scutellum bearing a contrasted spot of white scales **(C)**……….………***Naupactus leucoloma***

4' Elytra with homogenous color, lacking white bands on the side; the scutellum concolor, not bearing a contrasted spot of white scales……………5

5 – Body almost entirely covered with metallic greyish scales, hardly visible through the scales **(D)**………………….……….……….***Eremnus setifer***

5' – Body integument glabrous in appearance, only covered with very small and scattered scales **(E)**..……..................................***Eremnus chevrolati***

6 - Body integument red; rostrum bearing three distinct longitudinal carinae, separated from eyes and frons by a transversal furrow; pronotum with distinct granules **(F)** …….***Sciobius tottus***

6' Body integument black or dark brown; rostrum flat or with a longitudinal furrow; lacking transversal furrow near eyes …………………….………7

7 – Body integument black, shiny, bare scales; elytral declivity with tubercles on interstriae 1, 3 and 5 **(G)…**……………………….. ***Eremnus atratus***

7' – Body integument dark brown, densely covered with scales; elytral declivity lacking tubercles on interstriae ……………………………………….8

8 – Sides of the elytra bearing a short oblique strip of white scales near the hind femora; eyes very convex, distinctly bypassing the convexity of head capsule in dorsal view **(H)**…………………..…………………………………………………………………………….***Pantomorus cervinus***

8' – Sides of the elytra without distinct white strip of scales near the hind femora; eyes almost flat, moderately expanding from the side of the head in dorsal view……9

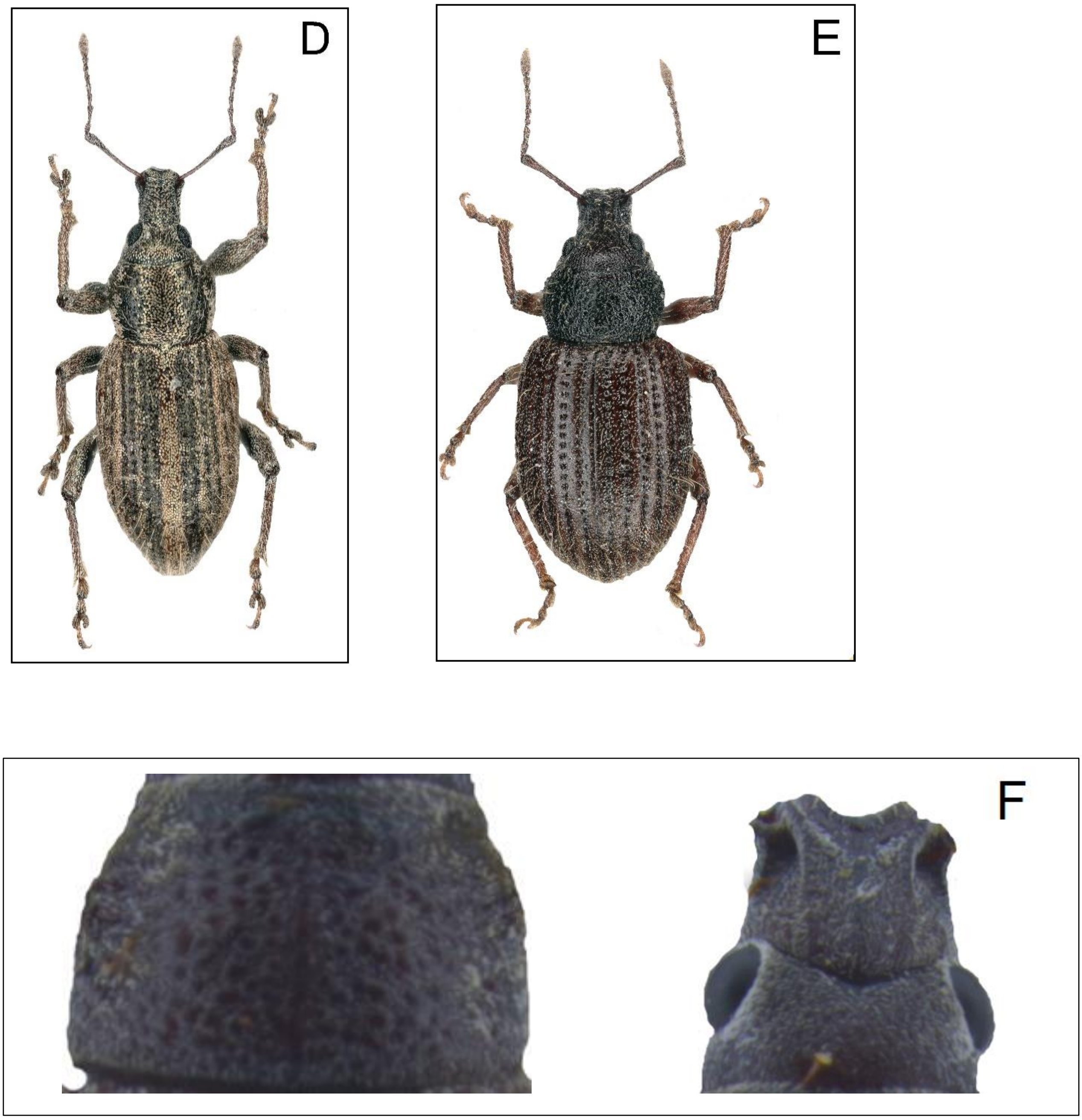
9 – Rostrum with a distinct longitudinal furrow; pronotum bearing erected setae on the sides; profemora unarmed; general shape of body short and rounded **(I) ……*Eremnus cerealis***

9' – Rostrum smooth, lacking longitudinal furrow; pronotum covered with scales but lacking erected setae; profemora with a distinct tooth; body shape elongate **(J)**…….***Eremnus occatus***


A

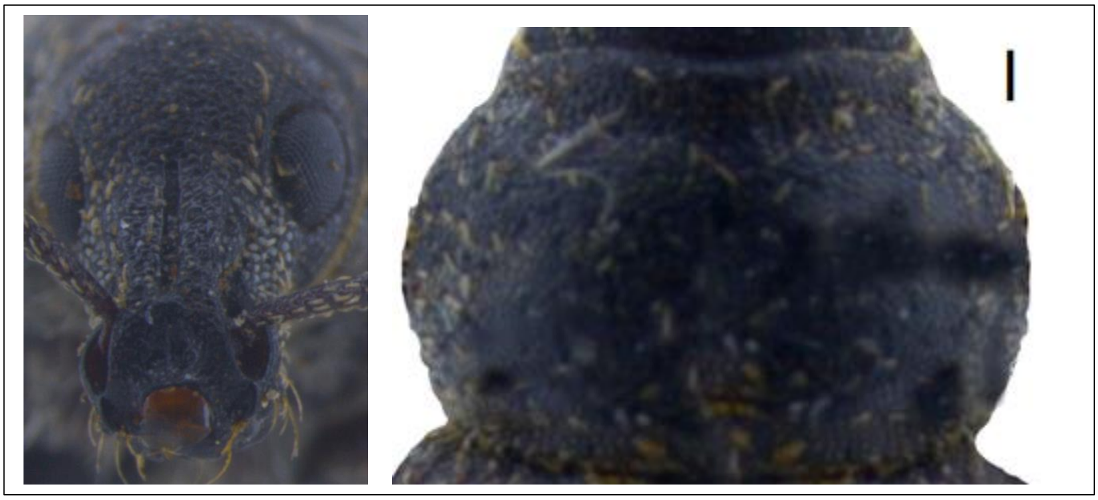

B


C


D
E
F


G


H


I


J