1 Chapter 1: General introduction and review of the literature

The redheaded cockchafer (RHC), *Adoryphorus couloni* (Burmeister)(Coleoptera: Scarabaeidae: Dynastinae) is a soil borne insect pest, native to Australia, found most commonly in the higher rainfall areas of the south-eastern states (Berg *et al.* 2013). It is a pest in its larval stages feeding on roots and organic matter found below the surface. This review will outline the key information available about the RHC including;

- pest status;
- economic importance;
- current and potential future distribution;
- morphology and lifecycle;
- nature and extent of the damage caused; and
- control techniques available.

Pest sampling strategies and the use of insect population density maps to encourage the use of site specific management will be discussed. The environmental factors which may have a relationship with RHC population densities and the potential for sensors which measure variation in the landscape (termed precision agriculture sensors) to assist in the detection or prediction of infestations will be explored. The final part of this review will provide an outline of each of the chapters in this thesis.

1.1 The redheaded cockchafer *(Adoryphorus couloni*)

1.1.1 Pest status of the redheaded cockchafer

The significance of the RHC has increased from being considered a minor threat in the 1960's to a major pasture pest of great importance to agriculture (McQuillan & Webb 1994). This is especially the case in the dairy industry (Miller & Newell 2013) where there has been an increase in the planting of perennial ryegrass (*Lolium* perenne), a particularly susceptible pasture species (Heap 1998; McQuillan *et al.* 2007; Berg *et al.* 2013). Within Australia It is difficult to assess the current area under attack by the RHC as there have been no recent comprehensive surveys conducted to ascertain its distribution. In 1993, Rath & Pearn (1993) reported over three million hectares of agricultural land in Victoria and four hundred and fifty thousand hectares of improved pastures in Tasmania had been infested by the RHC. Control of the RHC is difficult as there are currently no registered pesticides for use on this pest nor easily accessible biological control options (Mickan 2008; Berg *et al.* 2013). It has been acknowledged that there are substantial gaps in the knowledge surrounding the population dynamics of the RHC which has contributed to the difficulty in determining an effective control strategy (Miller & Newell 2013).

1.1.2 Economic Importance

It is difficult to determine, with any certainty, the economic loss sustained by producers affected by RHC infestations as the density of populations and the subsequent damage caused has not been well defined nor quantified. The absence of an apparently effective control measure also means that the cost of control versus damage is indeterminate in a traditional approach to calculating economic thresholds. A starting point might be to use the cost of re-establishing pasture. Rath and Pearn (1993) report that pastures in Tasmania were completely destroyed when the population density of third instar RHC larvae was 700-1100m^{-2}. It has been widely reported when the number of third instar RHC larvae is greater than 300m^{-2} this is enough to cause severe pasture damage and considerable loss of biomass (Douglas 1972; Rath & Pearn 1993). However, Mickan (2008) reports that third instar RHC larvae at abundance levels of 70m^{-2} is enough to cause substantial reduction in pasture growth. Estimated annual economic loss as a result of RHC infestations in Tasmania has been reported as over $1 million (Pauley & Miller 1993). In Victoria, economic loss due to RHC has been reported aa $100ha^{-1} for lamb production, $900ha^{-1} for dairying and $9,000ha^{-1} for turf production (Berg *et al.* 1993). More recently Berg *et al.* (2013) reported from unpublished survey data that producers in the South Gippsland lost on average $803ha^{-1} as a result of RHC damage due to the need to renovate pasture and purchase additional feed, as well through lost production.

1.1.3 Current and Projected Distribution in Australia

The RHC is commonly found in semi-improved and improved pasture in the Australian Capital Territory, New South Wales, South Australia, Tasmania and Victoria in Australia (Hardy 1971; Rath & Pearn 1993; Berg *et al.* 2013). The geographical distribution of RHC in Australia, as recorded from verified insect collections, is displayed in fig. 1. In nearby New Zealand (NZ), the RHC is only found on the South Island close to Christchurch but, since the first recorded siting of the pest in 1963 (Berg *et al.* 2013), its distribution has continued to slowly grow towards the Canterbury Plains (Brownbridge *et al.* 2009). Berg *et al.* (2013) suggests that the RHC is found mostly in areas where the annual average daily mean temperature is between 12-15°C. The majority of the RHC population distribution is within areas with an annual average rainfall of 500-800mm but they are also found in areas with higher rainfall of 1000mm per annum (Berg *et al.* 2013). It is anticipated that as native grasslands in both Australia and NZ are converted to improved pastures consisting of introduced species favoured by the RHC the distribution of the insect will increase.

With a two year lifecycle (discussed next section), RHC damage was initially only observed bi-annually (Stufkens & Farrell 1980a; Hardy 1981; Berg *et al.* 2013). However, in recent years it is believed overlapping populations now occur and damage to pastures can be observed in consecutive years (Douglas 1972; Stufkens & Farrell 1980a, Rath & Rowe 1993; Berg *et al.* 2013). Due to the hundreds of different cockchafer species present in Australia, many without their morphological characteristics described in the literature, it can be difficult for producers to identify RHC as the cause of the damage to their pastures (Berg *et al.* 2013). It was partly on the basis of this uncertainty as to which soil-dwelling scarab was causing damage to pastures that a preliminary study was undertaken across the Gippsland region of Victoria identify which species were present (discussed in Cosby *et al.* 2012 [Chapter 2]).

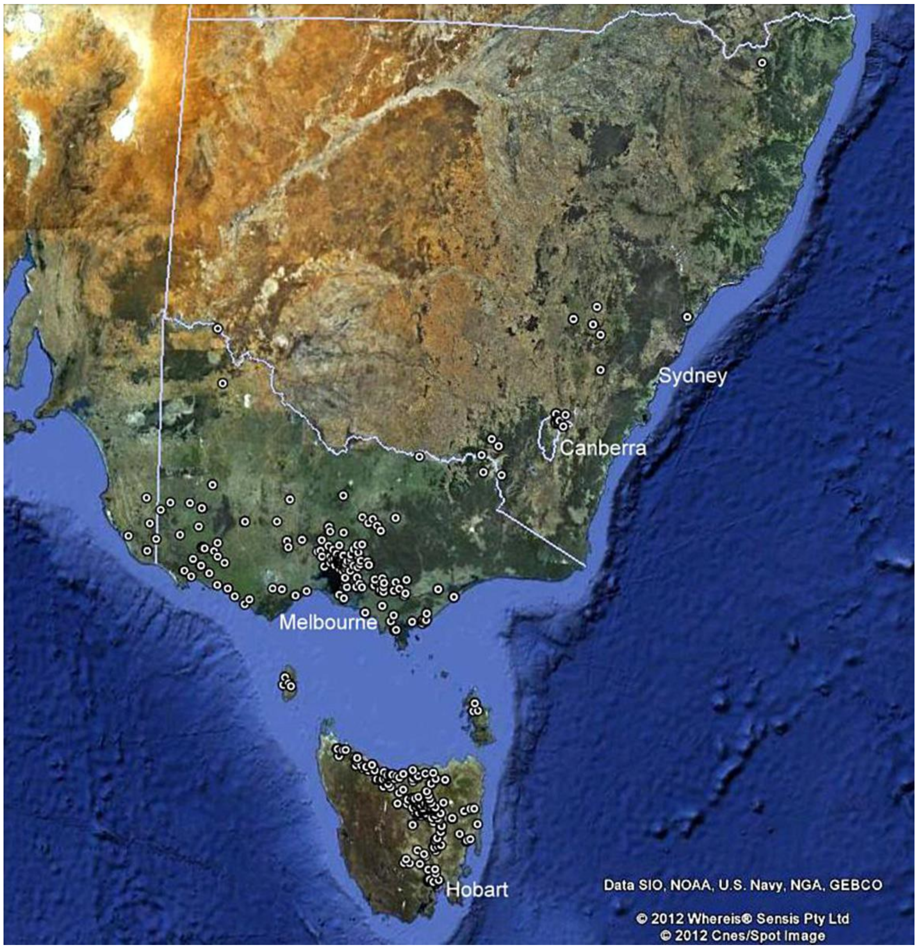

Fig. 1 The distribution of *Adoryphorus couloni* in Australia (indicated by circles) (Source: Berg *et al.* 2013).

1.1.4 Morphology and Lifecycle

The RHC predominantly lives below ground apart from a period of time it spends crawling along the surface of the pasture or flying as an adult beetle (Berg *et al.* 2013) between mid-August and October (Mickan 2008). The adult RHC is a beetle, 10-15mm in length and is shiny black to darkish reddish brown in colour (Berg *et al.* 2013) (fig. 2). The beetle copulates in the soil most commonly at shallow depths, however up to 120mm below the ground is possible (Stufkens & Farrell 1980a; Hardy 1981; Berg *et al.* 2013). The RHC oviposits, singly or in groups of 10-20 (Stufkens & Farrell 1980a; Hardy 1981; Berg *et al.* 2013). Eggs are white and approximately 2mm in diameter (Hardy 1981). The incubation period is influenced by temperature with eggs hatching in the soil, usually in late spring or summer. Under laboratory conditions, eggs required 29 days to hatch at 21°C (Hardy 1981).

The larvae of the RHC have a C-shaped body which is similar to most 'curl' grubs. They develop through three larval instars, as other Scarabaeidae do, with the insect taking approximately 5-8 weeks to complete both the first and second instar, normally beginning the third instar in late summer-early autumn (Hardy 1981). They remain in this third instar stage for approximately 12 months feeding in the upper 15cm of the soil (Rath & Pearn 1993; Mickan 2008). First instar larvae are 4mm long at hatching and reach a maximum of 30mm in length at the end of their third instar (Hardy 1981; Berg *et al.* 2013). The larvae have a reddish brown head capsule and transparent body, apart from the last quarter which is slightly swollen and darker in colour due to the contents of their hind gut, which account for close to half the weight of the larvae (McQuillan & Webb 1994; Berg *et al.* 2013) (fig. 3).

When third instar larvae are ready to pupate (December to March) they move further down the soil profile, as deep as 40cm, and create an earthen cell taking 3-8 weeks to complete the pupal process (Hardy 1981; Berg *et al.* 2013). The pupa of the RHC has not been reliably described in the literature (Berg *et al.* 2013). However, they are generally yellow brown or ginger brown and between 15-20mm in length. They remain in the pupal cell for 6 months as non-reproductive immature adult (McQuillan *et al.* 2007) before emerging and find their way to the surface to fly (Stufkens & Farrell 1980a; Hardy 1981; Berg *et al.* 2013). Details about flight activity is limited however it is thought that at dusk adult RHC emerge from the soil and fly for a short time and then return to the soil (Berg *et al.* 2013).

The damage to pastures is caused predominately by third instar RHC larvae which feed on roots and organic matter. Since the adult beetle does not appear to feed, it does not cause any damage underground or the above ground pasture. The adult neither feeds nor causes damage via its burrowing nor oviposition (Stufkens & Farrell 1980a; Hardy 1981; McQuillan & Semmens 1990; Berg *et al.* 2013).

Fig. 2 Adult female *Adoryphorus couloni* taken by Ian Faithful, Department of Environment and Primary Industries, Victoria.

Fig. 3 Third instar *Adoryphorus couloni.* taken by author.

1.1.5 Damage to pasture

Third instar RHC larvae causes the most damage to semi-improved and improved pastures by feeding on organic matter and root matter found below the surface. This leads to a weakening of the pasture root-system making it susceptible to being pulled up and overturned by grazing livestock, machinery and birds (Hardy 1981; Rath & Pearn 1993; Berg *et al.* 2013). If the infestations are extremely severe the RHC may sever the roots of plants, especially the more susceptible shallow rooted perennial ryegrass (*Lolium perenne*), causing reduced growth and even death of individual plants (Heap 1998; McQuillan *et al.* 2007; Berg *et al.* 2013). Damage is usually observed in mid to late autumn or early winter and ranges from small isolated patches to areas greater than 50 hectares (Hardy 1981). The damage caused by the RHC is also observed on turf farms, sporting grounds and lawns (Berg et al. 1993; Berg et al.

2013). Damage has also been recorded in wheat (Triticum aestivum) (Douglas 1972) although it is not considered a serious pest of cropping systems. The third instar of the RHC is the life stage which causes damage to pastures and is therefore the focus of the research in this thesis (Hardy & Tandy 1971; Hardy 1981; Rath & Pearn 1993; Candy & McQuillan 1998). As third instar RHC live below the surface it is difficult to detect infestations before significant damage has already occurred (Rath & Pearn 1993; Berg et al. 2013). This is a key issue which has driven the development of the current project. If areas of the landscape susceptible to RHC damage can be easily identified through the use of precision agriculture sensors, methods to prevent an infestation establishing, or steps to control the population, can be targeted to these regions. Although limited, the options currently available to producers to prevent damage or control RHC infestations will be discussed below.

1.1.6 Control options

There are many practices that are employed by farmers in an attempt to control third instar RHC damage and none of them are demonstrated to be reliable. The most frequently used tactic is to re-sow damaged pastures (Berg *et al.* 2013). The disturbance to the soil profile by ploughing and other traditional cultivation techniques may physically kill RHC larvae or expose them to predation by birds thereby reducing the insect population (McQuillan 1993; Rath *et al.* 1995). However, if all RHC larvae are not killed during this process they may adversely affect the establishment of a new pasture (Douglas 1972; Berg *et al.* 2013). Planting more resistant pasture species such as phalaris (*Phalaris aquatica),* lucerne (*Medicago sativa*), tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*) is another option (Heap 1998; McQuillan *et al.* 2007). Douglas (1972) thought that heavy grazing by livestock which reduces the amount of pasture present may reduce the preference of these paddocks to adult RHC when laying their eggs. They reported that in a paddock with a stocking rate of 3 wethers (castrated male sheep) acre^{-1}, 69 RHC larvae m^{-2} were observed compared to another paddock with 6 wethers acre^{-1} where there was only 1 RHC larvae m^{-2}. Either removing pasture through fodder conservation to decrease the amount of food available for consumption by the RHC (Heap 1998) or larval mortality resulting from trampling and habitat disturbance are potential control options (McQuillan 1993). However, when RHC damage is observed, it is generally recommended that producers remove or reduce livestock numbers in affected paddocks to prevent further destruction to the remaining sward (McQuillan *et al.* 2007; Berg *et al.* 2013).

1.1.7 Insecticides

There are currently no synthetic pesticides registered for use against the RHC and the development of any in the future is hindered by the need for any insecticide to be able to gain sufficient contact with insect below the surface (Berg *et al.* 2013). Douglas (1972) was the last author to report an investigation into an insecticide to use against the RHC in Australia however it was unsuccessful for reasons not stated. Stufkens and Farrell (1980b) trialled the effectiveness of the active chemical ingredient, lindane, to control the RHC in New Zealand. They found these chemicals to be effective for up to 5-6 months after application in killing first instar RHC larvae, but not third instars. Lindane, an organochlorine is banned for use in Australia because of its persistence in the environment and bio-accumulation in animal products (Australian Pesticides and Veterinary Medicines Authority 2010).

1.1.8 Fungal pathogens

There have previously been commercially available products which contain a fungal pathogen, DAT F-001 a strain of *Metarhizium anisopliae* (Metschnikoff), known to be pathogenic to RHC at low soil temperatures. Larval mortality of 60% to over 80% between the first application of DAT F-001, in mid-winter to the following late summer and autumn was reported (Rath *et al.* 1990; Berg *et al.* 2013). Autumn was deemed to the best time to apply the 'biological insecticide' to reduce larval RHC population with 94% mortality reported compared to 50% when applied at other times of the year (Rath 1992; Berg *et al.* 2013). DAT F-001 was marketed as Biogreen Granules Biological Insecticide™ (BioCare Technology Pty Ltd, Somersby, NSW, Australia) and became available in 1996. It was later renamed and re-registered as ChaferGuard Granules Biological Insecticide™ in 2005 and is no longer available for reasons unknown. Potential reasons are lack of demand with irregular infestations occurring in differing areas of Australia at different times, production challenges, cost, or the ability to successfully store the product (Berg *et al.* 2013).

1.1.9 Fungal Endophytes

There are many advantages and disadvantages of planting endophyte containing pastures. They are thought to improve establishment, productivity, and deter feeding of some insect pests but are also known to cause animal health issues such as ryegrass toxicity (Watson 2007). A new pasture species mix (meadow fescue (*Festuca pratensis*) and perennial ryegrass (*Lolium perenne)*) called Barrier Combo™, containing the fungal endophyte GrubOUT® U2 which is a strain of *Neotyphodium uncinatum*, is now commercially available in New Zealand and reportedly offers protection from pasture pests including RHC (CropMark Seeds 2013). However the effectiveness of the endophyte against the RHC has not been proven. An experiment conducted by Watson (2007) demonstrated that RHC larval populations were not affected by the alkaloids produced by wild-type, or AR37, novel endophyte perennial ryegrass (*Lolium perenne*) pasture. In this work investigators reported no significant levels of mortality or larval weight loss. More recently the same endophyte found in the GrubOut© pasture mix *Neotyphodium uncinatum* was trialled against second instar RHC larvae. The mortality of the second instar RHC larvae was not affected by the treatments in the experiments however feeding was reduced. The study concluded that there is a potential for the endophyte to control the RHC with the belief that over a longer period of time mortality may have increased (Bryant *et al.* 2010).

1.1.10 Nematodes

Another potential biological control option is a strain of the entomopathogenic nematode *Heterorhabditis zealandica* (Poinar), which is most commonly used in high value industries such as nurseries and turf farms (EcoGrow Pty Ltd. 2013; Berg *et al.* 2013). The effectiveness of this nematode against RHC however has not been reported in a reliable source (Mickan 2008; Berg *et al.* 2013) The high cost of this product at approximately \$A6,500ha^{-1} (EcoGrow Pty Ltd. 2013), also prices it out of reach of grazing enterprises if applied at farm or even paddock scale.

1.2 Site specific pest management at the sub-paddock or sub-farm scale

If areas susceptible to high densities of third instar RHC larvae could be easily identified, prevention or control techniques such as the entomopathogenic nematode biological control could potentially be applied in a site specific manner reducing the cost of the input. Likewise, endophyte containing pasture species could be sown in particular paddocks which are identified as been at a high risk of RHC damage.

At the farm or paddock scale, understanding the spatial distribution of an insect pest is important if a farmer is to use site specific management (SSM) to manage pests (Fleischer *et al.* 1999). In this context, SSM can be described as managing a section of the land on a smaller than a whole paddock or property spatial scale, i.e. only where the insect population reaches the economic threshold (ET), as opposed to the widespread uniform treatment of a property or paddock (Park & Krell 2005). To warrant SSM a farmer must find it economically or environmentally advantageous. The variability in the insect population within a paddock will need to be sufficient to justify SSM and be economically significant, easily identifiable and measurable (Plant 2001). The key issue for the RHC is being able to identify and measure infestations of this pest.

Mapping pest infestations for the purpose of implementing SSM has been explored for other insects (Park & Krell 2005; Park & Tollefson 2005). The western corn rootworm (*Diabrotica virgifera virgifera*)(LeConte) is similar to the RHC in that they cause significant economic damage whilst below the soil surface feeding on the roots of maize (Park & Krell 2005). However unlike the RHC there are effective control methods available. Farmers can rotate their corn crop with other non-host species, or treat the soil with an insecticide at planting or plant a variety of corn resistant to attack (Park & Krell 2005). The aim of Park and Krell's (2005) research was to develop maps of the expected corn rootworm distribution and indicate management zones where insecticide should be applied and/or resistant corn planted. This study relied on the current year's adult corn rootworm population to predict future densities as environmental variables such as soil moisture and elevation were not consistent indicators of infestations (Park & 2005). The maps produced were not overly useful as they underestimated the area where the corn rootworm population was over the ET and sampling cost was high. It was concluded that the cost of sampling may be reduced if environmental attributes could be correlated with corn rootworm population densities (Park & Krell 2005). One of the objectives of this thesis, in an effort to overcome the issue faced by Park and Krell (2005), is the identification of any potential relationships between RHC and environmental variables detected by some form of proximal or remote sensing device, and examine whether these correlations could be used to produce risk maps in Chapter 5.

Another insect where spatial mapping and SSM have been explored is the bean leaf beetle (*Cerotoma trifurcata*)(Foster), an important pest of soybeans. Park and Krell (2005) stated that the movement habits of the insect are important when determining whether mapping populations with any precision was possible. Similarly to the RHC, the bean leaf beetle only travels short distances making it a suitable target for the possibility of SSM (Park & Krell 2005). Whilst in the overwintered life stage, the distribution of the bean leaf beetle was found to be random across the paddock and therefore a blanket treatment based on the average population density was most appropriate. However, first and second generation bean leaf beetles demonstrated spatially significant aggregated populations (P <0.05) and a prescription map with three different ET's (as these change with the cost of control and market value of soybeans) was developed (Park & Krell 2005). For first generation bean leaf beetles based on ET-1, ET-2 and ET-3, 90%, 50% and 3% of the paddock, respectively would need to be treated with an insecticide. This article demonstrated a procedure for generating prescriptive 'treatment' maps for the bean leaf beetle and for delineating management zones (Park & Krell 2005). It would be useful to develop a similar protocol for the RHC. However, as RHC spend the majority of their lifecycle underground, sampling is much more difficult, unlike the bean leaf beetle where above-ground sweeping nets are used. Therefore it is important to design a sampling strategy which is time efficient for farmers and this is the focus of Chapter 6 of this thesis.

1.2.1 Insect sampling plans

The sampling design is crucial to producing maps of insect population densities (Fleischer *et al.* 1999). Sampling for soil borne pests such as the RHC is laborious and time consuming as they need to be removed from the soil and identified (Edwards 1991). To encourage the use of the risk maps of predicted RHC population (developed in Chapter 5), a sampling plan which minimises the effort required by farmers as well as damage to pasture should be designed. When designing a sampling plan the number and size of each sample and its location should be decided with reference to the knowledge of the target insect's spatial distribution (Naranjo & Flint 1994; Park & Tollefson 2006).

When evaluating the economic benefits of traditional pest management compared to SSM for the bean leaf beetle, Krell *et al.* (2003) found that uniform treatment in most circumstances would be adequate. However, if the cost of sampling could be reduced, and insect populations highly aggregated, SSM could potentially be worthwhile (Krell *et al.* 2003; Park & Krell 2005; Park & Tollefson 2006). The same theory could be applied to the RHC. This demonstrates the need to develop an appropriate sampling plan for the RHC which detects any spatial dependence if we are to encourage the use of any risk maps which are produced for the RHC. Park and Krell (2005) used a semi-variogram to determine the distance with which samples are spatially dependant. They found that samples should be taken less than 270m apart for the first generation adult bean leaf beetle and 174m apart for the second generation in order to detect spatially dependent, or more simply aggregated, populations.

A sampling program for the adult western corn root worm was the focus of Park and Tollefson's (2006) research. They found that the distribution of adult western corn root worms in two paddocks were spatially dependent. Using a semi-variogram they established that samples within 45m were spatially correlated. Additionally, 39-90% of the variability was due to spatial dependence. However, in this study the cost of sampling was more than the economic benefit obtained by implementing SSM. It was reported that, as the distance between samples increased or the number was taken was reduced, the cost of sampling could be substantially lower (Park & Tollefson 2006).

1.3 Soil and plant sensors used in precision agriculture and their potential for identifying areas of the landscape preferred by the redheaded cockchafer

It has long been recognised, but only recently published, that there is a gap in the knowledge surrounding the biology and ecology of the RHC (Miller & Newell 2013; Berg *et al.* 2013). Further research is also required to determine the biophysical factors which foster higher third instar RHC infestations (Berg *et al.* 2013) as this may assist in the improved management of the pest. It is possible that environmental variables derived from soil and plant sensors, such as those used in precision agriculture, may be linked with third instar RHC populations.

1.3.1 Soil characteristics which influence RHC presence and survivability

There have been no laboratory-based studies to determine the physiochemical characteristics of soil favoured by RHC (Berg *et al.* 2013). The most damaging infestations of RHC in the state of Victoria have been found in soils with a topsoil of greater than 15cm. Damaging population densities have also been found on lighter textured (sand and sandy-loam) over clay soils (Douglas 1972). Similarly to Victoria, the most affected pastures in Tasmania are those growing on lighter textured soils (Hardy & Tandy 1971). In Tasmania, RHC are observed in most soil types, but more frequently on well drained, self-mulching clays and loams on top of gravel and/or clay. In New Zealand, RHC have generally not been found on poorly drained soils (Watt 1984). This could be because scarabs which live in the underground, such as the RHC, survive better when the profile has the ability to drain freely (Berg *et al.* 2013) and this characteristic is generally associated with lighter textured soils. Waterlogged soils and surface water have been reported to lead to high mortality of RHC larval populations (Douglas 1972; Berg *et al.* 1984). In drought conditions a lack of moisture and therefore plant growth may lead to larval mortality because of limited food (Havelock & Fidler, 1936). Davidson *et al.* (1972) also explored the impact of soil moisture content on scarab larvae. At soil moisture contents of 6-12% in a loam and 5-10% in silt larvae survivability was high. However, in soils which had reached water saturation, 57% of the larvae died after 1 day and 93% after 4 days (Davidson *et al.* 1972). As discussed above, because the RHC spends the majority of its lifecycle underground, the texture of the soil and its water holding capacity are believed to influence the survivability of the larvae.

Soil temperature is also thought to have an effect on RHC, more so than air temperature, however a range of values which are optimum for survival or prove fatal have not been recorded in the literature (Berg *et al.* 2013). Havelock and Fidler (1936) conducted a study on the larvae of the brown chafer (*Serica brunnea* L.), which similarly spends the majority of its life underground, and the physical characteristics of their soil habitat. They concluded that different soil factors influence larvae mortality in different seasons. In winter, low soil temperatures which drive the larvae further down the profile where oxygen and food are limiting factors. An excess of soil moisture and warmer temperatures in autumn can not only cause larval death but foster the development of fungus and bacterial disease. To determine whether soil has an effect on the population densities of RHC, chapters 2,3 and 4 of this thesis examine whether there is a relationship between third instar RHC larvae and a device known as an 'EM38', an above-ground sensor which measures apparent soil electrical conductivity in the soil below.

1.3.2 The EM38 soil sensor

One method commonly used to map the spatial variability of 'the soil' at a scale of tens of metres is to use on-the-go electrical conductivity sensors such as the EM38 (Geonics, Ontario, Canada; Hossain *et al.* 2010; Serrano *et al.* 2010; Padhi & Misra 2011). This electromagnetic induction ('EM') instrument allows the operator to safely and rapidly measure the apparent electrical conductivity of the soil (soil ECa) which may be influenced by several characteristics of the soil (Hossain *et al.* 2010; Padhi & Misra 2011). An EM38, with an inter-coil spacing of 1m (distance between the transmitter and receiver coils) will respond to the apparent electrical conductivity of the soil to a depth of about 1.5 metres (Hossain *et al.* 2010; Padhi & Misra 2011). When operated in the 'horizontal dipole mode' the value given by the EM38 reflects the conductivity in the top 75cm of the profile (McNeill 1980b). The electrical conductivity of the soil is generally increased by growing levels of salinity, clay (cation exchange capacity) and moisture content (McNeill 1980a; Hossain *et al.* 2010; Serrano *et al.* 2010; Padhi & Misra 2011) and this suggests that it might be useful for mapping soil properties related to third instar RHC population densities. However, the exact combination of soil characteristics that drives the value obtained by the EM38 at a certain location is unknown and must be divined from detailed soil core assessments (Serrano *et al.* 2010). However, generally, the greater soil ECa values are associated with areas of heavier textured soils and high moisture content (Serrano *et al.* 2010; Padhi & Misra 2011). Whether soil ECa correlates to third instar RHC population densities is explored in chapters 2, 3 & 4.

1.3.3 Topographic attributes which influence RHC survivability

RHC larvae have been recorded at a range of elevations on a regional scale. McQuillan *et al.* (2007) incorrectly stated that RHC were not found in Tasmania at elevations greater than 200m above sea level (ASL) as there have been records of RHC at 370m-420m ASL (Rath & Rowe 1993; McQuillan & Webb 1994). In Victoria, RHC have been found over a range of elevations from sea level to 600m ASL and in New South Wales and the Australian Capital Territory at range of 340m-850m ASL (Berg *et al.* 2013). However, there has been no information reported regarding the preferred range of elevations for the RHC on a smaller scale, i.e. at a farm or paddock level. As there are a wide range of elevations where RHC can be found, when trying to identify areas of the landscape which are more susceptible to RHC infestation it might be more important to compare the difference in elevations across a property instead of its average altitude. It could be argued that the higher areas of a property have better soil drainage and therefore foster larger RHC population densities as they are not susceptible to waterlogging. The topographic attributes of the landscape and soil drainage characteristics are known to be correlated (Troeh 1964; Liu *et al.* 2008). Therefore this hypothesis is explored in Chapters 2 and 4 of this thesis where the relationship between the difference in elevation across a paddock and third instar RHC larvae is studied. It is recognised that, any relationship found will likely be complicated by any interaction between soil texture, temperature and elevation. A digital elevation model (DEM) can provide a large amount of information regarding the morphology of the landscape (Jenson & Domingue 1988). A DEM consists of the spatial distribution of elevation values sampled across a landscape (Moore *et al.* 1991). Elevation data can be collected through an on-ground paddock survey using a differential global positioning system (DGPS) (or Global Navigation Satellite System - GNSS), through the use of a satellite imagery or stereo photography (Moore *et al.* 1991), LIDAR survey (Bowen & Waltermire 2002) or from the shuttle radar (Rabus *et al.* 2003). Using a geographical information system (GIS) this raw data can be interpolated to create an elevation map of the property or paddock (Moore *et al.* 1991; Liu *et al.* 2008).

1.3.4 Characteristics of the pasture which may influence RHC survivability

The available pasture biomass may affect the viability and development of RHC larvae. The ideal conditions are ample, dense pasture in the reproductive stage which protects the soil surface and, if senescent, helps in the retention of moisture (Douglas 1972). Larvae feed on the roots of pasture and organic matter reducing growth and, in severe infestations, killing plants (McQuillan & Webb 1994; McQuillan *et al.* 2007; Berg *et al.* 2013). Therefore, areas of reduced pasture biomass may indicate areas which are currently suffering damage as a result of previous or prevailing RHC larvae feeding. Areas damaged by RHC can be small isolated areas of only square metres in a single paddock all the way through to large areas encompassing several paddocks or an entire property (Hardy 1981). Due to the sporadic nature RHC damage in a paddock it would be useful if a convenient above-ground sensor which has the ability to measure and map photosynthetically active biomass (PAB) could be used to survey paddocks at potential risk of infestation. Whether there is a relationship between an such a sensor which measures PAB and third instar RHC larvae is explored in chapters 2,3 & 4 of this thesis.

1.3.5 Active optical sensing of PAB

The normalised difference vegetation index (NDVI) is a numerical index (between 0 and 1) which quantifies the relative strength of the optical reflectance from a surface in the red and near infrared wavelengths of the electromagnetic spectrum. The NDVI is known to correlate to PAB (Flynn et al 2008; Trotter *et al.* 2010) and can be measured through a ground based survey technique known as active optical sensing (AOS). The CropCircle™ ACS210 (Holland Scientific Inc., Lincoln, NE, USA) and GreenSeeker™ RT500 (Ntech Industries, Ukiah, CA) are two examples of AOS which, as the term suggests, produce their own 'sensing' light. These tools emit red (650-660nm) and near infrared (780-830nm) (NIR) and the radiation reflected by the plant canopy is recorded (Holland *et al.* 2004; Flynn *et al.* 2008; Trotter *et al.* 2010). These small sensors are positioned approximately 0.6-1 m above the (pasture) surface and can be used in a hand held configuration for onsite measurements. Alternatively they can be mounted on an all-terrain vehicle (ATV) for a rapid, relatively non-invasive pasture survey across a paddock or even property scale (Flynn *et al.* 2008). The normalised difference vegetation index is calculated by using the recorded wavelengths of light (red and NIR) in the equation (NDVI = (NIR - Red)/(NIR + Red)) Rouse *et al.* 1974). The NIR reflectance band does have some limitations, and in turn so does the NDVI calculation. For dense canopies the NIR appears to reach a point of saturation and is not able to penetrate the canopy surface when the density (formally known as leaf area index, or leaf area per unit ground area) is larger than 3-5 (Lamb *et al.* 2002; Trotter *et al.* 2010) and this may potentially be an issue with the pastures studied in this research which consist of predominately dense perennial ryegrass (*Lolium perenne*).

1.4 Objectives and outline of this thesis

The overall aim of this research is to determine if precision agriculture tools, namely above-ground soil, plant and topographical measuring devices could be used to assist with the improved management and detection of third instar RHC populations on dairy farm pastures. This thesis examines the relationships between environmental variables measured by so-called precision agriculture tools and third instar RHC population densities. The tools evaluated include the EM38, CropCircle™, multispectral satellite imagery and the digital elevation model. Using the relationships found between these variables, risk maps and a sampling plan are produced and the economics of implementing these strategies is explored. A diagrammatic representation of the structure of the thesis is shown in the flow chart in fig. 4. This thesis is divided up into five inter-related objectives:

Objective 1 – To determine whether there are any relationships between third instar RHC and sensor-derived environmental variables.

Chapter 2 examines several properties in the south, east and west Gippsland regions of the State of Victoria (Australia) which were surveyed with an EM38 operated in the horizontal dipole mode, a CropCircle™ and DGPS. The aim of this research was to determine whether any correlations could be found between RHC population densities and these precision agriculture sensors. It is noteworthy that, consistent with the earlier discussion around the lack of knowledge of RHC characteristics, it was discovered during the physical sampling process that the damage observed by farmers in the east Gippsland region was not actually caused by the RHC as previously thought; no evidence was found of any RHC populations.

Objective 2 – To investigate the relationship between the individual measurements taken by the EM38 and CropCircle™ and third instar RHC larvae population densities.

The relationships between the EM38 and CropCircle™ sensors and RHC population densities were investigated further in Chapter 3. The two wavelength components of light recorded by the CropCircle™, red and NIR, were explored individually to determine which of these has a stronger relationship with third instar RHC larvae. Whether the EM38 operated in the horizontal or vertical dipole modes (effectively two different sensing depths) had a better relationship with third instar RHC population densities was also investigated.

Objective 3 – To use the relationships between sensor derived environmental variables and third instar RHC larvae to produce a range of models which predict population densities.

Further exploration of the relationship between PA sensors and their ability to predict third instar RHC populations occurs in chapter 4 with the development of univariate, bivariate and multivariate models to predict third instar RHC populations. The robustness of the models was assessed by looking at the mean absolute error of residuals and their statistical significance. The models produced in this chapter are of little use to farmers if they cannot use these relationships to easily identify which areas of the paddock are at the greatest risk of third instar RHC infestation. Chapter 5 uses the relationships between on-the-go sensors and third instar RHC larvae population densities to develop risk maps which display regions of the paddock which are most susceptible to infestation.

Objective 4 – To develop maps which delineate areas of the paddock which are at the greatest risk of third instar RHC infestation and assess the economics of producing them.

Using the relationships found between third instar RHC populations and PA sensors in previous chapters, the next step undertaken in chapter 5 was to produce maps which delineate the categorical risk of infestation within a paddock. Ordinal categories based on levels of third instar RHC population densities were created. Different maps were produced using actual RHC populations, environmental variables obtained from PA surveys and a combination of both. The aim of these maps is to allow farmers the option to implement SSM by perhaps using biological control agents, planting endopyhte pastures thought to be resistant to insect feeding or if an insecticide was to become available for RHC it could be used in areas only where it is required. These maps require current third instar RHC larvae population data to reliably describe the areas of the paddock which are subject to a certain level of third instar RHC infestation. The potential savings the farmer could make through utilising these risk maps to site specifically apply the biological control to areas at extreme risk of third instar RHC infestation is also discussed. To encourage farmers to sample for RHC a sampling strategy which minimises effort and disturbance to the pasture is needed and this is the aim of the next chapter.

Objective 5 – To design a sampling algorithm which minimises the sampling effort required to detect third instar RHC larvae and the cost of implementing the plan.

Chapter 6 looks at designing a sampling plan which outlines the minimum number of holes which need to be dug and searched for RHC to detect a certain category of infestation. The objective of this research is to minimize the effort required by farmers and physical disturbance to the paddocks to sample for third instar RHC infestations which may encourage them to implement SSM. The number of samples, the range of correlation and the location of the sites is determined. The cost of implementing this sampling plan to detect certain levels of third instar RHC infestations is also examined.

The key outcomes of this thesis and recommendations for future research are discussed in chapter 7.

A note to reviewers

The physical structure of this thesis is based around a series of papers prepared for peer-reviewed scientific publications. Some have been published, or are 'in press', while others have been submitted for publication and are undergoing peer review. As a consequence there are certain sections in each of the chapters which contain some repetition, especially with regards to the collection of data, description of the study site and key information surrounding the redheaded cockchafer. While the contents of each chapter have been preserved to reflect their respective published (or submitted) form, fonts and figure/table layouts and references, abbreviations and nomenclature have all been 'standardised' wherever possible to preserve consistency. On some occasions, extra text has been inserted into a chapter, within square brackets, to clarify the inter-relationship between a section in one chapter (paper) and an earlier chapter (paper).

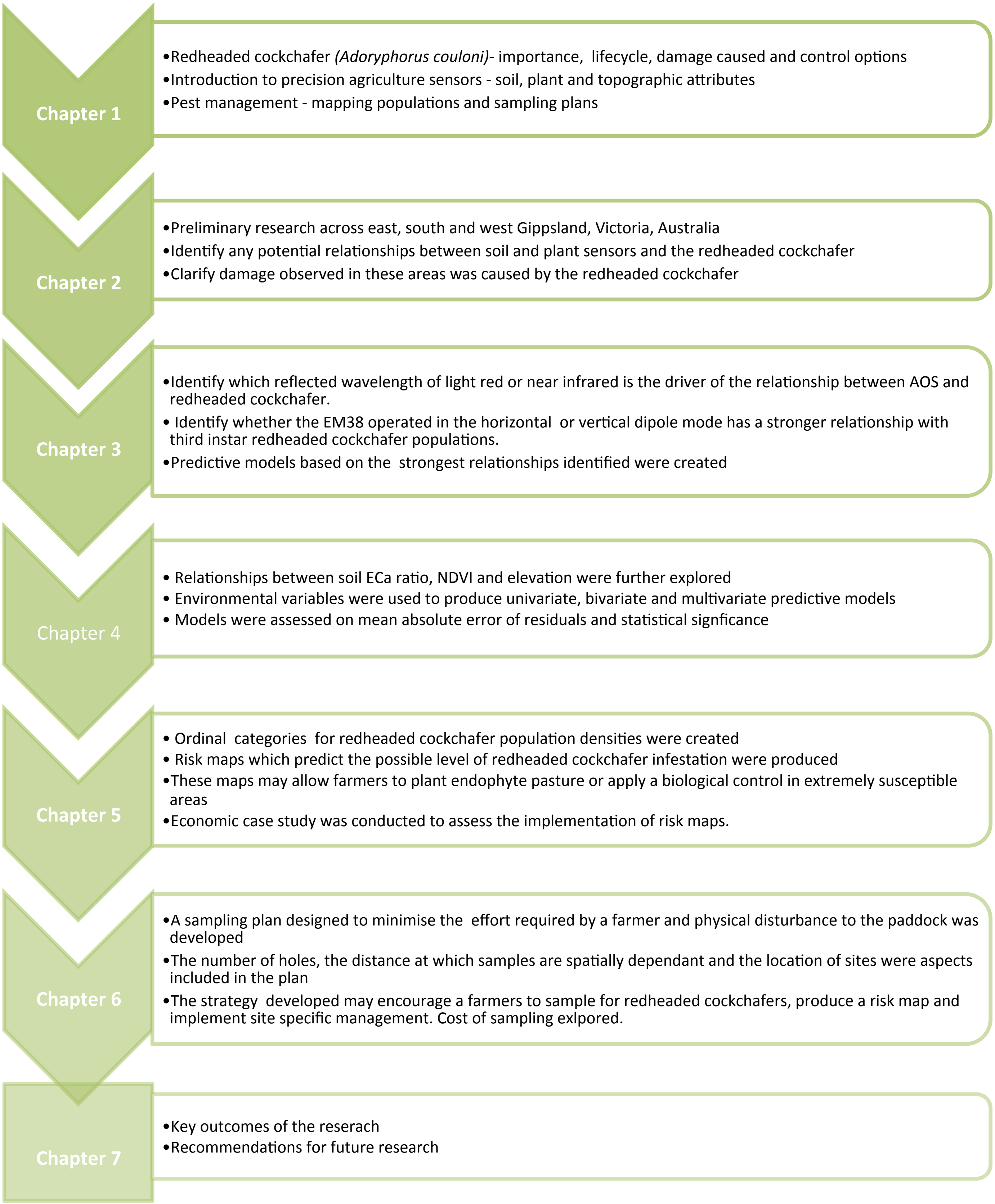

Fig. 4 Flow chart of thesis structure

Chapter 2: Detection of pasture pests using proximal PA sensors: a preliminary study investigating the relationship between EM38, NDVI, elevation and redheaded cockchafer in the Gippsland region.

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Published as a peer-reviewed, full paper in the Proceedings of the Australian Agronomy Society Conference, 2012.

Cosby, A., Trotter, M., Lamb D., Falzon G., Stanley J., Powell, K. and Bruce, R. (2012). Detection of pasture pests using proximal PA sensors: a preliminary study investigating the relationship between EM38, NDVI, elevation and redheaded cockchafer in the Gippsland region. In: Capturing Opportunities and Overcoming Obstacles in Australian Agronomy, Proceedings of 16th Australian Agronomy Conference, ed. I. Yunusa. Online Community Publishing.

Chapter 3: Mapping redheaded cockchafer infestations in pastures – are PA tools up to the job?

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Published as a peer-reviewed, full paper in the Proceedings of the 9th European Conference on Precision Agriculture, 2013.

Cosby, A., Trotter, M., Falzon, G., Stanley, J., Powell, K., Schneider, D., and Lamb, D. (2013). Mapping redheaded cockchafer infestations in pastures - are PA tools up to the job? In Precision Agriculture '13, Ed. John V Stafford, at the 9th European Conference on Precision Agriculture Lleida, Catalonia Spain 7-11 July, Wageningen Academic Publishers, pp 585-592.

Chapter 4: Associating third instar redheaded cockchafer *(Adoryphorus couloni)* (Burmeister) larvae population densities with precision agriculture sensors.

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Submitted as a peer-reviewed, full paper to the journal Precision Agriculture. Currently 'In Review'.

Chapter 5: Risk mapping of redheaded cockchafer (*Adoryphorus couloni*) (Burmeister) infestations using a combination of novel K-means clustering and on-the-go plant and soil sensing technologies.

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Submitted as a peer-reviewed, full paper to the journal Precision Agriculture. Currently 'Accepted for publication pending revisions'. These revisions have been incorporated in this chapter

Chapter 6: Developing a spatial sampling algorithm to improve the detection and estimation of redheaded cockchafer (*Adoryphorus couloni*) (Burmeister) populations in dairy pastures.

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Submitted as a peer-reviewed, full paper to the journal Precision Agriculture. Currently 'In Review'.

7 Chapter 7 - Discussion and Conclusion

The objective of the research presented in the preceding chapters of this thesis was to use precision agriculture sensors to improve the management of the insect pest, the redheaded cockchafer (*Adoryphorus couloni)* (Burmeister) (RHC). The hypothesis was that environmental variables derived from soil and plant sensors and a differential global positioning system (DGPS) would be associated with third instar RHC larval populations. These sensors could then be used to develop maps which highlight areas of the landscape which are at greatest risk of third instar RHC infestation. This hypothesis was supported through a series of experiments in the thesis;

- The preliminary study presented in Chapter 2 looked at 7 properties across the Gippsland region in Victoria. Each of the paddocks sampled on these properties was scanned with an EM38 to measure apparent soil electrical conductivity (ECa), CropCircle™ to measure the normalised difference vegetation index (NDVI) and DGPS for elevation. The results of this study indicated that the damage observed by farmers in east Gippsland was not caused by the RHC as there was no evidence of a population in this region. Also, there was no third instar RHC larvae found in west Gippsland with only adult remains found on these properties. Two paddocks surveyed on 2 different properties in south Gippsland were used in this research as they had third instar RHC larval populations. Three zones were created based only on soil horizontal ECa values and 20 samples were taken in each paddock. In both paddocks high third instar larval populations were observed in areas of higher elevation and lower soil ECa. However, in one paddock low NDVI favoured high third instar RHC populations and in the other high NDVI. This initial paddock study provided the basis for the following chapters where soil ECa, NDVI and elevation were also used to choose sample sites on a property with a history of severe RHC infestations in west Gippsland. Although an association between sensor derived environmental variables was established it required further exploration to see if extended across different properties at other times.
- Chapter 3 continued to explore the relationship between third instar RHC larvae and soil ECa and NDVI measured with a EM38 and CropCircle™ respectively. Chapter 3 looked at which of the measurements recorded by each instrument, horizontal (ECa-H) or vertical soil ECa (ECa-V) and red or near infrared (NIR) reflected wavelengths, drive the observed relationship between soil ECa, NDVI and third instar RHC larval populations. The exploratory analysis showed that there was a weak, positive non-linear relationship between the red reflectance band and third instar RHC larvae populations. There was no clear trend between NIR and third instar RHC populations. Both soil ECa-V and ECa-H had a negative non-linear relationship with third instar RHC population densities. ECa-V had a smaller median residual error than ECa-H and the red wavelength of light were chosen for statistical modelling. A generalised linear mixed model (GLMM) where red band values increase as do third instar RHC counts was statistically significant. As high red reflectance values are associated with areas of low photosynthetically active biomass (PAB) and large RHC infestations cause severe pasture damage it makes sense that this trend is observed. A generalised additive model (GAM) was developed for third instar RHC infestation and soil ECa-V, however

when adjusted for multiple comparisons it was not statistically significant. The trend observed was not a straight line and from the GAM it appeared that at lower soil ECa-V values third instar RHC population densities were higher. This could be explained as areas of low soil ECa are associated with lighter textured soils with a lower water content and these properties have been found to favour third instar RHC larvae infestations, possibly due to better drainage characteristics. The red reflectance GLMM was determined to have better a predictive capability than the soil ECa-V GAM when fit was assessed using the standardised residual sums of squares.

- Following on from chapters 2 and 3 where relationships with third instar RHC larvae and sensor derived environmental variables were established, chapter 4 develops univariate, bivariate and multivariate models using elevation, soil ECa ratio and NDVI to predict potential RHC infestations rather than describe the current population density. The concept of soil ECa ratio was used in this research as in chapter 3 it was established that there was little difference in the relationship observed between soil ECa-H and ECa-V and third instar RHC larvae. This way the model could use both measurements captured by the EM38 in an attempt to relate the physical characteristics of the soil and third instar RHC population densities. The univariate elevation model had the greatest predictive capability and confirms research undertaken in earlier chapters that areas of higher elevation have greater third instar RHC larvae population densities probably as they are less susceptible to waterlogging. The NDVI univariate model was not statistically significant when adjusted for multiple comparisons and had the lowest predictive capabilities of the three variables. This was reiterated when the bivariate model of soil ECa ratio and elevation was found to have a greater ability to predict third instar RHC larvae population densities when compared to the multivariate model which included NDVI. Although NDVI did not appear to substantially contribute to the predictive capabilities of the models in this chapter, it could be used in a different way. That is as a detection tool rather than a predictive one.
- Chapter 5 uses the relationships established in earlier chapters between third instar RHC larvae and NDVI, soil ECa ratio and elevation to develop maps which predict population densities on a categorical basis. This chapter develops 5 ordinal third instar RHC population density categories which can be used to divide a paddock up into areas which are at different levels of potential risk of RHC infestation from 'trace' to 'extreme'. Three risk maps were produced; the first using third instar RHC counts and their spatial location only, the second sensor data (soil ECa ratio, NDVI and elevation) and spatial location, and the third using third instar RHC counts, spatial locations and sensor data. The map produced which was considered most useful to farmers was the model which used third instar RHC counts, spatial locations and sensor data as it had the highest cross validation accuracy estimate. These maps could be used by farmers to locate areas of their property at greatest risk of an 'extreme' RHC infestation and they may choose to treat these areas, and others which they consider important, with the few control options available (endophyte pasture or biological agent). An economic case study was undertaken for the study site and it was determined that the blanket sowing of the endophyte pasture and site specific application of the biological control had the potential to save a farmer money, when compared to the current method of control; re-sowing pastures each year. Additionally, if a chemical control was to come onto the market it could also be used in an

economically and environmentally friendly manner. The greatest limitation to the implementation of these maps by farmers is that they require the input of current and historical RHC population data to be produced which can be costly and laborious.

- A spatial sampling algorithm was developed in chapter 6 with the aim to minimise the resources required for RHC sampling as well as pasture disturbance. This follows on from the need identified in chapter 5 for current third instar RHC population data to produce risk maps. Environmental data obtained from sensors, known to correlate with third instar RHC larvae, is also required for use in the algorithm. The sampling algorithm developed determined how many holes would need to be dug to detect a certain category of third instar RHC infestation, the location of the sample sites and the distance at which they are correlated. The sampling strategy developed is more likely to be used by farmers as it is time efficient with a set number of holes which need to be dug in each paddock based on the level of infestation they would like to detect. The cost of sampling for third instar RHC infestations based on the category of damage a farmer wants to detect was also outlined in this chapter. The total cost to conduct PA sensor ground surveys, sample for and control (widespread planting of endophyte pasture and site-specific application of the biological agent) an 'extreme' third instar RHC larvae infestation for the 9 hectare study area was $26,376.72.

The biggest limitation to the research presented in this thesis is that field work could not be replicated over more than one year. Above average rainfall (165.8mm) in November 2011, nearly twice the 125 year average (Bureau of Meterology, 2014) was observed on the study site and several areas of the property were left waterlogged for a period of time. It is likely that soil remained saturated for the first half of 2012 as above average rainfall was observed in each month from January to June, totalling 199.7mm more than the 125 year average (Bureau of Meterology, 2014). It is believed that these rainfall events lead to a great reduction in RHC populations and when sampling in March 2012 no RHC larvae were found. Later visits to the study site in Lardner, West Gippsland, Victoria, Australia and frequent conversations with the owner of the property established that the RHC population at this property and also in the surrounding regions had diminished. Although, RHC were not a problem in the Gippsland region in 2012-13 it is believed that if below average rainfall and drought conditions occur in the coming years, this insect pest will again cause significant damage to pastures, and this reduction in population is only temporary.

Another limitation that this research project faced was the lack of essential knowledge surrounding the basic biology and ecology of the RHC (Berg *et al.* 2013). This includes the timing of adult emergence, flight activity and any influences on the development of RHC through its life cycle. The economic threshold for the pest is not firmly established and it is difficult to determine at what population density control should be applied to prevent further damage.

7.1 Future research

A number of future research opportunities have been identified to further the management and control of the RHC:

1. Laboratory and paddock studies which study the lifecycle and each life stage of the RHC should be conducted. There are substantial gaps in the knowledge regarding the time and duration of flight activity by the adult RHC, how they choose where to lay their eggs and how mobile larvae are in the soil profile. If more was known about the biology of the RHC it may be determined whether there is certain life stage which could be more easily controlled than another.
2. The economic threshold, which is the population density where RHC begin to cause damage to pasture, needs to be firmly established. This would give farmers a better understanding of when they should invest in controlling the RHC and may lead to an adjustment of the population density categories established in this research.
3. There are many more environmental covariates and their relationship with the RHC that could be investigated. The ideal pH, soil temperature, nutrient availability, moisture content and preferred pasture species could be explored. If a relationship could be established with other environmental covariates these could be added as data layers to the models developed in this research to increase their accuracy.
4. As there are limited options available for farmers to control the RHC there is an opportunity to develop effective controls whether it be chemical or biological. If an efficient and cost effective control is developed the risk maps developed in this research may be able to be utilised by farmers to target potential or currently highly infested areas of the landscape.
5. A phone application which assists with the identification of insect pests including the RHC could be useful for farmers to identify the cause of any damage and to avoid any misidentification. This way any control applied will be for the correct pest and therefore wastage of a chemical or biological control will not occur.

7.2 Conclusion

The research contained in this thesis has contributed to the current knowledge of the whether environmental variables measured by precision agriculture sensors can indicate areas of the landscape which are preferred by the RHC. It has been established that there is a relationship between soil ECa, the PAB and topographic variables such as elevation, slope and flow accumulation. The development of a map which highlights the risk of RHC infestation across a property will assist farmers in controlling a population, especially if an economically viable control option becomes available. Farmers will be encouraged to collect the data required for these maps as the sampling algorithm developed minimises the effort required and disturbance to pasture. The ability to use remotely sensed data to develop risk maps eliminates the need for a ground survey to be conducted also saving the farmer time. Overall the findings presented in this thesis will assist in the prediction and detection of third instar RHC population densities and also the application of control options in areas where it is most needed.

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