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Validity of realized vs. fundamental host range of insects used as biocontrol agents of invasive alien weeds: Eucalyptus weevil (Gonipterus scutellatus) as a test case

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Validity of realized vs. fundamental host range of insects used as biocontrol agents of invasive alien weeds: Eucalyptus weevil (*Gonipterus scutellatus*) as a test case.

By

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A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science.

School of Animal, Plant and Environmental Sciences,

Johannesburg, March 2010

Declaration

This report was supervised by Prof. M.J. Byrne. I declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Solomon Wakshim Newete

8th day of March 2010.

Abstract

The conservative method of host specificity testing dictates that a potential biological control agent which shows polyphagous behaviour in the laboratory will be rejected, even though in a natural situation it may be monophagous or nearly so. To distinguish one from the other the performance of eucalyptus weevil, (*Gonipterus scutellatus*) was tested on 14 *Eucalyptus* and one *Syzygium* species in the laboratory, and the field. The weevil revealed different levels of polyphagy, depending on how the host plants were presented; as cut leaves, bouquets or sleeved-branches; or in choice or no-choice combinations. However, the fundamental host range was broader than the realized host range. *Eucalyptus smithii* and *E. urophylla* were the most preferred hosts (contrary to the literature), while *E. saligna* and *Syzygium myrtifolia* were immune to feeding and oviposition.

Nevertheless, adult feeding and oviposition was more selective in the field, and the larvae are less discriminating than the adults. Finally, the weevil is shown to have a narrow host range within two sections of the subgenus *Eucalyptus*, sufficiently restricted if it was ever to be considered as a biocontrol agent.

Dedication

I dedicate this work to my family and particularly my wife Azmera D. Mebrhatu, my son Nathan S. Newete and my brother Yewhannes W. Newete for their moral support and help during the course of my research.

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Chapter 1.

General Introduction

1.1 Overview

When alien invasive plants find their way into a distant new environment, they arrive without their natural enemies. In the first phase of their arrival they encounter several environmental constraints, from both physical and biotic agents (Mack *et al*., 2000). Many introduced plant species will perish as a result before they establish. However, over time a proportion of the new arrivals will overcome the various barriers to establishment and eventually become naturalized (Richardson and van Wilgen, 2004). It is often very difficult during this time of their lag phase to distinguish the potentially invasive species of introduced plants (Mack *et al*., 2000). After an initial phase of slow growth, alien plants increase exponentially by proliferating through disturbed ecosystems and habitats. This rapid propagation into the new environment is often driven by the fact that such new arriving species enjoy freedom from their natural enemies (pathogens and herbivores) that used to check their population density in the country of origin (van Wilgen *et al*, 2004; Blumenthal *et al*., 2009). Invasive alien plants alter the native species community composition by changing fire frequencies, soil chemistry or nutrient cycling and water resources (Mack *et al*., 2000). Among the principal terrestrial exotic plant invaders in South Africa are the genera *Acacia*, *Hakea*, *Pinus* and *Eucalyptus* and some of such notorious terrestrial invasive plants are *Eucalyptus camaldulensis*, *Jacaranda mimosifolia*, *Opuntia* species and *Prosopis* species (Richardson and van Wilgen, 2004). Among the major aquatic weeds in the country are water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), kariba weed, (*Salvinia molesta*), parrot's feather (*Myriophyllum aquaticum*) and red water ferns, *Azolla filiculoides* (Richardson and van Wilgen, 2004).

The conventional way to bring alien invasive plants under control has often employed mechanical and chemical methods. However, the achievements of such management methods are often small, despite the enormous resources and efforts demanded (Zimmermann and Olckers, 2003). The use of chemicals is expensive and environmentally unfriendly, and is facing growing limitations (Messing and Wright, 2006).

Biological control of alien invasive weeds, with its long history as management tool, has achieved more attention and popularity as an alternative method of control, being is potentially safer, with little environmental impact, self-perpetuating and cost effective (Fowler *et al*., 2000a). The principle of classical biological control hinges on host specificity. Ecosystems contain communities of organisms that occupy niches often limited by feeding specializations. Host specificity is a subset of the niche and can be described precisely (van Klinken and Edwards, 2002). Insect herbivores often specialize in a narrow range of hosts (McFadyen, 1998), and the fact that such insects can suppress and control exotic plant invaders forms the basis of their extensive use in the biological control (Schärer and Schaffner, 2008).

However, in classical biocontrol, candidate agents are imported from the country of the invader weed, and the major concern of the method is that such imported biocontrol agents may have a negative potential impact on non-target native plants. To ensure the safety of introduced control agents, several protocols and regulations are in place and are followed by governments and international organizations such as FAO (Food and Agricultural Organization of United Nations) (McFadyen, 1998). Since the early 20^{th} century, especially after the striking success of prickly pear control in Australia, the most widely accepted procedure in biocontrol programs has been the test of potential control agents for host specificity before their release in the field (McFadyen, 1998).

Host specificity testing of candidate agents is conducted in the laboratory in a closed environment as choice and no-choice tests by exposing the agent to non-target plants that are potentially at risk (van Driesche and Bellows, 1996). However, the host range may vary in response to stress driven by the environment (Galway *et al*., 2003), and insects usually make use of fewer hosts in the field than they are capable of exploiting in the laboratory (McEvoy, 1996). Such conservative methods of host specificity testing dictate that a potential biological control agent showing polyphagous behaviour in the laboratory will be rejected, even though in a natural situation it may be monophagous or nearly so (Balciunas *et al*., 1996). Even more problematic are those species that show reduced feeding or development on non-target hosts in the laboratory but are known to be monophagous in their native range, which make practitioners wary of committing an error of judgment when advocating release of a species polyphagous in the laboratory and consequently opt for caution by recommending rejection of the candidate agent (Messing and Wright, 2006). The converse scenario of monophagy in the laboratory converting to polyphagy after release in the field, or host switching, has never been shown despite some claims to contrary (Fowler and Withers, 2006; van Klinken and Edwards, 2002). Extrapolating one to the other is a key issue for ensuring the safety of biological control agents released into a new habitat, where they could attack non-target plants.

The Eucalyptus weevil (*Gonipterus scutellatus* Gyllenhall) was considered as a test case of laboratory vs. field host specificity in this study. The weevil specifically feeds on eucalyptus species (Tooke, 1953; Carbone and Rivera, 1998; Rivera *et al*., 1999). It has spread to many parts of the world where eucalyptus plantations exist (EPPO, 2005). In many literature reviews it is indicated that *E. globulus* and *E. viminalis* are the most preferred and attacked host plants of eucalyptus weevil (Hanks *et al*., 2000; Dungey and Pots, 2003; Millar *et al*., 1998; Loch, 2008). However, this may not be true for different countries across the continents that the weevil has reached, because *G. scutellatus* is indicated to represent a species

complex (pers. comm. R. Oberprieler, CSIRO (Commonwealth Scientific and Industrial Research Organization)). There are at least two species of this weevil namely *Gonipterus scutellatus* and *Gonipterus gibberus* Boisduval both of which are native to south-east Australia (EPPO, 2005). It is not always clear which of these two species of weevils has spread from Australia, and hence it is difficult to conclude that the host range and host preferences of the weevil are the same across the world, wherever the weevil is present. Therefore, determination of the host range of *G. scutellatus* in South Africa is an interesting question in itself.

The objectives of this research are to investigate:

1. the host range of *Gonipterus scutellatus* in South Africa, and
2. the fundamental and realized host range of a phytophagous insect, using *G. scutellatus* as a model biological control agent of an alien invasive weed.

1.2 Biological control

The two oldest events in biological control, where humans deliberately used natural enemies to control pest organisms, occurred in China in about 324 BC, when people relocated a mass of *Oecophylla smaragdina* ants into orchards of citrus tree, against pest caterpillars and large boring beetles (Hajek, 2004), and in Yemen in 1200 AD, when ants were applied to reduce the pests of date palms (Neuenschwander *et al*., 2003). The next documented practice of biocontrol was in 1888 in California, where an Australian ladybird beetle was introduced to control the cottony cushion scale, *Icerya purchasii* Maskel, in citrus orchards. And few years later, in 1892, the introduction of *Rhodolia cardinalis* Mulsant, into the Cape Colony and Egypt marked the first application of biocontrol for the continent of Africa (Neuenschwander *et al*., 2003).

The fast-growing world trade and tourism sectors that involve rapid and easy movement of people have facilitated mass relocation of species around the globe,

either accidently or deliberately. For instance, in South Africa 17 of the 40 declared most aggressive alien plant invaders were deliberately introduced for forestry and agro-forestry purposes, among which are *Prosopis* species, *Acacia saligna*, *Acacia melanoxylon*, *Pinus pinaster* and *Pinus radiata* (Zimmermann and Olckers, 2003). On arrival in their new environment these alien species enjoy ecological release as they escape from their coevolved natural enemies that would normally check their population, and consequently penetrate into disturbed and open niches by recruiting large number of seeds, out-competing native plants and eventually become aggressive pests that destroy the existing indigenous species and ecosystem (Mitchell and Power, 2003).

Alien invasive plants have extensively covered many agricultural, forest and range lands. They are ranked second as a global threat to biodiversity after direct habitat destruction, and they are the worst nightmares of conservation management practitioners (Mack *et al*., 2000; Richardson and van Wilgen, 2004). The invasion of alien plants into different biota is estimated to be covering 3% of the planet's total land surface area, excluding areas under agriculture and ice cover (Ricciardi, 2007). They cause huge damage to biodiversity and natural ecosystems, and the global cost of damage from invasive alien plants and their control programs is estimated to be around US$314 billion annually (Le Maitre *et al*., 2004). In South Africa over 10 million ha (6.8% of the total landscape) are occupied by invasive alien plants, which are responsible for the loss of about 7% (3300 million m^3) of mean annual runoff from water resources through transpiration of woody plants (van Wilgen *et al*., 1998; Zimmermann and Olckers, 2003).

An estimated 2.5 million tons of chemicals were applied per annum to control pests as of 1990, at a cost of $20 billion worldwide, but still the challenge remains (Hajek, 2004). Herbicides by far constitute the largest proportion out of the total (85%) amount of chemicals applied (Wilson and Tisdell, 2001). Despite the cost and environmental hazards the demand for pesticides is still growing, particularly

in developing countries (in Asia and Africa), with increased concentrations applied per ha. This is partly because of population growth and the greater and more extensive cultivation of lands, and partly to overcome pest resistance. India, Sri Lanka and China are among the countries recording an increased use of insecticides (Wilson and Tisdell, 2001). In the United States crop production is reduced by 37% as a result of weeds, insects and plant pathogens, and despite the increased use of pesticide, crop loss due to insect pests doubled from 7 to 13% between 1945 and 1989, while the loss due to weeds showed no change even when herbicide use increased 100-fold (Pimentel, 2005).

As a result of environmental hazards from the residue of broad-spectrum inorganic compounds that leads to pollution by arsenic, copper and sodium salt, the increased cost of selective organic herbicides (Briese, 2004), increased health hazards and development of resistant weed strains due to frequent chemical applications, there is a growing concern over chemical use and restrictions are increasing with time (Messing and Wright, 2006), while alien plant invaders are running out of control.

In California, agricultural damage due only to exotic pests costs $3 billion (Messing and Wright, 2006), while the overall cost to the country as a result of biotic invasion is estimated to be over $137 billion annually (Pimentel, 2005). In South Africa the losses in agricultural productivity and from control programs of alien weeds is worth tens of billions of rand annually (Maitre *et al*., 2000). The cost of clearing of such alien plant invasions is estimated to be US$0.86 billion over 20 years (Le Maitre *et al*., 2000). A recent economic impact assessment estimated a loss of $1.4 billion incurred by one tree species, the black wattle, *Acacia mearnsii* (Le Maitre, 2004).

Against this background of limitations of chemical pest control, the demand for an alternative method of invasive alien plant control has increased. Biological control is regarded as the best alternative and as a green approach in the control of alien

invasive weeds (Cory and Myers, 2000), which has achieved greater momentum following the first major success in the early 20th century against the prickly pear, *Opuntia spp.*, in Australia (Cory and Myers, 2000). Since then over 1000 introductions of weed biocontrol agents from about 400 species of invertebrates or fungi has taken place worldwide against 133 weed species (Julien and Griffiths, 1998; Fowler *et al*., 2000a). The scientific study underpinning biological control has transformed pest management perspectives from an industrial to an ecological model that is potentially sustainable, effective, self-perpetuating, cost effective and environmentally safe and can be integrated with other management practices, with minimum non-target and pollution impacts (McEvoy and Coombs, 2000; Zimmermann *et al*., 2004).

1.2.1 Success of biological control

Classical biological control is the deliberate importation of biocontrol agents for long-term pest management (van Lenteren *et al*., 2003) and its success can be defined as "complete" (when no other method of control is required at least in the region where the agent is established); "substantial" (where other methods are needed but the effort is reduced, e.g. less herbicide or less frequent application) and "negligible" (where despite the damage inflicted by the agent, control of the weed is still dependent on other control measures) (Hoffmann, 1995). The aim of biocontrol programs is not to eradicate weeds, but to keep their population below an economic threshold, at a density level similar to that in the pest's home range, and this often makes it irrelevant to raise a question such as what will happen to the agent after using all the target weeds (Hill *et al*., 1999). Apart from *Cactoblastis cactorum*, which has successfully controlled the invasive prickly pear in many parts of the world, the other often mentioned example in successful biocontrol programs is the exotic forb Klamath weed, *Hypricum perforatum* L., in the USA (Julien and Griffiths, 1998). Klamath weed had invaded about a million ha of range land in northern California by mid 1900 but was reduced to 1% of its

invasion density by the beetle *Chrysolina quadrigemina* (Pearson and Callaway, 2003).

High establishment rate of introduced agents is the first measure of success in biocontrol (Syrett *et al*., 2000). An establishment rate of 60% is often quoted as the general indicator of biocontrol success (McFadyen, 1998). The global establishment rates of introduced biocontrol agents still remain at an average of 55% (McEvoy and Coombs, 2000). However, the failure of establishment doesn't necessarily indicate the weakness of the biocontrol method, because imported agents can fail to establish for a number of reasons, such as phenological mis-matching, eco-climatic matching and biotic factors such as predation in the area of introduction (Syrett *et al*., 2000). For example an arctiid moth, *Pareuchaetes pseudoinsulata* Rego Barros, released against *Chromolaena odorata*, failed to establish in South Africa due to predation of its eggs by ants and chrysopids (Syrett *et al*., 2000). Establishment rates also vary from one species to another and from one region to the next (Syrett *et al*., 2000). However, these rates can be raised through a systematic and proper selection of agents (McEvoy and Coombs, 2000). It has been possible, for instance, to achieve an establishment rate of 81% of imported biocontrol agents in the state of Oregon, USA (McEvoy and Coombs, 1999). Above all, what matters most in biocontrol projects is the proportion of programs that achieve successful control. For instance in South Africa six out of 23 target weeds are under complete control and another 13 under substantial control, yielding a combined success rate of 83% (Hoffmann, 1995). Some of the most active countries that historically achieved high rate of success in biocontrol targeting weeds, ranked by the number of releases and weed species targeted, are the United States, Australia, South Africa, Canada and New Zealand (McFadyen, 1998).

1.2.2 Dangers of biological control

Unlike chemical control, where spraying can be contained or changed as a response to impacts, the practice of biocotrol is irreversible and involves potential risk to non-target, native plant species in the new environment of introduction. Negative impacts arise when biocontrol agents spread and colonize indigenous plants, causing long-term damage or temporary economic damage when at some stage they attack non-target native plants although they cannot develop and complete their life cycle on them (Briese and Walker, 2002). To reduce the potential impacts of a released agent on non-target plants, modern biocontrol programs against weeds employ only host-specific (or at least stenophagous) control agents and implement several other safety measures, such as complying with legislation on agent importation, screening and selection, studying the ecology of both the agent and the target and conducting host specificity tests before release (Thomas and Willis, 1998).

However, a few cases are reported of non-target impacts of biocontrol agents after release, and most of these occurred in the past when vertebrates, snails and generalist arthropods were selected, based on experience and observations of field scientists in the home range of the control agents. Most of these introductions were at the time when an established legislation or government oversight of biological control was lacking (Messing and Wright, 2006).

Dennill *et al*., (1993) showed host expansion of the Australian gall wasp, *Trichilogaster acaciaelongifolia* Froggatt, in South Africa from its two known host plants that originate in south-eastern Australia, *Acacia longifolia* and *A. floribunda*, to *A. melanoxylon* and *Paraserianthes lophantha*. *Acacia melanoxylon*, which overlaps in geographical range with *A. longifolia* in Australia, might sustain occasional galling in its native range, but this remained unnoticed possibly due to a high level of infestation of the main host, *A. longifolia*. While the new association

between the wasp and *P. lophantha*, which occurs in Western Australia in contrast to *A. longifolia*, is worth noting as a case of host shift, *P. lophantha* has not been exposed to the wasp before and hence was not known as a host plant (Dennill *et al*., 1993). Thus, the widely accepted view among biological control practitioners is that a host-specific herbivore does not exhibit host range expansion or shift, and no example of evidence to date of such an occurrence exists (McFadyen, 1998). Roderick and Navajas (2003) indicated that insects introduced as biocontrol agents might only shift their host plants on an evolutionary timescale and that this generally results in the formation of a new species. For instance, the moth genus *Hedylepta* has five species that feed on banana in Hawaii, which probably evolved since the introduction of banana into Polynesia within the past 1000 years (Roderick and Navajas, 2003). Another example of such speciation or genetic divergence in a herbivorous insect involving a host shift, is that of *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), which took hundreds of years to shift and adapt from its native hawthorn host plant (*Crataegus* L.) to the introduced apple (*Malus pumila L.*) in the region of Hudson Valley, New York (Feder, 1998).

Therefore, host specificity testing is regarded as the best assurance for detection of potential non-target impacts of introduced biocontrol agents after release. However, even with such codes of conduct and regulations requiring strict quarantine and host specificity tests, and the use of only monophagous insects to control invasive alien weeds, there are still some examples of non-target attack, constituting 3% of the total 400 releases worldwide in the last 100 years (McFadyen, 1998).

The most common example of biological control mentioned as failure is that of the prickly pear, *Opuntia cacti*, in Australia in the early 1900s by the moth *Cactoblastis cactorum* Bergroth, native to South America. Unlike the other three species in the genus *Cactoblastis*, *C. cactorum* was an insect known to be oligophagous, using a wide range of *Opuntia* species in its native geographical

range (Zimmerman *et al*., 2000). However, despite the fact that it is not host-specific, its release as a biocontrol agent was safe and successful in Australia, where no *Opuntia* species are native (Zimmerman *et al*., 2000). After this initial success it was introduced to many parts of the world including the Caribbean, from which it spread to Florida in the United States (it is not yet clearly known how it spread) (Zimmerman *et al*., 2000), where currently it attacks five native species of cacti including the rare semaphore cactus, *O. spinosissima* (Johnson and Stiling, 1998). *Cactoblastis cactorum* is sometimes used as an example of a failure in biological control despite the fact that the agent was not deliberately introduced in to Florida as a biocontrol agent (Stiling, 2000; Zimmerman *et al*., 2000). Another example is the seed-feeding weevil, *Rinocyllus conicus* Froel, introduced to North America against the Eurasian thistle, *Carduus spp*., but now attacking two native non-target *Cirsium* thistles (Louda *et al*., 1997). However, it was known to be oligophagous and the risk of damage to native plants was predicted, but the weevil was still introduced and released (Louda *et al*., 2003). These might be considered cases of improper application of biocontrol agents rather than failure.

1.3 Selection of biocontrol agents

The success of biocontrol depends on three essential factors: the intensity of the damage each individual agent imposes on the host plant; the ecology of the agent, which determines the establishment and population density in the environment of introduction; and the weed ecology, which enables prediction of the potential of the agent to reduce their population (McFadyen, 1998). For instance, the key to the success of *C. quadrigemina* against Klamath weed populations was not only attributed to its host specificity but also to its strong negative interaction with the host (Pearson and Callaway, 2003).

The technique of agent selection gradually changed after 1920 when testing of selected economic plants started (Briese and Walker, 2002). Modern biocontrol of

weeds involves selection and screening of host-specific phytophagous insects through phylogenetic screening techniques (Wapshere, 1974). However, an agent with very few alien non-target alternative plants as hosts is to some extent still advocated, as in the case of *Acacia floribunda*, which may be secondarily attacked by the gall forming wasp *Trichilogaster acaciaelongifoliae*, whose primary target is *A. longifolia* (McGeoch and Wossler, 2000). To ensure the safety of the selected agent for biological control therefore requires host-specificity testing, where the potential agent must be subjected to a wide range of target and non-target plants, beyond just the species of economic and agricultural value that used to be tested in the past (Thomas and Willis, 1998).

1.4 Host specificity testing

Host specificity is the combination of host range breadth, the level of preference for each host for feeding or oviposition, and the rate of growth and reproductive performance on each host (van Klinken, 2000). Host specificity tests predict the potential risk of a candidate agent to non-target plants in the release environment (McEvoy, 1996). For over 70 years herbivorous insects in biocontrol programs have been tested against all potential host plants, and the method forms the corner-stone of biological control by evaluating the performance of the agents based on parameters such as adult and larval feeding, survival, oviposition, larval development, pupal weight and host preference (Barratt *et al*., 2007). The technique identifies the most host-specific agents and ensures their safety to the environment after release, since a sudden host shift to include a novel plant as a host has never been recorded and such evolutionary change in feeding rather favours the move to be from generalist to specialists rather than vice versa (Futuyma *et al*., 1995). During laboratory host-specificity testing the fundamental or physiological host range of the control organism is determined under suitable environmental conditions by excluding all the potential factors that limit the agent's genotypic expressions (van Klinken, 2000). Therefore, the challenge

remains whether the biocontrol practitioner is able to properly define the fundamental host range or not (McFadyen, 1998).

The centrifugal phylogenetic method formalized by Wapshere in 1974 is a systematic selection criterion for all the plants that must be included for testing on the basis of their taxonomic relatedness to the target plant, and to the present day this phylogenetic technique remains the primary criterion of selecting plants for host-specificity testing (Briese and Walker, 2002). The system outlines the criteria by selecting a series of plants starting from those closely related to the target plant to those progressively distantly related plants, until the fundamental host range has been circumscribed. In case the technique of centrifugal phylogeny fails, as a safeguard Wapshere has also extended the criteria to include cultivated plants with little or no experience of relevant natural enemies, plants that are known to be attacked by insect species closely related to the control agent under study or any plant on which the agent has previously been recorded (Wapshere, 1974). Other components that need to be considered to reduce the potential risk involved in biological control, apart from the phylogenetic relatedness of non-target plants to the agent, are the biogeographic overlap and ecological similarity of the native plants to the host plant of the agent (Briese and Walker, 2002).

In most cases phytophagous insects only attack non-target plants if their chemical composition is similar to that of the target weed, and hence those with completely different phytochemicals from the target host plant, or taxonomically very isolated ones are much safer and may not be of a great concern in suffering attack from the control agent (Mitchell, 1988; Pemberton, 2000). For instance, during post-release risk-assessment analysis conducted on 117 control agents (112 insects, three fungi, one mite and one nematode) released against 55 weed species in the USA, Hawaii and the Caribbean since 1902, all of the non-target native plants (40 plants of the total 41 non-target attacked plants) attacked by the established control organisms were those closely related to the target weed (Julien and Griffiths, 1998).

Laboratory experiments are conducted in cages in isolation, where the control agent is exposed to selected non-target plants. Adult feeding and oviposition and larval survival and development to maturity are measured in no-choice tests, which determine whether the adult or larvae feed on the non-target plant or starve to death. The situation in the field, where an agent at some stage of its life cycle will use a non-target plant (although this will not support development and oviposition), can be exhibited during the laboratory testing, and that defines the fundamental host range of the control agent (van Klinken, 2000). Choice tests on the contrary are designed to present a semi-natural situation, where agents are tested in single large cages with target and non-target plants presented simultaneously to measure the level of preference (either in pairs or multiple choice) (van Lenteren *et al*., 2003). These tests were initiated in the 1970s and 1980s, when no-choice tests increasingly resulted in the rejection of some potentially important control agents, because it is misjudged by the use of these non-target plants that are not used in the field (Barratt *et al*., 2007). Both cases, choice or no-choice tests, result in a wider host range than the actual one that will be exhibited in the field under natural conditions, and this emphasises the importance of including field-tests in host-specificity testing by biocontrol practitioners (McEvoy, 1996) even though it is often neglected for reasons such as cost.

1.5 Insect host selection

Host preference of insects is attributed to behavioural response to chemical, visual and tactile cues from the plants encountered (Bernays and Chapman, 1994; Briese and Walker, 2002), which provide insects with positive and negative signals that enable them to identify the correct host (Bernays, 1989). Most plant species employ secondary chemical compounds as a defence mechanism, and those with a higher level of defensive chemicals (condensed tannins and phenolics) are more

resistant to insects than plants with lower concentrations, (Stenberg *et al*., 2008). Thus, plants with higher levels of protein or nitrogen and lower concentrations of secondary compounds provide foraging efficiency and a high rate of larval development, and will most likely be accepted as the most suitable insect host preferences (van Lenteren *et al*., 2006). Plant odour, colour and anatomical and morphological characteristics are also factors that influence insect host choice; e.g. insect host searching can be reduced by hairy structures in plants (Bernays and Chapman, 1994; van Lenteren *et al*., 2006).

In many cases, host plant choice is carried out by the mobile female insect searching for oviposition sites since larvae are generally less mobile than adults. The most palatable host plant should be selected by the female to ensure larval survival, and therefore growth rate of the larvae on the host plant species is partly attributable to the female oviposition preferences (Thompson, 1988). In some insects, however, the adult female is immobile while the larval stage is an active crawler capable of dispersing, assisted by vectors such as wind, from one plant to the next until it settles on the most suitable host. In such cases the potential risks to non-target plants can be established through host-specificity testing by properly identifying the host preference of the neonate larvae to settle on different test plants in addition to their rate of survival and the potential of the adults to reproduce (Barratt *et al*., 2007).

In general, the overall host plant choice by the female for oviposition can be affected by several factors, such as the plant and its ecological community (phytochemicals, morphology, spatial distribution and biotic associations) and other features of the insect such as starvation, egg load and its phenotypic and genotypic constraints. Consequently a clear picture of host preference in “paired-choice” tests might not emerge in the laboratory. Insects may do less well on a non-target species when compared to the target but still manage to develop (at a slower rate), pupate and emerge (at a lower weight) on the wrong host (Olckers *et*

al., 1995). This conundrum may be solved to some extent by creating a performance index for the agent on each non-target species and generating a risk analysis for the potential release (Olckers, 2000). Precisely what each risk analysis reveals is unclear, however, because post-release evaluation of biocontrol agents is so rare, these analyses are rarely if ever tested (McEvoy and Coombs, 1999). This project is designed to evaluate the risks involved to non-target plants, thereby testing the usefulness of this contentious tool.

1.6 The Eucalyptus weevil (*Gonipterus scutellatus*)

The Eucalyptus weevil, *Gonipterus scutellatus* Gyllenhal, (Coleoptera: Curculionidae) is native to south-east Australia and found exclusively on eucalyptus species (Tooke, 1953; Carbone and Rivera, 1998; Rivera *et al*., 1999). Both larvae and adults preferably attack the younger and tender leaves, shoots and buds, but larval feeding is more severe (Tooke, 1953; Loch and Floyd, 2001). Among many insects that attack eucalyptus plantations, the eucalyptus weevils are by far the most notorious defoliators and at high populations cause enormous economic problems, by destroying the foliage and retarding the growth of the branches, eventually killing the plant, particularly the susceptible species (Hanks *et al*., 2000).

1.6.1 The biology of *Gonipterus scutellatus*

Life history: The colour of the adult ranges from dark to orange brown, while the larva is yellowish with black spots and a pair of dark stripes running dorso-laterally along the body (Tooke, 1953). The mature larva has a yellowish-green colour (Mally, 1924; Paine *et al*., 2000). The adults are strong fliers and can easily disperse long distances by clinging to moving objects (Mally, 1924; Tooke, 1953). They live 2-3 months in summer and spend the winter under the bark of the host plant (Tooke, 1953; Hanks *et al*., 2000). The female eucalyptus weevil deposits an average of nine eggs in a dark-brown protective case or capsule that is made of

excreta, and 20-30 of these capsules are deposited per female on younger leaves and shoots (Hanks *et al*., 2000). Geography and climate determine the number of generations a female can have, but is usually one or more generations occur per year (Carbone *et al*., 2006). For instance, *G. scutellatus* has one generation in spring and another one in summer in Australia, Argentina (Rivera *et al*., 1999), South Africa (Tooke, 1953) and Italy (Carbone *et al*., 2006), whereas in the San Felipe Region of Chile the eucalyptus weevil reproduces at annual rate of 3-4 generations (Fuentes *et al*., 2008). The neonate larvae emerge by biting through the base of the egg capsules after about 2 weeks (Hanks *et al*., 2000; Carbone *et al*., 2004) and during the first instar feed on the surface of the foliage. At later stages they start feeding from the edges towards the center of the leaves (Hanks *et al*., 2000). The neonate matures to a full larva through four instars in 4-6 weeks before reaching the pupal stage, which occurs in the soil for another 30-40 days, and emerge as adults usually during the spring (Loch, 2008). *Gonipterus scutellatus* prefers temperatures between 10-30°C and the eggs fail to develop at temperatures below 6.5°C (Carbone *et al*., 2006), and oviposition is inhibited at temperatures below 5.5°C (Hanks *et al*., 2000).

1.6.2 Host range and geographical distribution

Gonipterus scutellatus feeds only on eucalyptus species (Tooke, 1953). Because of their enormous economic importance, eucalypts have been extensively planted for pulpwood and timber production in many parts of the world, and along with this the eucalyptus weevils have spread almost into all regions, building serious pest population where their host eucalyptus have been introduced. Their current geographical distribution is as indicated in Figure 1.1.

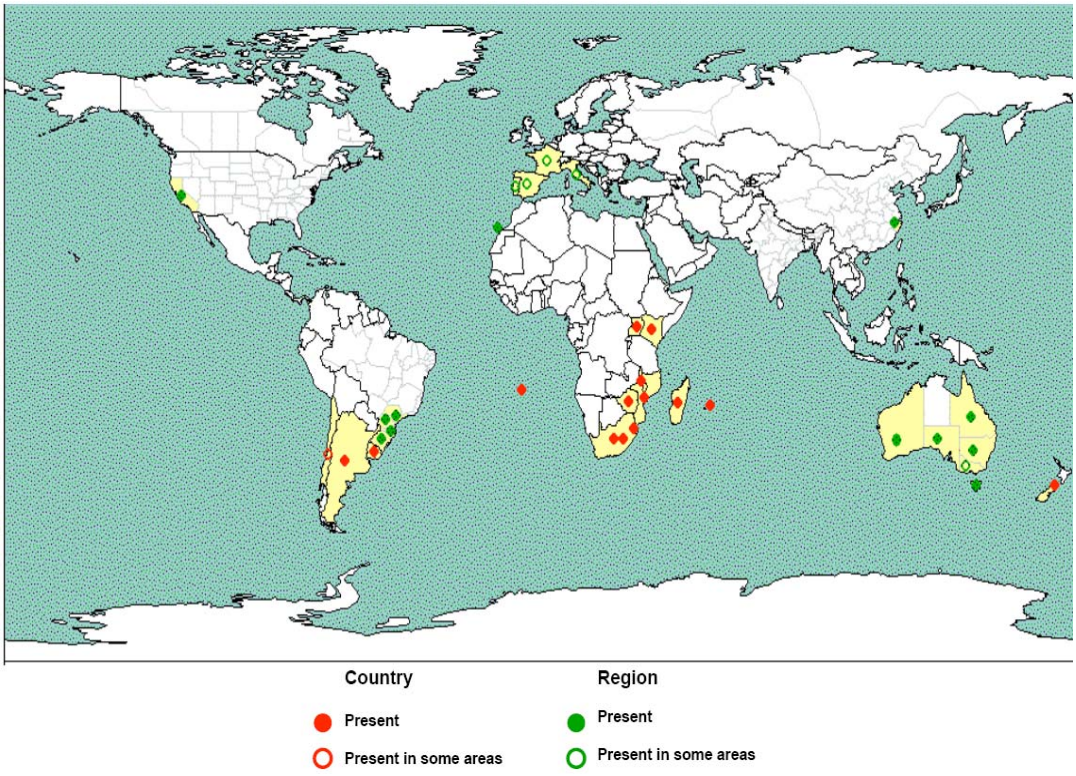

Figure 1.1: Geographical distribution of *Gonipterus scutellatus* (EPPO, 2005).

However, in its native range it is of less importance as a pest, with only a few instances of temporary defoliation and outbreaks recorded in native forests and plantations in its place of origin, south-east Australia (Loch and Floyd, 2001). This is due to the presence of natural enemies such as *Anaphes nitens* Siscaro (Hymenoptera: Mymaridae), which exclusively parasitize and kill the weevil's eggs (Loch, 2008). Nevertheless, in the 1990s *G. scutellatus* developed into the most severe pest of *Eucalyptus globulus* plantations in south-western Australia, giving rise to the suggestion that it was introduced into the region with seedlings of *E. globulus* from the south-eastern part of the country (Loch, 2006; Loch, 2008). The species of *Gonipterus* present in south-western Australia, *Gonipterus scutellatusD* is endemic to NSW and is different from the weevil that naturally occurs in the eastern part of the mainland (*Gonipterus scutellatusF*), and hence the parasite *A. nitens* that occurs naturally in south-eastern Australia is unlikely to be effective in controlling the *Gonipterus scutellatusD* outbreaks (pers. comm. R.

Oberprieler; Mapondera *et al*., 2008) (Fig. 1.2). Eucalyptus weevils were first introduced accidently into South Africa in 1916 (Malley, 1924; Tooke, 1955), and rapidly became a pest causing damage on eucalyptus plantations (Tooke, 1955). According to the early studies of *G. scutellatus* in South Africa *E. viminalis*, *E. punctata* and *E. gobulus* are the most susceptible species, of which *E. viminalis* is the preferred host (Malley, 1924; Tooke, 1955). Currently ca. 700 000 ha of the 1.4 million ha of plantations in the country, comprise eucalyptus trees (Gebeyehu *et al*., 2005).

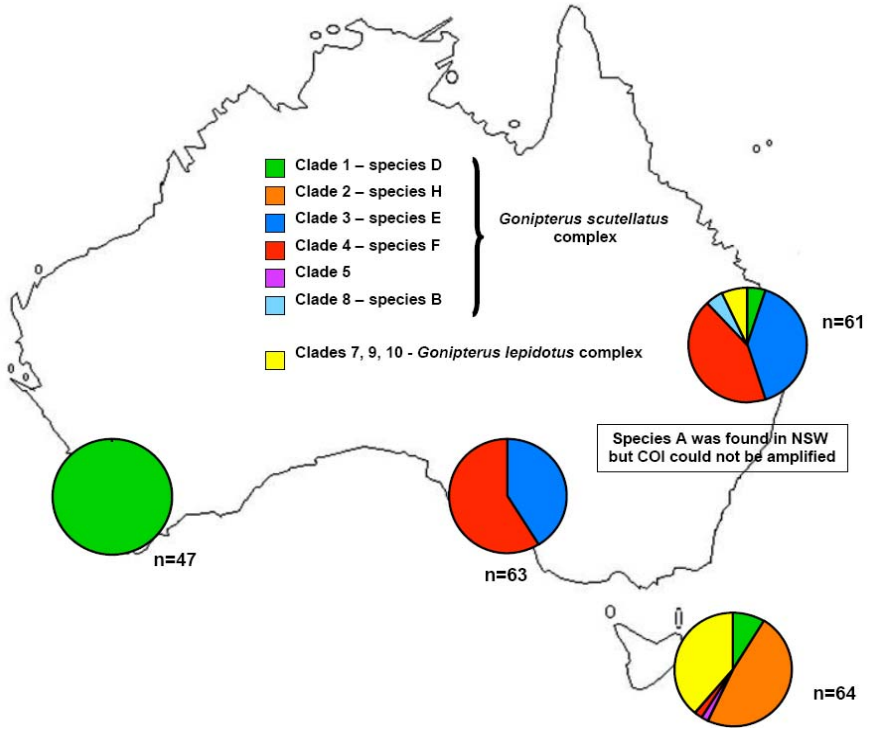

Figure 1.2: Regional composition of the *Gonipterus scutellatus* species complex in Australia (Mapondera *et al*., 2008).

Although, the selection of eucalyptus species for commercial plantations has been under progressive change from the 1980s onwards, the main species planted remained either the pure stands of *E. nitens*, *E, macarthurii*, *E. grandis*, *E. dunnii*, and *E. smithii*, or hybrids of *E. grandis* with *E. nitens*, *E. urophylla* and *E. camaldulensis* (Morris, 2008). Of the few insect pests known to attack eucalyptus

in South Africa, *G. scutellatus* is the most severe defoliator (Gebeyehu *et al*., 2005).

Eucalyptus globulus spp. *globulus* and *E. viminalis* are indicated as the preferred hosts of eucalyptus weevil (Dungey and Pots, 2003; Hanks *et al*., 2000; Loch, 2008; Millar *et al*., 1998). However, the eucalyptus weevil feeds on a variety species of eucalyptus and causes different levels of damage on different species in different countries. For instance, studies conducted in southern Tasmania on seven naturally co-occurring eucalyptus species indicated more eggs of *G. scutellatus* (an insect which is also native to Tasmania) on *E. pulchella*, *E. tenuiramis* and *E. amygdalina* than on *E. viminalis, E. ovata*, *E. globulus* and *E. oblique* (Clarke *et al*., 1998). All these species are native to Tasmania, and the first three are endemic (Duncan, 1990). Furthermore, Dungey and Pots (2003) indicated in their field study that some hybrid species of eucalyptus are more susceptible to the weevils than the pure species. However, this result was not due to *G. scutellatus* but rather *G. rufus* which they had misidentified as *G. scutellatus* (pers. comm. R. Oberprieler). The resistance of eucalyptus species to pests is attributed to the chemical composition of their foliage (Fuentes *et al*., 2008), as indicated by the indirect relationship between the concentration of a monoterpene, (1,8-cineol) or sideroxylonal, found in the oil of the leaves of *E. camaldulensis*, *E. sideroxylon* and *E. milliodora*, and the differential susceptibility of these species to insect herbivory (Fuentes *et al*., 2008). The host preference of the eucalyptus weevil is not entirely the same across countries and host species. Table 1.1., indicates the differences in susceptibility of some *Eucalyptus* species. This host preference variation across countries seems largely due to "*G. scutellatus"* being a species complex. For instance the weevil species present in southern and central California, San Felipe region (Chile), Italy and Spain are *G. scutellatusD*. Whereas the weevil species found in Mauritius and Kenya are *G. scutellatusF* (pers. comm. R. Oberprieler, CISRO). Bernard Slippers has suggested that there are at least two species of the weevil in South Africa (pers. comm. FABI, Pretoria).

Table 1.1: Difference in susceptibility of eucalyptus species to the *Gonipterus scutellatus* species complex across different geographical regions.

	Country of introduction	**Preferred host range of Eucalyptus weevils**	**Resistant *Eucalyptus species* (not attacked)**	**References**
	N. America, California (southern and central California).	*E. globulus, E. viminalis* and *E. territicornis*	*E. cladocalyx, E. amaculata, E. polyanthemos, E. saligna* and *E. trabutti*	(Hanks *et al*., 2000)
	S. America, Chile (San Felipe region)	*E. gobulus* spp. *globulus, E. camaldulensis, E. viminalis, E. robusta, E. punctata, E. maidenii* and *E. smithii*	*E. saligna* and *E. citriodora.*	(Fuentes *et al*., 2008)
Europe	Italy	*E. globulus* spp. *globulus,*	*E. cinerea, E. gunnii, E. polyanthemos, E. stuartiana* and *E. rostrata.*	(FAO, 2007)
	Spain	*E. globulus* spp. *globulus, E. oblique, E. longifolia, E. grandis,* and *E. proinqua*		FAO, 2007)
Africa	Madagascar	*E. cornuta, E. viminalis, E. punctata, E. globulus* spp. *globulus, E. uringera,* and *E. camaldulensis*		(FAO, 2007)
	Mauritius	*E. robusta, E. territicornis* and *E. kirtoniana.*		(FAO, 2007)
	Kenya	*E. globulus* spp. *globulus, E. maidenii* spp. *globulus, E. robusta* and *E. smithii*	*E. saligna,* and *E. citriodora*	(FAO, 2007)

1.7 Eucalyptus plantations

There are over 500 species of eucalyptus native to Australia, excepting two species, *Eucalyptus urophylla* in Timor and *E. deglupta* in Papua New Guinea, Irian Jaya, Indonesia and the Philippines (Campinhos, 1999). *Eucalyptus* species range from sea level to 1800 m in altitude and favour mainly acidic soils in a wide range of environments extending from hot, humid, tropical lowlands to cool, temperate highlands (Campinhos, 1999). They are the most extensively grown trees in the world, occupying almost all environments due to the diversity of species in the genus, which enables selection of species that can grow in a wide range of altitudes, climate and soil exceeding their natural range of occurrence (Campinhos, 1999). For instance *E.* g*lobulus* can grow beyond its natural limits (latitude 38.5° S and 43.5° S under 1000 m) in Ethiopia at latitude 12° N and 2500 m altitude and in Peru at latitude 5-10° S and 3000 m altitude (Campinhos, 1999).

The total world eucalyptus wood production covers about 14 million ha (8% of the world is total productive planted forests) (Morris, 2008). An estimated 10 million ha in the tropics are planted to eucalyptus of which one million ha falls to India and about three million ha to Brazil (Turnbull, 1999); while in temperate climates, Chile has planted about 300,000 ha. These figures were estimated over a decade ago and there has been a huge demand increase both for pulp and wood eucalyptus product since then, as illustrated in South Africa, where the total eucalyptus plantation cover expanded from 477, 000 ha in the 1990s (Turnbull, 1999) to 700,000 ha after 2000 (Gebeyehu *et al*., 2005). In fact, the eucalyptus plantation is by far the fastest-expanding sector of industrial forestry in the world (Wingfield *et al*., 2008) with the majority of such plantations being in Brazil, South Africa, Spain and Portugal (Turnbull, 1999). The increasing demand for pulp and wood has been growing at a rate of 11.2% annually since 1980s (Campinhos, 1999). Eucalyptus trees also attract the attention of local communities and farmers by providing extensive benefits in a shorter period than indigenous plants. For instance, many

people in Ethiopia are completely dependent on eucalyptus for fuel and house building materials (Turnbull, 1999).

The first introduction of eucalyptus into South Africa was in the late 19th century, when they were imported to grow mining poles, and by 1940 about 149 species were already established (Forsyth *et al*., 2004). Most of all the early introductions of eucalyptus were for railroad ties, mining poles and milled lumber. However, this has recently been extended to a number of new roles including an economic source of biomass, hard wood veneer, high-quality cellulose pulp for paper production, as windbreaks in agricultural areas, for aesthetic value, fire-wood, honey production and insect repellents (Paine *et al*., 2000).

1.8 The controversies around eucalyptus trees

The common perception of alien plants by ecologists and biodiversity experts is that they have a negative impact on natural ecosystems and environments when introduced into new regions. Although it is not the objective of this research report to discuss this matter broadly, it is worth comparing the arguments around this notion in brief.

Eucalyptus trees are the main sources of paper, providing an excellent-quality bright fiber highly suitable for copying, writing, printing and tissue papers and almost dominating other sources of pulp, particularly in the tropics and subtropics, like *Acacia*, *Gmelina* and *Pinus* (Campinhos, 1999). Eucalyptus also provides the best source of fire wood. For instance, in Brazil, the largest producer of pulp wood in the world, most of the eucalyptus is used to provide high-quality industrial charcoal for iron and steel production (Campinhos, 1999). In most developing countries, where large portions of the populations live in rural areas, the main source of energy is wood and most of the indigenous trees have been removed for fuel. In such circumstances eucalyptus trees, with the shortest rotation period (an

average of seven years) and a wide variety of species to choose from, have a competitive advantage over other, native plants. Eucalyptus trees can grow in degraded soils, where indigenous trees fail to establish, and are planted widely as a quick fix to the shortage of fire wood, e.g. in southern China, where eucalyptus trees, because of their short rotation, have been successfully grown on degraded lateritic soils (Turnbull, 1999). In fact, most attempts to grow indigenous plants to meet the local demands for firewood and for other benefits require much effort and are often unsuccessful due to the lack of available knowledge on their biology, ecology and silviculture, a situation that makes eucalyptus even more attractive due to the richness of information or techniques of propagation and different environmental requirements (Feyera *et al*., 2002).

In contrast to this benefits, many people or experts argue that eucalyptus trees degrade the soil, consume a large amount of water and lead to drying of local water resources, out-compete native plants for water, light and nutrients, lead to soil erosion, promote fire in natural forests, reduce biodiversity (Turnbull, 1999) and produce allelopathic chemicals that inhibit the growth of other plants under their canopies, and even if eucalyptus trees are accepted for plantations they are regarded as "a green desert without biodiversity, monotonous, and not aesthetically pleasing" (Jagger and Pender, 2003). However, these arguments, and particularly those of biodiversity and the allelopathic effect, are contradicted by Feyera *et al*. (2002), who showed that many indigenous plants in Ethiopia grow under the canopy of eucalyptus stands. In fact, such indigenous plant cover per ha is larger in the eucalyptus plantations than in those of other exotic trees such as *Pinus patula* and *Cupressus lustitanica* (Table 1.2).

Table 1.2: Density of regenerated native woody plants in different exotic tree plantations and ground cover with forbs and graminoids at Degaga, Ethiopia (Feyera *et al*., 2002).

Plantation stand/species	Density of native sp. (stem ha^{-1})	Density of ground cover (%) from native species
E. saligna (29 years old)	23,630	89
E. globulus (24 year old)	17,430	100
Pinus patula (23 year old)	5,760	40
Cupressus lustitanica (27 year old)	4,750	0
Adjacent natural forest	-	100

NB. - indicates data not present.

The list of contradicting views about the planting of eucalyptus trees is long, but the huge socio-economic value of eucalypt plantations is undeniable, as is the lack of competitive and vigorous indigenous plants that provide the same services. For instance, in South Africa eucalyptus and pines constitute almost the total forestry plantations, employing about 100,000 people and contributing over 2% of the GDP (US$300 million per annum), and a further US$1.6 billion is generated from industrial products that are based on forestry plantations most of which are exported (Le Maitre *et al*., 2002). However, eucalyptus plantations threaten the water resources of the country due to their large water consumption and high rate of evapo-transpiration, particularly in riparian ecosystems and along water catchments. One such example is the afforestation of water catchments in the province of Mpumalanga, which led to a complete drying-up of streams within 6-12 years after planting (Forsyth *et al*., 2004). Despite such negative impacts the demand for eucalyptus plantations has not ceased, and is continuing to grow at even higher rates, particularly in tropical and subtropical developing counties where deforestation and soil degradation is failing to support local communities with wood for fire (Turnbull, 1999).

Therefore, eucalyptus plantations will not be abolished in the foreseeable future. However, they can be planted in demarcated areas with proper management so as to reduce their impacts on natural resources such as water. An estimated 198 exotic plant species are considered as serious environmental problems in South Africa and regulated in terms of the Conservation of Agricultural Resources Act, 1983 (Act No. 43 of 1983) (Zimmermann and Olckers, 2003), which was amended in March 2001. *Eucalyptus camaldulensis* and *E. grandis* are among the invasive alien plants that require proper control measures (Forsyth *et al*., 2004). Thus, it is important to investigate the pathogens and insects that attack and reduce the productivity of eucalyptus trees. It is in this context that this study focuses on the intensity of damage caused by the eucalyptus weevil and its host preferences among several *Eucalyptus* species grown in South Africa.

Chapter 2.

Materials and Methods

2.1 Introduction

This study was conducted in the laboratory at the University of the Witwatersrand, Johannesburg, the Forestry and Agricultural Biotechnology Institute (FABI) nursery at the University of Pretoria, and the "Tom Jenkins" eucalyptus plantation in Pretoria belonging to the Pretoria Zoo. The plants at the FABI nursery were about eight years old and grown from identified eucalyptus seeds imported from the CSIRO, Australia, for experimental purposes. The "Tom Jenkins" plantation had 23 identified species of eucalyptus, planted to provide food for the koala marsupial at the National Zoological Gardens, Pretoria, and managed by the same institution. There are many eucalyptus trees in and outside of Wits campus, but since identification of the species of these trees is notoriously difficult, the FABI nursery and "Tom Jenkins" plantation were chosen to conduct the experiments of this study. However, two of the test plants used, *Eucalyptus dunnii* and *Syzygium myrtifolia*, were growing at Wits University and identified by experts.

The laboratory test was designed as choice and no-choice trials of cut leaves in Petri dishes and bouquets in cages. To determine the realized host range of *G. scutellatus*, a field survey of the weevils' performance was carried out in parallel to the laboratory trials at the "Tom Jenkins" plantation under natural conditions. No-choice confinement trials were also conducted on sleeved branches at the FABI nursery. These no-choice trials were run, because chemistry of cut leaves and bouquets can change due to the physical injury caused by cutting, and as a result the response of the weevils might also change (Pare and Tumlinson, 1999). The difference in palatability of host plants to insects is primarily based on the genotype of the plants, such as the level of phenolic glycoside, the main determinant of host quality, and the level of nutrients (foliage nitrogen level) as a

secondary factor that determines herbivore performance on host plants (Osier and Lindroth, 2001). For instance, in an experiment to evaluate the effects of such compounds on the performance of gypsy moths (*Lymantria dispar*) on artificially defoliated saplings of quaking aspen, *Populus tremuloides*, a high concentration of foliar phenolic glycoside affected the relative performance and growth rate of the herbivore negatively, while nitrogen level was positively correlated with insect performance (Osier and Lindroth, 2001). Also when plants suffer physical damage such as herbivore feeding or cutting of branches, they release larger amounts of volatile chemicals, and the level and identity of the volatile compounds released varies with plant species (Pare and Tumlinson, 1999). Plant secondary metabolites (PSMs) are chemical compounds that do not perform any other function apart from defence against herbivores, and their level of concentration increases during physical injury (Edwards and Wratten, 1983; Pass *et al*., 1998). Plants with high concentrations of secondary compounds are more resistant to beetle attack than those with less foliar concentration of such chemicals (Stenberg *et al*., 2008). For instance, Fuentes *et al*., (2008) indicated the existence of an inverse relationship of foliar monoterpene (1,8-cineol) concentration and the susceptibility of *Eucalyptus camaldulensis* to herbivory.

Insect host selection is a behavioral response stimulated by cues of a visual, olfactory, gustatory and physical nature of the plants (Heard, 2000). Insects use such cues during host selection by prioritizing factors affecting them in a hierarchal fashion, starting with habitat location and followed by host location, host acceptance and host use (Heard, 2000). Laboratory trials in confinement can alter this natural behavioral process of host selection and are likely to result in false positives or false negatives (McEvoy, 1996; Briese and Walker, 2002). As such, Petri dish trials can be designed as choice and no-choice tests for larvae due to their small space requirement, whereas larger arenas such as cages are better suited for adult feeding and ovipositon testing (van Lenteren *et al*., 2006).

In general, host specificity testing of phytophagous insects in restricted areas has its advantages and disadvantages. Larval no-choice tests in the laboratory help to determine test plants that would support larval feeding and survival until maturity is attained, and for the adult they are used to evaluate interests in feeding and oviposition (Barratt, *et al*., 2007). Insects have a hierarchal choice of host plant preferences. When the preferred host is available in a suitable habitat, other plants in the host range remain as the least preferred plants, but in the absence of the preferred host the next plant in the hierarchy becomes the most preferred one (Conlong *et al*., 2007). This hierarchy can be determined through no-choice and choice trials in the laboratory. Such no-choice trials can, however, sometimes lead to false positive results due to the absence of the correct feeding or oviposition cues (Heard, 2000). However, the absence of any chance to escape makes the negative results even more robust and increases the conservative effect of the method (Briese and Walker, 2002). Results from no-choice tests help to understand the behavioural response of a candidate agent in situations where the target weed is absent or less abundant (Heard, 2000). Results of no-choice tests are also robust in feeding and survival trials of larvae incapable of moving from one tree to another, as in *G. scutellatus*, which will either eat or starve to death (Barratt *et al*., 2007). Larval and adult choice tests of *G. scutellatus* were thus conducted on cut leaves and caged bouquets to determine host preference by comparing feeding and oviposition. Choice tests also allow evaluation of the pattern of insect performance on non-target plants, in the presence of the target plant (Heard, 2000). In this study, *E. globulus* was considered to be the preferred host based on assumptions made in the literature (Dungey and Pots, 2003; Hanks *et al*., 2000; Loch, 2008; Millar *et al*., 1998) and was paired with each non-target plant in the choice tests.

The open field survey was conducted to examine the field host range of the weevil in a natural environment, in order to check and evaluate the false positive results obtained from the closed environment of laboratory no-choice tests.

2.2 Experimental procedures

Fourteen species of *Eucalyptus* and one closely related species of *Syzygium*, which belongs to the same family, Myrtaceae, as *Eucalyptus*, were tested for host preferences of the eucalyptus weevil, *Gonipterus scutellatus*, in the laboratory, and 23 species of eucalyptus were tested in the field survey, between December 2008 and March 2009. Of the 15 species used for the laboratory test, only ten were included in the survey since the five remaining species were not grown at the site of the field survey. Instead, 13 other eucalyptus species cultivated in the field where the survey was conducted were included to determine the host range of the weevil in the region. *Eucalyptus globulus* spp. *globulus* was assumed to be the preferred host throughout the experiment, since this species is most often mentioned in terms of host preference of the beetle (Hanks *et al*., 2000; Dungey and Pots, 2003; Millar *et al*., 1998; Loch, 2008).

All plant material was collected from the FABI nursery and the "Tom Jenkins" plantation except of *E. dunnii* and *Syzygium myrtifolia*, which were sampled at Wits University. All adult weevils and larvae, except for those mentioned in the field survey, were collected from the same place in Centurion, from the eucalyptus trees planted along the Old Johannesburg Road to Pretoria, the R101, next to the South African Air Force Center (S26°17´47.7" and E030°35´46.0"). Data were recorded on a weekly basis during each set of experiments on larval feeding, adult feeding and oviposition. Larval and adult survival was assessed after two weeks, except larval survival at the FABI nursery, which was measured after one week. This difference was due to the high temperatures in the nursery, which at the time of the experiment reached 28 - 30°C, with hot sunny days and frequent rainfall, causing most of the larvae to die before they reached the second week of monitoring. After two failed trials of larval survival over two weeks, the larval survival at the FABI nursery was recorded after one week during relatively mild temperatures, of about 25°C, and no rain.

2.2.1 Laboratory tests

The physiological host range of the weevil was determined in the laboratory in Petri dishes and cylindrical cages of cotton mesh with a diameter of 63 cm and a height of 60 cm. In the no-choice test, each plant species was presented on its own whereas in the paired-choice test the preferred host, *E. globulus*, was placed together with another non-target species in a single cage or Petri dish, for all combinations of all the non-target plants that sustained some sort of attack during the no-choice tests. The number of replicates for each test was six. The larvae of *G. scutellatus* have four instars that differ in the size of head capsule. Body size was used as a crude surrogate for head capsule width and used to classify larvae into four size classes.

Cut-leaf test

Leaf tests were conducted for larval feeding and survival only and for the investigation of feeding rates on different species of eucalyptus in the no-choice test, two leaves per plant species of about the same size, were placed in each of six Petri dishes with moist filter paper. These dishes were inoculated with two larvae of size classes 1-3, i.e., a pair of larvae of the same class in each of two Petri dishes, with a total of 12 larvae in the six replicates. In the paired-choice test one leaf from the preferred host plant species (*E. globulus*) and a similar-sized one from a non-target plant were placed in each Petri dish and inoculated with larvae of size classes two, three and four. Three larvae of each size class were placed in each Petri dish for a total of six replicates. Feeding was measured as mm^2 of the leaf epidermis grazed by the larvae, estimated by overlaying 1 mm^2 transparent graph paper every day after changing the leaves, and the larval survival was recorded after two weeks.

Caged bouquet test

Both larvae and adults were tested in no-choice and choice tests in cages. Branch-tip "bouquets", about 30 cm long, were cut, rinsed of potential predators and debris and then placed in flasks of water to maintain turgidity. In no-choice tests, each plant species was caged individually and inoculated with four insects, two males and two females. The number of replicates for each plant species was six. In the paired-choice tests, one branch of *E. globulus*, as the preferred host, and one of a "non-target" *Eucalyptus* species were placed together in a single cage, covering all non-target plants that sustained some attack during the "no-choice" tests. The bouquets were changed every week to prevent wilting of the leaves and corresponding changes in their palatability to the weevils. Defoliation or the proportion of leaf damage, was estimated on a scale of 0-4, where 0 represented no feeding, 1<5%, 2=6-25%, 3=26-50% and 4=51-100 %.

2.2.2 Field-confinement tests

The no-choice tests in the caged bouquets trials were repeated on trees growing in the FABI nursery. A branch tip 30 cm in length of six randomly selected trees per species was sleeved in a cylindrical cage of cotton mesh (35cm of height and 15cm of diameter) on the tree. In the test of larval survival a pair of larvae of size classes three or four was placed in each sleeve, while in test of adult survival two males and two females were place in each sleeve. The oviposition test was repeated with a single pair of adults as the initial trial failed to result in any oviposition, as the limited space provided by the sleeve and the level of foliage damage caused by four adults could have prevented the females to oviposit in the sleeves

Table 2.1: Taxonomic grouping (from Brooker, 2000) of *Eucalyptus* species used in the field and laboratory to test the host preference of *G. scutellatus*. (* = absent, ** = sleeved in Tom Jenkins plantation, *** = tested at Wits University).

Genus	Subgenus	Section	Species		
			Bouquet test plants (Wits lab)	**Sleeved branches (Field test, FABI)**	**Field survey (Tom Jenkins)**
Eucalyptus	*Eucalyptus*	*Maidenaria*	*smithii*	*smithii*	*smithii*
			nitens	*nitens*	*nitens*
			viminalis	*viminalis* **	*viminalis*
			globulus	*globulus*	*
			dunnii	*dunnii****	*
			macarthurii	*macarthurii*	*
					nicholii
					ovata
					dorrigoensis
					scoparia
					goniocalyx
		Latoangulatae	*grandis*	*grandis*	*grandis*
			urophylla	*urophylla*	*
			saligna	*saligna*	*saligna*
					botryoides
					robusta
					punctata
					propinqua
		Exsertaria	*camaldulensis*	*camaldulensis*	*camaldulensis*
			tereticornis	*tereticornis*	*tereticornis*
		Eucalyptus			*obliqua*
		Pseudophloius			*pilularis*
	Symphyomyrtus	*Adnataria*	*paniculata*	*paniculata***	*paniculata*
					sideroxylon
	Alveolata		*microcorys*	*microcorys***	*microcorys*
	Corymbia	*Septentrionales*	*citriodora*	*citriodora***	*citriodora*
					maculata
Syzygium			*myrtifolia*	*	*myrtifolia****

2.2.3 Field surveys

The assessment of weevils feeding performance in an open field test was conducted at the Tom Jenkins plantation during February and March 2009. Branch tips of 50 cm length were scored for adult feeding and oviposition and for larval feeding activity. Trees in this plantation are planted in plots each including all the 23 eucalyptus species, each species grown in one row per plot containing 12 trees spaced 3 m apart from neighbouring trees. Twelve plants from two plots, six from each, were randomly selected and surveyed for larval or adult feeding and for oviposition.

2.2.4 Risk analysis

The relative preference of the weevil for each non-target plant (Designated as R) was quantified as the proportion of its performance for different parameters (survival, feeding and oviposition) of that on the most preferred host, *E. smithii* (Table 2.4 and Appx. A and B). The overall potential risk of attack by the weevil to each of the 15 non-target plants was determined by calculating the product of the relative preference of the no-choice larval survival (R^1) and the relative female choice for oviposition in the multi-choice field survey (R^4) (Baars *et al*., 2003). The choice of these two parameters was based on the assumption that larvae able to survive on a test plant in a no-choice test are likely to reach maturity and reproduce, and yield viable adults, and that a host selected for oviposition under field conditions, is likely to support larval development. However, in this study larval survival (R^1) was substituted by larval feeding (Table 2.4), since the survival trial was not carried out long enough to permit assessment of larval survival to pupation. The level of larval feeding recorded on non-target plants during such no-choice trials also implies that the larvae can survive, and hence these two variables (larval survival and larval feeding) can be used interchangeably.

In the calculation of the relative performance of the weevil in the risk analysis, all results with zero values were replaced by 0.001 for calculation purposes. In the case of species for which some data were lacking, as with *E. globulus*, *E. urophylla*, *E. dunnii*, and *E. macarthurii* in the field survey, the values of the corresponding species from the caged-bouquet trials were used and divided by the value obtained for *E. smithii* in the same test, because *E. smithii* was found to be the preferred host in these trials (Table 2.4).

2.2.5 Species identification

Because the eucalyptus weevil has at least two species in South Africa (pers. comm., Bernard Slippers, FABI), it was important to ensure that the species identity of the weevil used in the laboratory and the field trials was the same. Weevils used in the laboratory experiment were all collected from the same site (Centurion), and ten weevils were collected from each field site, Centurion and the "Tom Jenkins" eucalyptus plantation in Pretoria. Voucher specimens of these samples were submitted to B. Slippers (FABI) and R. Oberprieler (CSIRO) for identification.

2.2.6 Statistical analysis

All the data in the experiments except for those of choice tests were transformed to approximate a lognormal distribution, as the log of base ten plus one, $\log_{10}(x + 1)$, where x is a number in the actual raw data and the constant number "1" is added to prevent the values reaching zero, which does not give a log value. The transformed data were tested using Analysis of Variance (ANOVA) followed by Duncan's new Multiple Range Test (MRT). This test, for multiple comparisons of data, uses the studentized range statistic q_r to compare the differences between means in responses to *G. scutellatus* in terms of feeding, oviposition and survival to the different test plants. The results from both larval feeding choice tests in Petri dishes and adult feeding and oviposition choice tests in cages were analysed using

the Mann-Whitney non-parametric U test for comparing two independent sets of samples. The Mann-Whitney U test is an alternative to the t-test when the data are ordinal, and, unlike the t-test, does not assume normality of the data distribution. STATISTICA six sigma (Statsoft Release 7, 2006) and Microsoft Office Excel (2007) were the computer packages used for data analysis.

2.3 Results

The results are presented in tow sections, the laboratory and field results and the risk analysis. In the laboratory and field results, the larval and adult performance of the eucalyptus weevil was compared in terms of survival, feeding intensity and oviposition on different eucalyptus tree species, presented in tables and graphs. The suitability of the test plants to support feeding and oviposition of *G. scutellatus* was then assessed as a risk analysis.

2.3.1 Laboratory and field results

The larval feeding intensity on leaves of different *Eucalyptus* species in Petri dishes showed significant differences between tree species ($F_{(14,\ 75)}$=5.18, P<0.001). *Eucalyptus smithii* suffered the highest level of damage, with a mean of 22.1 cm^2 (at 95% CI: 7.2-6.8), followed by *E. tereticornis* and *E. dunnii*, although there were no significant differences between these three plant species (Table 2.2). This pattern of larval feeding preference changed when compared to the sleeved-branch trials, in which larvae were exposed to the end of living branches. The feeding damage was significantly different across all species ($F_{(13,\ 70)}$=12.75, P<0.001), but this time *E. tereticornis* was the top of the rank in terms of damage intensity, followed by *E. citriodora*, *E. urophylla*, *E. viminalis* and *E. globulus*. Some species, such as *E. nitens* and *E. paniculata*, were well used by the larvae in confined trials on both cut leaves and sleeved-branches, but no feeding was reported in the field survey (Table 2.2).

Table 2.2: Host preference of *Gonipterus scutellatus* larvae for different *Eucalyptus* species as determined by feeding intensity in laboratory and field trials.

Test plants	Mean larval damage (95 % C.I)		
	Cut-leaf[x]	Sleeved-branch[y]	Field survey[y]
E. smithii	22.1 (7.2, 68.0) e	3.3 (2.8, 3.9) cd	4 (4, 4) g
E. urophylla	11.7 (0.9, 153.5) cde	4.6 (4.1, 5.2) de	-
E. viminalis	2.7 (0.3, 22.7) bcde	4.4 (3.6, 5.5) de	3.7 (3.0, 4.5) g
E. grandis	1.1 (0.1, 9.1) abc	4.1 (3.1, 5.3) de	4 (4, 4) g
E. tereticornis	18.7 (11.0, 31.8) de	5.0 (5.0, 5.0) e	2.2 (1.5, 3.0) def
E. camaldulensis	3.3 (0.3, 35.8) bcde	3.9 (3.1, 5.0) de	2.2 (1.6, 3.1) def
E. nitens	8.2 (0.7, 94.9) cde	2.5 (1.9, 3.1) bc	0 a
E. dunnii	14.8 (4.7, 46.4) de	2.2 (1.4, 3.4) b	-
E. globulus	2.3 (0.2, 32.1) bcde	4.4 (3.6, 5.5) de	-
E. microcorys	0.9 (0.1, 7.9) abc	3.7 (2.9, 4.9) de	1.8 (1.8, 1.8) cdef
E. macarthurii	0.4 (0.0, 4.1) ab	2.2 (1.4, 3.4) b	-
E. citriodora	1.6 (0.1, 43.7) bcd	4.8 (4.4, 5.3) e	2.3 (1.7, 3.1) ef
E. paniculata	0.4 (0.07, 2.4) ab	3.3 (2.1, 5.1) cd	0 a
E. saligna	0 a	0 a	1.1 (0.3, 0.3) ab
S. myrtifolia	0 a	-	-

NB: Means compared by One-way ANOVA; those means in the same column followed by the same letter(s) are not significantly different ($P > 0.05$; Duncan's multiple range test).

[x] Feeding scores in cm^2.

[y] Feeding categories defined in chapter 2 section 2.2.1.

- Species not present in the field.

Eucalyptus saligna and *Syzygium myrtifolia* were immune from larval feeding across all three larval trials. A very low level of feeding was recorded on *E. saligna* during the field survey, but this was not significantly different from that on *E. paniculata* on which no feeding was recorded.

There were significant differences in larval survival on different plant species in the cut leaf trial ($F_{(14, 75)}$= 4.05, P<0.001). *Eucalyptus smithii* showed the highest larval survival, although it was only significantly different from those of *E. saligna*, *E. paniculata*, *E. grandis* and *S. myrtifolia* (Figure 2.1A). Larval survival in sleeved branches also showed significant differences between test plants ($F_{(13, 70)}$=3.70, P<0.001) (Fig. 2.1B), but only *E. saligna* and *E. nitens* yield high larval mortality.

Adult survival was high in the caged-bouquet trial. Although a significant difference was indicated ($F_{(13, 70)}$=24.60, P<0.001), the only plant species on which the adult survival was significantly different was *S. myrtifolia* (Fig. 2.1C), indicating that the beetles are not resistant to starvation. In the sleeved branch trials there were no significant differences in adult survival ($F_{(13,70)}$=1.08, P<0.394), due to the fact that in this trial *S. myrtifolia*, the species that did not support larval survival in the caged-bouquet trial, was not included (Fig. 2.1D).

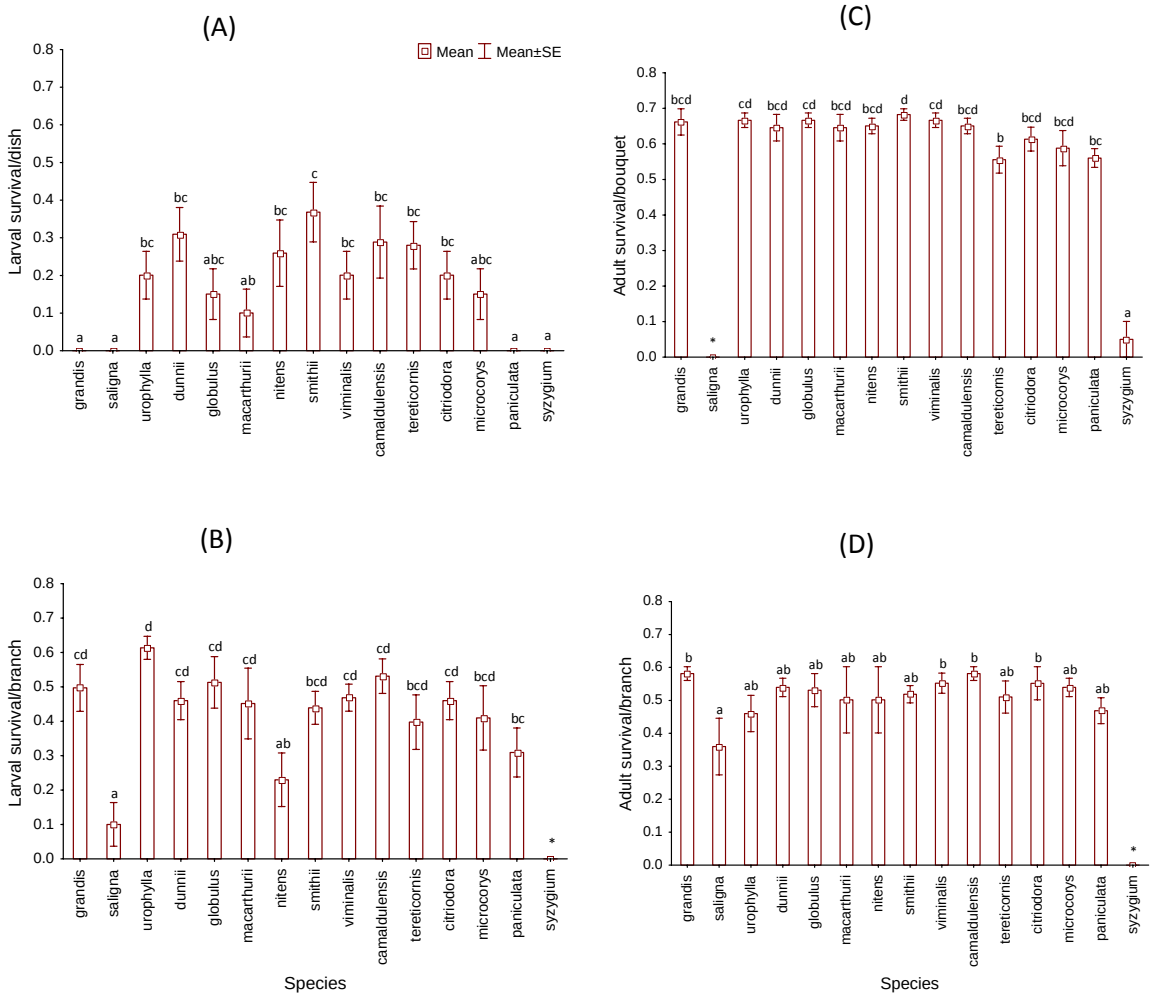

Figure 2.1: Larval and adult survival of *Gonipterus scutellatus* in different treatment of different *Eucalyptus* species, two weeks after inoculation. (A) larvae on cut leaves; (B) larvae on sleeved branches; (C) adults on caged bouquets (D) adults on sleeved branches. Means compared by One-way ANOVA and those followed by the same letter(s) are not significantly different (P>0.05; Duncan's multiple range test). NB: * species not tested; Y-axis = log transformed proportions of survivals.

Adult feeding in both the caged bouquets and the sleeved branches was significantly different between tree species ($F_{(13,\ 70)}$=15.16, P<0.001 and $F_{(13,\ 70)}$=23.57, P<0.001, respectively). *Eucalyptus saligna*, *E. citriodora* and *S. myrtifolia* showed no adult feeding in either trial (Fig. 2.2). The pattern of feeding between the trials did not differ much, except that *E. macarthurii* in the sleeved-branch test did not significantly differ from the species on which adults showed no

feeding. Although feeding on *E. smithii* was not significantly different from that on *E. urophylla*, *E. grandis* and *E. globulus*, it was the most extensive in both trials (caged bouquets and sleeved branches). Feeding on *E. smithii* was significantly more extensive than *E. viminalis* in the caged-bouquets (Fig. 2.2A) but not in the sleeved-branch test.

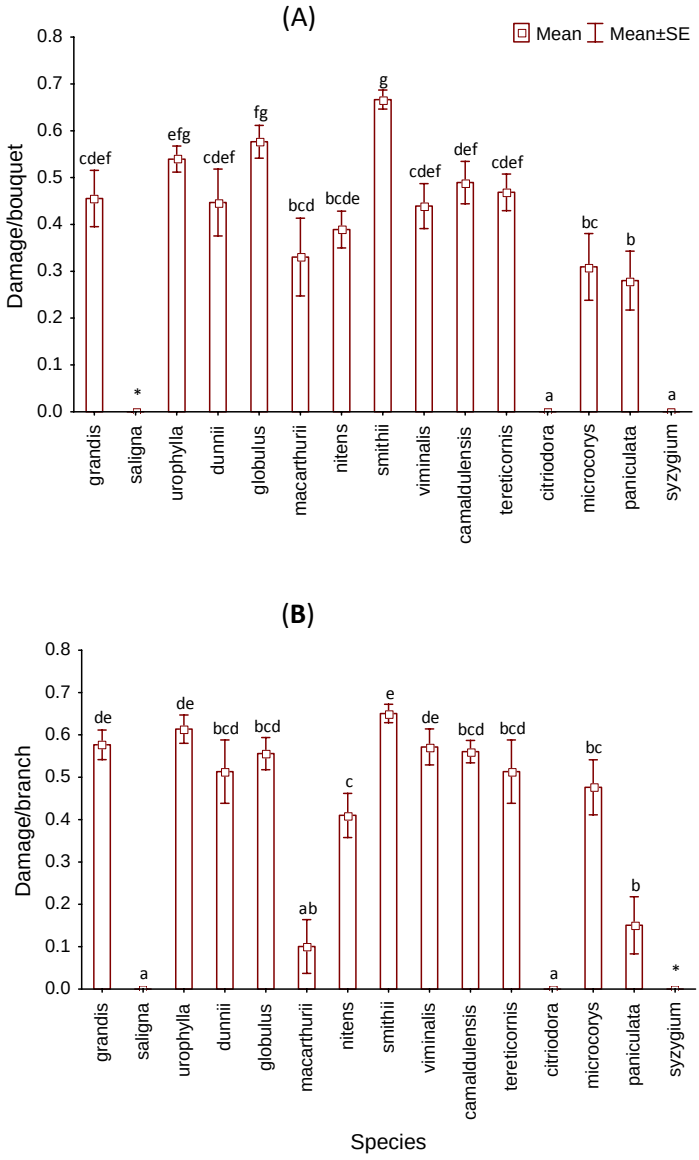

Fig. 2.2: Comparison of adult feeding of *Gonipterus scutellatus* in different treatments of *Eucalyptus*. (A) Adult feeding on caged bouquets; (B) adult feeding on sleeved branches. Means compared by One-way ANOVA and those followed by the same letter(s) are not significantly different (P>0.05; Duncan's multiple range test). NB: * species not tested; Y-axis = log transformed proportions of damage per bouquet or branch.

Survey results

There were significant differences in adult feeding on different tree species in the field survey ($F_{(22, 253)}$=31.28, P<0.001) (Fig. 2.3A). Eight of the 23 plant species surveyed were not used by the weevils, and, moreover, feeding on another three species that showed some damage was not significantly different from that on species that were untouched. Most adult feeding occurred on *E. smithii*, *E. grandis* and *E. nitens* in the field (Fig. 2.3A).

Significant differences in oviposition were recorded across the tree species ($F_{(22, 253)}$=27.84, P<0.001) (Fig. 2.3B). No eggs were deposited on 13 species and only very few on another three, but the latter result was not significantly different from the species that supported no oviposition. *Eucalyptus smithii* and *E. grandis* received significantly higher number of eggs than all other species (Fig. 2.3B).

Choice tests

In the paired choice test, *E. globulus* was assumed to be the preferred host and was therefore tested in combination with each of the other 13 *Eucalyptus* species and with *Syzygium*. Larval choice was assessed in a cut-leaf test and adult choice of feeding and oviposition on caged-bouquets.

Larvae fed on almost all the species in the trial except *E. dunnii*. The larval feeding intensity was significantly greater on *E. globulus* in only two paired tests and significantly smaller in two other tests (Table 2.3). Both *E. urophylla*, with a mean feeding intensity of 50.9±16.7, and *E. viminalis* (11.2±3.8) were significantly more damaged by the larvae than *E. globulus*. The larvae were less discriminating than the adults (Table 2.3).

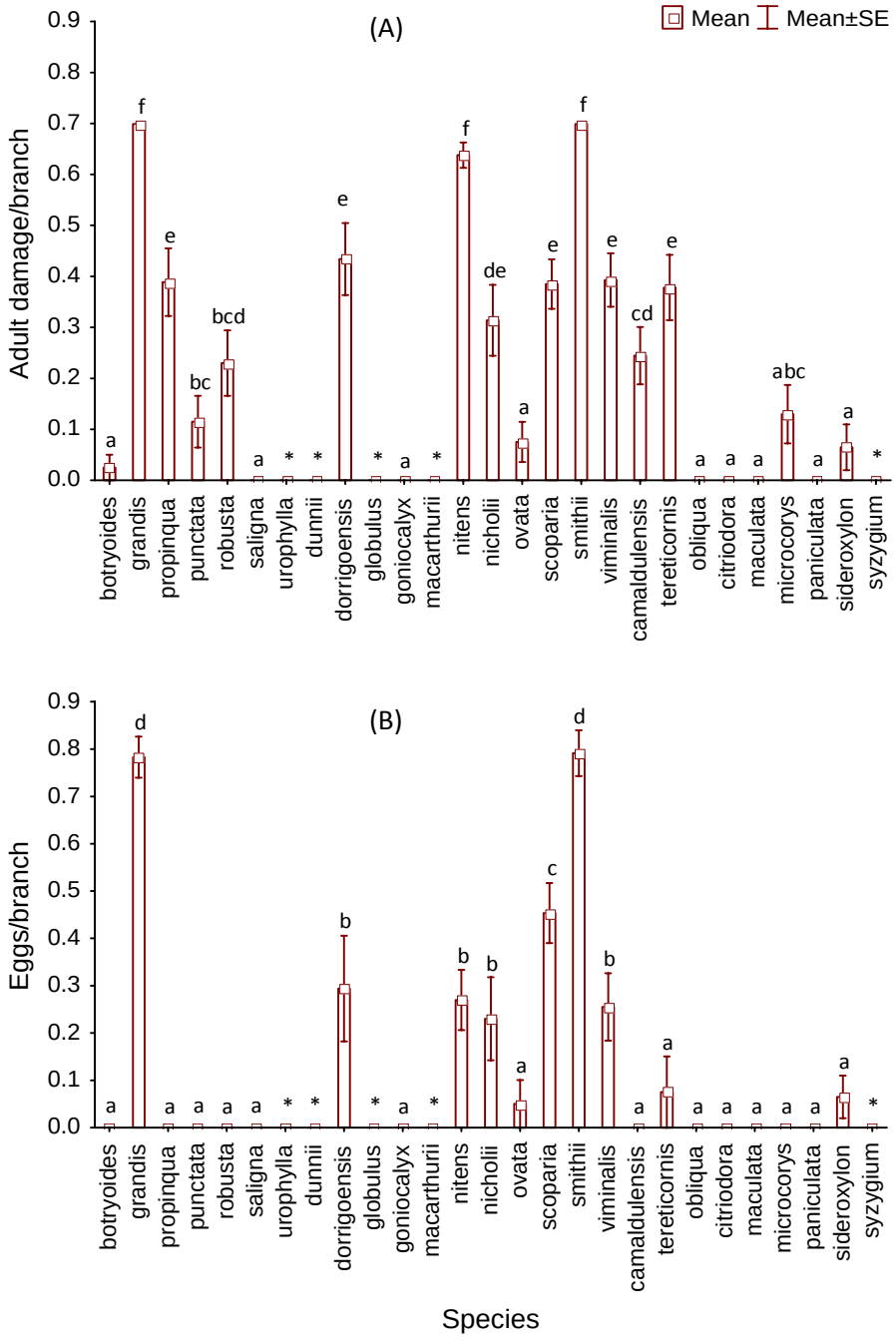

Figure 2.3: Differences in adult *Gonipterus scutellatus* performance on *Eucalyptus* species growing in a plantation, in a natural multi-choice environment. (A) Mean adult damage and (B) mean number of eggs deposited per branch. Means compared by One-way ANOVA and those followed by the same letter(s) are not significantly different (P>0.05; Duncan's multiple range test). NB: * species not tested; Y-axis = log transformed proportions of damage per branch.

The adult pattern of feeding showed significantly more damage on six species when paired with *E. globulus*, and only two pairings showed significantly more feeding on *E. globulus*, one of which was *E. saligna*, corresponding with the larval result of the larval test. Another four species showed no significant difference in feeding between species. The only test plant that was not used by the adult weevils was *E. citriodora*, but it was used by the larva in both choice and no-choice trials, including the field survey (Tables 2.2 and 2.3).

In the oviposition trials ten out of 13 tree species pairs showed no significant differences in the number of eggs laid, while there were significantly more eggs laid on *E. smithii* (6.7±2.6), *E. urophylla* (5.0±2.0) and *E. viminalis* (5.0±1.9) than on *E. globulus* (Table 2.3). In eight of the paired tests no eggs were laid on *E. globulus*, of which in four there were no eggs deposited on either of the tree species. *Eucalyptus macarthurii*, *E. nitens*, *E. citriodora*, *E. paniculata* and *E. saligna* received no eggs. In the field survey no eggs were laid on these species, except for *E. nitens*, on which a few eggs were deposited. No data were recorded for *E. macarthurii*, since it was not present at the survey site.

2.3.2 Risk analysis

The potential risk that *G. scutellatus* poses to each of the non-target tree species included in the experiment was assessed by evaluating the performance of the weevil in terms of larval feeding and oviposition with respect to the preferred host (as R for relative performance) (Olckers, 2000; Baars *et al.*, 2003). *Eucalyptus smithii* rather than *E. globulus* is concluded to be the most preferred species. The suitability and the chance of the non-target test plants being attacked was then calculated as the product of R^1 (larval feeding from the no-choice sleeved-branch trial) and R^4 (relative oviposition preference in the field survey) (Baars *et al.*, 2003).

Table 2.3: Host selection of *Gonipterus scutellatus* larvae and adults during paired choice tests as determined by their mean (± SE) feeding intensity and oviposition on different species of *Eucalyptus* in caged bouquets and cut-leaves.

Test plant species	Cut-leaf test	Caged-bouquet test	
	Mean larval feeding (cm^2)	Mean adult feeding[y]	Mean oviposition
E. nitens	13.5±5.1 a	1.7±0.2 b	0 a
E. globulus	17.4±6.2 a	1±0 a	0 a
E. saligna	1.1±0.2 a	0 a	0 a
E. globulus	15.3±2.6 b	1.5±0.6 b	0 a
E. viminalis	11.2±3.8 b	3.5±0.2 b	5±1.9 b
E. globulus	7.8±3.2 a	0.3±0.3 a	0 a
E. macarthurii	20.3±9.6 a	0.7±0.3 a	0 a
E. globulus	21.3±0.4 a	1±0.37 a	0.2±0.2a
E. camaldulensis	13.9±5.9 a	2.3±0.4 a	2.8±1.3 a
E. globulus	20.9±6.0 a	1.7±0.3 a	1±0.8 a
E. paniculata	7.3±1.9 a	1.5±0.4 a	0 a
E. globulus	22.6±10.0 a	1±0.6 a	0 a
E. tereticornis	19.4±4.2 a	3.2±0.3 b	3±1.8 a
E. globulus	26.8±7.7 a	0.8±0.3 a	0.2±0.2 a
E. microcorys	8.1±3.7 a	1.7±0.3 a	0.2± 0.2 a
E. globulus	24.8±6.2 a	2.2±0.5 a	0.2± 0.2 a
E. citriodora	15.7±3.6 a	0 a	0 a
E. globulus	21.2±8.7 a	3±0.4 b	0 a
E. urophylla	50.9±16.7 b	3.5±0.3 b	5±2.0 b
E. globulus	10.4±5.3 a	0.8±0.3 a	0 a
E. grandis	10.5±4.8 a	2.5±0.3 b	1.8±1.3 a
E. globulus	24.5±3.7 a	1±0.37 a	0 a
E. syzygium	-	-	-
E. globulus	-	-	-
E. smithii	10.5±4.7 a	3.7±0.21 b	6.7±2.6 b
E. globulus	16.6±5.9 a	0.7±0.5 a	1±1 a
E. dunnii	0 a	3.2±0.6 a	2.3±0.8 a
E. globulus	17.9±4.9 b	1.5±0.2 b	1.2±0.4 a

NB: Means compared by non-parametric Mann-Whitney U test; those paired test in the same column followed by the same letter(s) are not significantly different ($P>0.05$; Mann-Whitney U test).

[y] Feeding categories defined in chapter 2 section 2.2.1.

- Species not tested (species indicated as immune from the no-choice trial).

To compare the risk analysis as determined from the physiological and realized host ranges, the products of both larval feeding and oviposition were quantified for the laboratory (R^1*R^2) and field survey results (R^3*R^4**)** (Table 2.4).

Laboratory results indicated that *E. camaldulensis* is likely to be at high risk of attack by the weevil, with a 94% chance of supporting oviposition, whereas the risk analysis calculated from the field performance showed that it is probably safe from attack and the chance of it supporting feeding is only about 0.01%. Similarly, the laboratory results showed that *E. citriodora* would suffer feeding from *G. scutellatus*, since it has over 100% probability of being attacked, when such a risk is only 0.01% according to the field survey (Table 2.4). According to the overall risk analysis (Table 2.4), *E. camaldulensis*, *E. microcorys*, *E. citriodora*, *E. paniculata*, *E. saligna* and *S. myrtifolia*, all with under 0.03% chance of supporting the weevil (and note that these results have been over-estimated due to the addition of 0.001 to replace zero values), are very unlikely to be attacked by *G. scutellatus.* The remaining nine species, all with a 24% or greater chance of being attacked, are likely to suffer attack by the weevil.

Table 2.4: Risk assessment of attack on non-target test plants by *G. scuttelatus* as determined by the weevil's preference for oviposition and larval feeding in sleeved branch trials and a field survey in relation to *E. smithii* (the preferred host).

Test plants	Sleeved-branch-test			Field survey			Overall risk of attack to non-target plants (R^1*R^4)
	Relative Larval feeding (R^1)	Relative oviposition (R^2)	Relative risk of attack R^1*R^2	Relative larval feeding (R^3)	Relative oviposition (R^4)	Relative risk of attack R^3*R^4	
E. smithii	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E. urophylla	1.39	1.56	2.17	0.53[b]	0.81[b]	0.43	1.13
E. viminalis	1.33	1.25	1.66	0.93	0.35	0.33	0.47
E. grandis	1.24	0.0006[a]	0.0007	1.00	0.98	0.98	1.20
E. tereticornis	1.51	0.94	1.43	0.55	0.24	0.13	0.36
E. camaldulensis	1.18	0.94	1.11	0.55	0.0002[a]	0.0001	0.0002
E. nitens	0.76	0.0006[a]	0.0005	0.0003[a]	0.37	0.0001	0.28
E. dunnii	0.67	1.3	0.87	0.67[b]	0.36[b]	0.24	0.24
E. globulus	1.33	0.88	1.17	0.10[b]	0.74[b]	0.07	0.98
E. microcorys	1.12	0.0006[a]	0.0007	0.45	0.0002[a]	$0.9*10^{-4}$	0.0002
E. macarthurii	0.67	0.68	0.46	0.02[b]	0.45[b]	0.01	0.30
E. citriodora	1.46	0.69	1.01	0.58	0.0002[a]	0.0001	0.0003
E. paniculata	1.00	0.0006[a]	0.0006	0.0003[a]	0.0002[a]	$0.6*10^{-7}$	0.0002
E. saligna	0.0003[a]	0.0006[a]	$1.8*10^{-7}$	0.28	0.0002[a]	$5.6*10^{-5}$	$5.94*10^{-8}$
S. myrtifolia	0.00005[a]	0.0002[a]	$0.1*10^{-7}$	0.0003[a]	0.0002[a]	$0.6*10^{-7}$	$0.1*10^{-7}$

[a] Test plants with zero values are replaced by 0.001 for calculation purpose.

[b] Species lacking data (those plants not growing in the site of survey) have been replaced by oviposition results from the caged bouquet trials to calculate their relative suitability to *G. scutellatus*.

NB: the relative values R^1, R^2, R^3 and R^4 were calculated by dividing each value of the test plants for larval feeding and oviposition by the respective value of *E. smithii*.

2.4 Discussion

The concentration of defensive secondary chemicals produced in response to feeding damage by insects varies with the genetic constituents of plants, environmental factors and their interactions (Osier and Lindroth, 2001; Stenberg *et al*., 2008). Plants respond to physical injuries or defoliation by changing the level of volatiles of secondary chemical compounds (Pare and Tumlinson, 1999), and the concentration of such volatiles released by plants varies in different patterns of foliar damage. For instance, Hartley and Lawton (1987) compared undamaged and damaged leaves from the same plant and found a lower water content and higher level of phenolics in the damaged leaves, with the highest phenolic concentration being in younger leaves. They also found an increase in the level of phenolics by 12% for mined leaves, 25% for chewed leaves and 9% for artificially damaged leaves, respectively, and artificially damaged leaves had a significant increase of protein-precipitation compared to the control leaves. Most *Eucalyptus* species have various types of toxic secondary chemical compounds that inhibit herbivore attack (Rapley *et al*., 2008; Pass *et al*., 1998). The leaves contain terpenoides, tannin and other phenolics that constitute 40% of the leaf dry matter (Pass *et al*., 1998).

2.4.1 The difference between cut-leaf, bouquet and sleeve trials

The patterns of *G. scutellatus* performance in the three types of the laboratory trials were very different. The physical damage caused by cutting a leaf could be the reason why the weevil's performance in some cases varied between the laboratory and field trials. For instance, Wratten *et al*. (1984) showed that artificially damaged foliage of birch trees (*Betula* species) in laboratory feeding trials was free from insect herbivory, as a result of increased level of chemical compounds. Similarly, Haukioja and Hanhimaki (1985) indicated a retarded development with a decreased pupal weight in insects feeding on damaged birch foliages. In contrast, some insects preferably used damaged leaves even though these have an increased level of phenolics (Hartley and Lawton, 1987). Apart from plant secondary

metabolites (PSMs) that are released in increased concentration during leaf-cutting or other damage, host plant preferences may be altered by factors such as water content of the damaged foliage. For instance, Hartley and Lawton (1987) suggested from literature review, that insects avoided mining in cut leaves, because of their lower water content.

Feeding of *Gonipterus* larvae was lower in the cut-leaf trials on *E. paniculata*, *E. macarthurii*, *E. microcorys*, *E. grandis*, *E. citriodora*, *E. globulus* and *E. viminalis* than in the sleeved-branch trial (Table 2.2). This indicates that the larval host preference could not be solely determined from the larval feeding choice on cut leaves, since the feeding behaviour could have been altered due to a change in foliar concentration of secondary compounds (Pare and Tumlinson, 1999). Age difference of the leaves can affect feeding preference, since the larvae prefer to feed on soft, young leaf surfaces (Tooke, 1953), therefore in this study young branch tips and leaves were presented to the weevils. Larval survival rate often differed between cut-leaf and sleeved-branch trials, although these were not conducted for the same period of time (two weeks and one week, respectively). For instance, most larvae on *E. grandis* in the sleeved-branch test survived, while those in the cut-leaf test all died, suggesting that toxicity level in the leaves may have increased in response to the damage caused by cutting. Plant cell vacuoles store secondary compounds such as phenolics, flavonoids, quinines and alkaloides as glycosides, which can get hydrolysed and released as potentially toxic aglycones when plant tissues are wounded (Edwards and Wratten, 1983). Rapley *et al*. (2008) found that foliar tannins are negatively correlated to the larval survival of *Mnesampela privata* (Lepidoptera: Geometridaey) and the percentage of *E. globulus* branches defoliated by the caterpillars.

The response of foliage to artificial or larval damage varies between species, from rapid to extended release of secondary toxic chemical compounds over several weeks and or months. Different PSMs can also act very differently on insect

herbivores the major difference being between severe, fast-acting toxins and feeding inhibitors such as tannins, which also perform differently under different temperature regimes (pers. comm. Oberprieler). In species such as subterranean clover, there was a release of isoflavone aglycones within just one minute of leaf damage (Edwards and Wratten, 1983), whereas in *E. globulus* the level of tannins increased over three months defoliation (Rapley *et al*., 2008). Such potentially toxic compounds could explain why there was some larval feeding on *E. grandis* tested on cut leaves under room temperatures, as little as an average damage of 1.1cm^2, but that feeding rate declined after the initial herbivory until eventually all larvae died (Table 2.2).

The most severe damage to *Eucalyptus* species by *G. scutellatus* is caused by the larvae, which can lead to complete defoliation in large infestations. Therefore the impact of toxic compounds from the PSMs of injured leaves could be more profound on the larvae than the adults, which always have the option to fly elsewhere to choose undamaged leaves. As such there was no significant difference in adult survival between caged-bouquets and sleeved-branches. The only two species showing low adult survival were *S. myrtifolia* (Fig. 2.1C) and *E. saligna* (Fig. 2.1D). Similarly comparison of caged-bouquets and sleeved-branches for adult feeding showed no significant differences. In both cases the pattern of feeding intensity was the same, with *E. citriodora* and *E. paniculata* receiving the least or no damage (Fig. 2.2). Even though the toxicity levels in damaged foliage vary and might kill adult insects in shorter period as in *E. saligna* and *S. myrtifolia*, adult weevils can survive longer than the larvae in the absence of food (Fig 2.1). Thus a period of two weeks to monitor adult survival rate may not be long enough, to see the full effect of secondary toxic compounds released from foliar damage or cut leaves.

2.4.2 Fundamental vs. realized host range

Since the larvae of eucalyptus weevil are virtually incapable of moving from one plant to the next, host selection is essentially performed by the adult female, and hence emphasis was placed on the oviposition results to determine the realized host range of the weevil. This is due to the fact that female's strategy of oviposition is generally correlated to the mobility of the larva (Bergman, 2000). In this study, some plant species on which the adults were neither feeding nor laying eggs were found to support feeding and survival of larvae in both laboratory and field experiments. For instance, *E. citriodora* showed different levels of larval feeding in all three trials (Table 2.2). This indicates that biocontrol agents could cause economic damage to non-target plants at some stage of their life cycle, even though they may not mature to adults and reproduce, as concluded by Briese and Walker (2002).

Accepting or rejecting biocontrol agents based on laboratory risk assessments only is not without a cost, since it could result in either rejecting a valuable controlling agent or releasing an insect with a potential risk to non-target plants. It is therefore important to approach this dilemma from two perspectives. The host range of *G. scutellatus* is broad, but the realized is smaller than the fundamental range, and adult feeding was found to be more selective than that of the larvae. For instance, *E. paniculata* showed some level of larval feeding in cut-leaf and sleeved-branch trials and adult feeding in caged bouquets (Fig 2.2), but there was no larval feeding (Table 2.2) or adult feeding or oviposition recorded in the field survey under natural conditions (Fig. 2.3). Similarly, *E. microcorys* sustained larval and adult survival and feeding and oviposition (Fig. 2.2, Table 2.3) in laboratory trials, while field survey results showed only larval feeding. One possible cause of larval feeding recorded on *E. citriodora* and *E. microcorys* from the field could be of larvae crawling in overlapping branches of neighbouring trees, or that larvae were translocated to these species during frequent clearing and pruning of the trees by

the caretakers of the plantation. Briese and Walker (2002) also found some damage on non-target plants by the leaf beetle *Deuterocampta quadrijuga* and suggested that such short-term damage risk might be higher to plants occurring together with the target species but not persistent.

Larval feeding intensity on *E. tereticornis* was high, particularly in the cut-leaf and sleeved-branch trials but also in the field survey (Table 2.2). There was also a considerable number of eggs laid on it in both choice (Table 2.3) and no-choice laboratory trials. However, only very few eggs were laid on the species in the field, and not significantly different from those species on which no eggs were laid with no oviposition (Fig. 2.3A). Almost the same pattern of larval feeding and female oviposition was shown by *E. camaldulensis* in the laboratory, except that no eggs were found on it in the field survey. Based on the fact that host plant selection is carried out by the adult female, oviposition is the most important factor to determine the host range of immobile larvae (Bergman, 2000). Even though the *Gonipterus* larva is not immobile, it is incapable of moving from one tree to the next for host selection. Thus, the importance of *E. tereticornis* and *E. camaldulensis* as hosts of *G. scutellatus* is likely to be overestimated by the laboratory host-specificity results, and including *E. paniculata*, *E. microcorys*, *E. camaldulensis* and *E. tereticornis* in the host range of *G. scutellatus* based on the laboratory performance on these species overestimates the realized host range of the weevil.

Therefore, the species that marginally suffered oviposition in the fundamental host range test supported no oviposition in the field survey, except *E. tereticornis* (which was not significantly different from those with no oviposition) (Fig. 2.3). Based on the number of species that sustained some level of oviposition as well as feeding in the laboratory, the fundamental host range of *G. scutellatus* inferred in this study includes 11 species, of the 15 plants tested. Ten species were surveyed in the field tests, and only four sustained adult feeding and oviposition (*E. smithii*, *E.*

grandis, *E. viminalis* and *E. nitens*), three supported only adult feeding and the remaining three sustained neither adult feeding nor oviposition (Fig. 2.3). Hence, the realized host range of *G. scutellatus* determined in these trials is less than half of the fundamental host range.

2.4.3 Host plant preference

The most preferred host plants are expected to show almost the same pattern feeding in the laboratory and in the field as well as of oviposition. Of the 23 species of *Eucalyptus* tested in this study, only ten were assessed for host specificity both in the laboratory and in the field survey. *Eucalyptus smithii* with almost consistently the highest level of adult feeding damage and oviposition, emerged the most preferred host plant of *G. scutellatus* (Figs. 2.2, 2.3). Although *E. urophylla* was not surveyed in the field, based on the results of the laboratory trials (Table 2.3) it could be second in rank of host preference, followed by *E. grandis* and *E. viminalis* or *E. scoparia*.

The variation between the *Eucalyptus* species in terms of the weevil's performance is very striking. Some of the species were severely damaged, while others suffered little or no damage. For instance *E. saligna*, and *S. myrtifolia* consistently did not support feeding, survival or oviposition of *G. scutellatus*. The reason why *E. saligna* and some others are little utilized by *G. scutellatus* may lie in the nature and/or concentration of their foliar PSMs, which defends the plant from attack.

Eucalyptus nitens sustained adult feeding damage as high as that of the preferred host *E. smithii*, and as *E. grandis* in the field survey (Fig. 2.3). However, oviposition on *E. nitens* was significantly lower than on the other two species in the field survey as well as in caged-bouquet and sleeved-branch trials, and also adult feeding was lower in the laboratory than in the field survey (Figs. 2.2 and 2.3A). The higher level of adult feeding in the field survey on *E. nitens* could be

due to the characteristics of the plant. For instance, the *E. nitens* trees were old and had much older leaves than the other species surveyed, which were coppicing and sprouting new leaves. This difference might explain why no larval feeding was recorded in the field survey as compared with the laboratory trials (Table 2.1). *Gonipterus scutellatus* is known to generally feed on younger leaves, shoots and buds (Carbone and Rivera, 1998; Rivera *et al*., 1999), even though the adults are more capable of feeding on older foliage (particularly in *E. globulus*) than the larvae are especially in the first instars (pers. comm. B. Slippers). Tooke (1953) also indicated from some field observations that *G. scutellatus* preferred older leaves than the younger juvenile foliage of *E. globulus* and *E. maidani.* Therefore, both young and older leaves were used for all adult testing on *E. globulus* following which Tooke's observations were confirmed in this study. However, no specific analysis was conducted on leaf age in relation to host preference.

Both the fundamental and the realized host range from the test plants in this study showed that the most preferred host of *G. scutellatus* are *E. smithii, E. urophylla* and *E. grandis*, even though *E. urophylla* was not surveyed in the field. On the other hand, *E. saligna, E. microcorys, E. paniculata, E. citriodora* and *S. myrtifolia* were immune or the least attacked species. Several other studies also indicated that *E. saligna* and *E. citriodora* as resistant to attacks from *G. scutellatus* (Rivera and Carbone, 2000; FAO, 2007; Fuentes *et al*., 2008). Among those species that were only tested in the field survey (not in the laboratory trials), *E. robusta, E. botryoides, E. maculata, E. pilularis, E. sideroxylon, E. ovata, E. goniocalyx, E. obligua, E. propinqua* and *E. punctata* showed little or no feeding or oviposition, except for some adult feeding recorded on *E. robusta, E. propinqua* and *E. punctata*. Thus, except the last three species, these might also be considered as resistant to the attack of the weevil (Fig. 2.3).

Chapter 3.

General discussion

Gonipterus scutellatus is species complex comprising at least ten closely similar (cryptic) species. Since none of these can currently be identified as representing the true *G. scutellatus*, they are tentatively name *G. scutellatus* A to D (pers. comm. R. Oberprieler; Mopondera *et al*., 2008) *Gonipterus scutellatusD* occurs naturally only in Tasmania, the island to which *Eucalyptus globulus* spp. *globulus* is native and endemic. This *Gonipterus* species has also been introduced in Western Australia where over 160,000 ha of *E. globulus* plantations, introduced from south-east Australia, are grown for the pulp production industry (Loch and Floyd, 2001). *Gonipterus scutellatusD* is the species that does the most damage to *E. globulus* spp. *globulus* in Tasmania and Western Australia, and elsewhere where plantations of *E. globulus* spp. *gobulus* are present (pers. comm. R. Oberprieler).

The voucher specimens from this study submitted to R. Oberprieler and B. Slippers, for identification were found to all belong to one species labeled *G. scutellatusF*. These species naturally occurs in eastern Australia, particularly in New South Wales (NSW) and parts of Victoria (pers. comm. R. Oberprieler; Mapondera *et al*., 2008) (Fig. 3.1). All the *Eucalyptus* species utilized by this weevil as its most preferred host plants in this study, except *E. urophylla*, are native to Australia and widely distributed in its natural range. For instance *E. smithii* occurs in NSW and parts of Victoria (pers. comm. R. Oberprieler), *E. viminalis* occurs in southern and north-eastern Victoria as well as eastern coast of NSW (Ladiges and Ashton, 1974), *E. grandis* is widely cultivated in forest plantations in Queensland and NSW (Burgess, 1988), and *E. scoparia* occurs along the border of Queensland and NSW (Brereton *et al*., 2009) (Fig. 3.1). Some of the resistant species, such as *E. saligna*, are also found along the eastern coast (Burgess, 1988), while *E. citriodora* is in north-eastern Australia (Booth, 1990).

Eucalyptus urophylla is native to Indonesia, where it is endemic to the seven islands in the eastern part of the country (Payn *et al*., 2007) and a plant species of an economic importance.

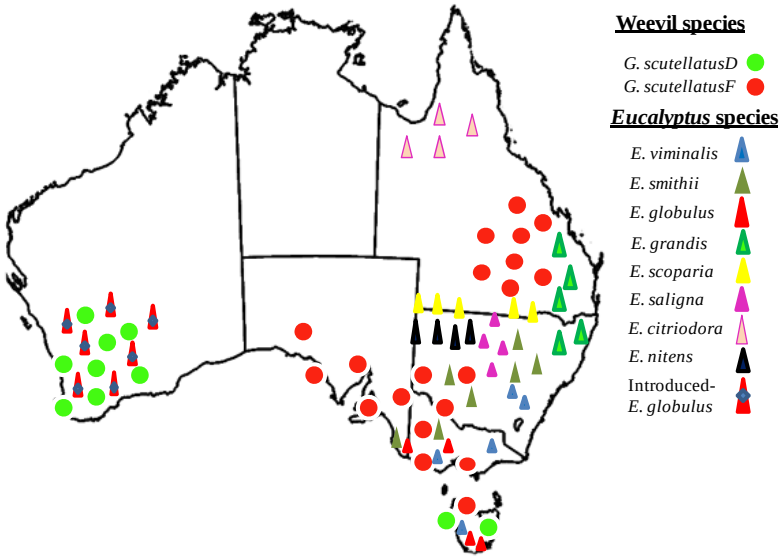

Figure 3.1: Natural occurrence and distribution of *Gonipterus scutellatusD* and *G. scutellatusF* and some *Eucalyptus* species in Australia (Adapted from Mapondera *et al*., 2008). N.B Some *G. scutellatusF* localities illustrated may require authentication (R. Oberprieler, pers. comm.).

Therefore it is not surprising that the most preferred host plants of *G. scutellatusF* in this trial are also those species that occur within its geographical distribution in Australia, from which both the weevil and the plant species could have been introduced into Africa (e.g. South Africa) and possibly in parts of Europe. One suggestion why *E. globulus* did not emerge as the preferred host in this study, as suggested in the literature (Dungey and Pots, 2003; Hanks *et al*., 2000; Loch, 2008; Millar *et al*., 1998), is because the identity of the weevil studied in these experiments is unclear. For example, Clarke *et al*., (1998) found the highest oviposition preference of the weevil to occur on three peppermint species (*E. pulchella*, *E. tenuiramis* and *E. amygdalina*), while *E. globulus* and *E. viminalis* were the least favoured as oviposition hosts. However, the weevil species they studied was not *G. scutellatus* but actually *G. rufus*, a species not even belonging to the *G. scutellatus* complex (pers. comm. R. Oberprieler). On the other hand,

Rivera and Carbone, (2000) found *E. globulus* and *E. viminalis* as the most preferred hosts in Kenya, California, Chile, Italy, and Spain. Once again the weevil tested in the last four countries is *G. scutellatusD* (pers. comm. R. Oberprieler).

The laboratory and field confinement trials conducted here show *E. urophylla* as being one of preferred hosts of *G. scutellatusF* (Table 2.2, 2.3). So far there is no evidence of *G. scutellatus* damage on *E. urophylla* in Indonesia (pers. comm. R. Oberprieler), and no literature including *E. urophylla* in host specificity tests for this weevil (Fig. 1.1). However, from the results of this study it is likely that *E. urophylla* is at risk of attack from *G. scutellatusF* should this species find its way to Indonesia.

Plants taxonomically very close to the target weed are at greater risk of attack by biocontrol agents due to their generally similar chemical composition (Pemberton, 2000). In these trials, *G. scutellatusF* selected its host plants mainly from one taxonomic group within the genus *Eucalyptus*. All the species that sustained larval and adult feeding and oviposition in the laboratory or the field belong to the subgenus *Eucalyptus*, and the most preferred hosts to the sections *Maidenaria*, and *Latoangulatae*. In contrast host preference of *G. scutellatusF* was low in the sections *Exsertaria* and in the subgenera *Symphyomyrtus, Alveolata* and *Corymbia* (Table 3.1). Thus *E. smithii*, *E. scoparia*, *E. dorrigoensis* and *E. viminalis*, from the section *Maidenaria*, and *E. urophylla* and *E. grandis*, from the section *Latoangulatae*, were the most preferred species (Table 2.3, Figs. 2.2, 2.3), while *Eucalyptus globulus*, *E. dunnii*, *E. nitens*, *E. nicholii* and *E. macarthurii* (from the section *Maidenaria*) supported adult feeding and oviposition (Table 3.1). In contrast, species in the section *Exsertaria* (*E. camaldulensis* and *E. tereticornis*) sustained only some feeding and oviposition in the laboratory, while *E. citriodora*, *E. paniculata* and *E. microcorys* were resistant to the weevil (Table 3.1). Similarly, *E. maculata* and *E. sideroxylon*, which also belong to subgenera *Corymbia* and *Symphyomytrus*, respectively, were found to be resistant to the weevil (Fig. 2.3).

According to field observation presented by Malley (1924) *E. viminalis*, *E. globulus* and *E. punctata* are recorded as the preferred hosts in South Africa, followed by *E. robusta* and *E. sideroxylon*. *Eucalyptus propinqua* was observed to have "slightly scalloped leaves" indicating feeding, but no larvae and eggs were found. *Eucalyptus maculata*, *E. botryoides*, *E. citriodora*, *E. obliqua*, *E. pilularis* and *E. saligna* were among the species that were recorded as not being attacked Malley (1924). Tooke (1953) also showed a similar pattern of host preference in South Africa, except that he included *E. smithii* as one of the preferred hosts, while *E. sideroxylon E. maculata E. saligna*, *E. oblique, E. ovata* and *E. microcorys* were recorded as "slightly attacked" species. Despite Tooke's suspicion that more than one species of *Gonipterus* might have been introduced into South Africa, all the aforementioned host ranges in the country were assessed by considering *G. scutellatus* as one species. Although the general pattern of host range in the present study seems to be in agreement with Tooke's and Malley's findings, there are some discrepancies with regard to some of the plants tested. For instance, in this study *E. punctata* and *E. globulus* were not the preferred hosts as indicated by Malley (1924). *Eucalyptus saligna* was found to be entirely immune to any attack by the weevil, unlike the results indicated by Tooke (1953).

In this study some of the plants species in the field survey, in the section *Latoangulatae* (*E. propinqua*, *E. punctata*, *E. robusta* and *E. botryoides*), also sustained adult feeding but no oviposition, since they were not included in the laboratory trials their suitability as potential hosts of *G. scutellatusF* was not also determined. However, these species are indicated as preferred hosts of *G. scutellatus* in some recent literature (Fuentes *et al*., 2008; FAO, 2007) (Table 1.1) in addition to Malley (1924) and Tooke (1953), with the exception of *E. botryoides* which Tooke recorded as slightly attacked, and Malley considered to be immune. Rivera and Carbone, (2000) identified *E. propinqua* to be among the species for which *G. scutellatus* showed a marked preference in the field. However, the exact species of "*G. scutellatus"* in that study was not determined. The only *Eucalyptus*

species in the section *Latoangulatae* consistently found to be immune or resistant to *Gonipterus* attack in the laboratory trials and field survey of this study was *E. saligna*. Several other studies also showed *E. saligna* and *E. citriodora* to be the most resistant species to *G. scutellatus* damage (Fuentes *et al*., 2008; Hanks *et al*., 2000; Rivera and Carbone, 2000). The geographical distribution of *E. saligna* in its native range is along the eastern coast of Australia, overlapping with the most preferred host plants of *G. scutellatusF* (such as *E. smithii* and *E. grandis*) (Fig. 3.1). However, because *E. saligna* is such a resistant species, while most of the other species in the section *Latoangulatae* support *G. scutellatus* feeding at some stage of its life cycle, it is tempting to suggest that the taxonomy of *E. saligna* might require revision.

Risk assessments of non-target plants

Despite overestimated values of larval feeding as a result of replacing the zero values by 0.001, the possibility of *E. camaldulensis, E. microcorys, E. citriodora, E. paniculata, E. saligna* and *S. myrtifolia* being attacked by *G. scutellatusF*, as quantified by the product of R^1 and R^4 (the relative larval feeding preferences in no-choice test and relative oviposition preference in field survey, respectively), is below 0.03%. Thus it can be concluded that these species are likely to be immune to damage by *G. scutellatus*. The remaining nine species are indicated to have a 24% or higher chance of being attacked. Although results from the caged-bouquet were used to calculate the potential risk values for all the test plants not present in the field survey site, their actual risk assessment was based on the performance of *G. scutellatusF* in the laboratory and field confinement trials at FABI (products of R^1 and R^2 from Table 2.4), the results of which indicated that these species are also vulnerable to the weevil. Thus, these species (*E. globulus, E. dunnii, E. urophylla* and *E. macarthurii*) should be included with the species that are indicated to be under threat from attack by *G. scutellatusF*.

Table 3.1: *Eucalyptus* species supporting adult feeding and oviposition in the field survey.

Genus	Sub-genera	Section	Species	Weevil activity
Eucalyptus	*Eucalyptus*	*Maidenaria*	*smithii*	A+O
			scoparia	A+O
			dorrigoensis	A+O
			viminalis	A+O
			globulus	A+O*
			nitens	A+O
			nicholii	A+O
			dunnii	A+O*
			macarthurii	A+O*
			ovata	-
			goniocalyx	-
		Latoangulatae	*urophylla*	A+O*
			grandis	A+O
			propinqua	A
			robusta	A
			punctata	A
			saligna	-
			botryoides	-
		Exsertaria	*tereticornis*	A
			camaldulensis	A
		Eucalyptus	*obliqua*	-
		Pseudophloius	*pilularis*	-
	Alveolata		*microcorys*	A
	Symphyomyrtus	*Adnataria*	*paniculata*	-
			sideroxylon	-
	Corymbia	*Septentrionales*	*citriodora*	-
			maculata	-
Syzygium			*myrtifolia*	-

A: adult feeding, O: oviposition, -: no feeding or oviposition.
*: adult feeding and oviposition in the laboratory; not present at the site of the survey.

Baars *et al* (2003) have performed a similar risk analysis and found the risk of attack by *Falconia intermedia* on non-target species, *Lippia wilmsii, Lippia* sp. A and *Lippia* sp. B, by *Falconia intermedia* to be 6, 10 and 21% respectively, when related to *Lantana camara*, the preferred host, while the other non-target species sustained a chance of attack of less than 2.9%. Likewise, Olckers (2000) conducted a risk analysis relative to the target species *Solanum mauritianum*, calculating feeding risk as the product of host preference and food acceptability and establishment risk as the product of oviposition preference, adult survival and the probability of host plant to be selected for oviposition. Olckers (2000) found that three non-target *Solanum* species had a relative risk of attack between 16 and 40%, 12 species of less than 4% and only one species sustaining 19% of supporting viable population of the insect tested. Baars *et al* (2003) and Olckers (2000) concluded that few non-target plants had sustained even slight attack from the respective potential biocontrol agents. Nevertheless, based on their risk analysis in these circumstances the risk is small and incidental. These non-target plants could only be at risk when agent infestations are large, and target host plants are intensively defoliated, which could lead to spill-over onto the related non-target plants that sustained some attack during host-specificity testing. Both authors concluded that, since biocontrol agents do not eradicate their target host species, if the non-target plants that could serve as alternative hosts for biocontrol agents are not in close proximity to the target host plant and are of a minor economic and aesthetic importance, the biocontrol agents tested could be released.

Chapter 4.

Conclusion

Environmental factors can have an effect on the response of insects to host plants (Heard, 2000), and the host range of insects is often reduced in the field as a result of various environmental constraints that are not reflected in the laboratory trials (McEvoy, 1996). For instance, Briese and Walker (2002) showed that the target plant (*Heliotropium amplexicaule*) and a closely related exotic species (*H. indicum*) were the two species on which the control agent, *Deuterocampta quadrijuga*, can complete its life cycle, while field observations and open field tests under natural conditions in Argentina indicated that *H. amplexicaule* was the only target species of *D. quadrijuga*. As such, field host-specificity testing enables further assessment of the fundamental host range, such as false positives produced from the laboratory trials, when decisions to release a particular agent are taken (Briese and Walker, 2002). Based on the fact that *H. indicum* was included in the host range in the quarantine test but was an exotic plant species and that field observations and tests showed *D. quadrijuga* to be limited to only one host species, the potential biocontrol agent was approved for release against *H. amplexicaule* in Australia (Briese and Walker, 2002).

Open field testing to determine the realized host range of biocontrol agents has often been put aside or is less used for a number of reasons, among which are that such tests are carried out in the country of origin, which incurs high costs (Barratt *et al*., 2007). However, the cost of finding a potential agent and rejecting it on the basis of laboratory results only is also an expensive option either, in addition to the cost that the environment could sustain as a result of not releasing a biocontrol agent. For instance, the seed bruchid, *Bruchidius villosus* Fabricius, was released in 1981 in New Zealand and in 1990 in Australia against broom, *Cytisus scoparius* (Fowler *et al*., 2000b; Syrret *et al*., 1999), based on choice tests only. Even though

the closely related alien plant tagasaste, *Chamaecytisus palmensis*, was included in the choice test, it is now attacked by the biocontrol agent released (Heard, 2000). This does not demonstrate a host shift after release but rather an indication of insufficient and in proper host specificity testing that relied on only choice-test trials and in which tagasaste sustained no attack.

The fundamental host range of *G. scutellatusF* as determined from its feeding and oviposition performance in the laboratory, includes several species of the sections *Maidenaria* and *Latoangulatae* as first-choice host plants. To a limited extent, with a marginal acceptance mainly for larval feeding, the weevil also accepted plants in the subgenera *Alveolata* (*E. citriodora*), *Corymbia* (*E. microcorys*) and *Symphyomyrtus* (*E. paniculata*) but completely refused *E. saligna* and *S. myrtifolia*. Thus, of the 15 species tested against *G. scutellatusF* in the laboratory, 12 species supported larval and adult feeding plus survival and ten species sustained oviposition. In the contrast, in the open field survey (excluding the five species that were not surveyed), only seven of ten species supported adult feeding and four received a significant level of oviposition (Fig 2.3). In the survey 13 additional eucalyptus species were assessed (Table 2.1), of which ten received no oviposition, but seven sustained adult feeding (Fig. 2.3). This indicates that adult feeding and oviposition is more selective than larval feeding and that *G. scutellatusF* is more selective in the field than in the laboratory.

Gonipterus scutellatusF has a broad host range in the field and feeds on more than one species in at least two different sections (*Maidenaria* and *Latoangulatae*) of the subgenus *Eucalyptus*. Hanks *et al*. (2000) and Dungey and Pots (2003) also indicated that *G. scutellatus* uses a variety of *Eucalyptus* species, although the exact species of *Gonipterus* in their trials was not identified. Li *et al*. (2004) defined herbivorous insects that specialize in a narrow host range or have an extended host range of not more than three plant families, as oligophagous. Most of the major defoliator pests of eucalyptus plantations attack more than one species

in the genus *Eucalyptus*. For instance the weevil *Gonipterus gibberus* feeds exclusively on *Eucalyptus* plants but on several species (EPPO, 2005), and the weevil *Gonipterus rufus* (pers. comm. R. Oberprieler) preferably attacks the three peppermint species (*E. pulchella*, *E. tenuiramis* and *E. amygdalina*) in the genus *Eucalyptus* (Clarke *et al*., (1998). Similarly other beetle pests of eucalyptus plantations such as the chrysomelid beetle, *Cadmus excrementarius* (Coleoptera: Chrysomelidae) is a pest of *E. globulus* spp. *globulus* in Western Australia, and seven other host species from the sub-genera *Eucalyptus*, and *Corymbia* (Dos Anjos *et al*., 2002). *Paropsine* chrysomelid beetles are also major pests of eucalypts plantations in Australia. For instance *Paropsine tomaria* is a common pest of *E. grandis*, *E. cloeziana*, and *E. pilularis in* Queensland and NSW, and of *E. camaldulensis*, *E. dunnii*, and *E. pilularis* in NSW (Nahrung, 2006). The pest *Chrysophtharta cloelia* (Coleoptera: Chrysomelidae) is another beetle that *attacks E. grandis*, *E. pellita*, and *E. urophylla* in Queensland, and *E. grandis* and *E. dunnii* in NSW (Nahrung, 2006). While *Chrysophtharta bimaculata* is the pest of *E. regnans*, *E. oblique*, *E. delegatensis*, and *E. nitens* (Raymond, 1995). Insect pests of *Eucalyptus* other than the beetles also attack several species of plantation eucalypts. For instance *Thaumastocoris peregrines* (Hemiptera: Thaumastocoridae), is a pest on 26 species of *Eucalyptus* in South Africa (Nadel *et al*., 2009).

Therefore, it is possible to suggest that all these major pests of eucalyptus which feed on more than one species of *Eucalyptus*, but within the genus *Eucalyptus* including *G. scutellatus* are oligophagous herbivores, but not polyphagous. The *Trichilogaster acaciaelongifoliae* which attacks both *Acacia longifolia* and *Acacia floribunda* in Australia was introduced to South Africa in 1982 to control the invasive alien species of *Acacia longifolia*, but it is now attacking *A. floribunda* (McGeoch and Wossler, 2000), and to some extent *A. melanoxylon*, and *Paraserianthes lophantha* in South Africa (Dennill *et al*., 1993). Thus, since *G. scutellatusF* strictly feeds on the genus *Eucalyptus*, and more specifically on only

two sections of the subgenus *Eucalyptus* in South Africa, it could be used as a biocontrol agent to control a specific *Eucalyptus* species in the absence of conflict of interest. For instance, *Cactoblastis cactorum* is an oligophagous insect that attacks several species of *Opuntia* in its native geographical range. But it was released as biocontrol agent against the alien invasive species of *Opuntia* in Australia, resulting in dramatic success, since the only non-target plants attacked or at risk were also invasive alien species in the genus *Opuntia*, of no economic interest (Zimmerman *et al*., 2000).

The *Eucalyptus* hosts of *G. scutellatusF* correlate with its native range in Australia (Fig. 3.1), suggesting that host selection behaviour is a stable character even in translocated populations. However with the recently emerging new sub-species of the weevil it is very important to properly identify right species of *G. scutellatus* to determine the correct realized host range of the insect. Barratt *et al*. (2009) has indicated that identifying the right species or varieties that exist as species complex of an insect during biocontrol agent selection is an important aspect that should receive proper attention. For instance, in the 1960s a snail parasite, *Sepedon sauteri* Hendel (Diptera: Sciomyzidae), was introduced several times to Hawaii to control the target liver-fluke-snail, *Galba viridis*, but afterwards field collections identified the presence of different biotypes of the parasite, of which two that had established did not even belong to *S. sauteri* and are believed to attack non-target snails but never the target liver-fluke-snail (Barratt *et al*., 2009). It therefore seems that in studies that identified *E. globulus* and *E. viminalis* as the most preferred host of *Gonipterus*, as in Chile or Ventura (California), the species of the weevil involved is not *G. scutellatusF* type (Fig. 3.1).

In conclusion, unless *G. scutellatusF* is controlled effectively in South Africa, high levels of infestation on *Eucalyptus* species such as *E. smithii*, *E. urophylla*, *E. grandis*, *E. scoparia*, *E. viminalis* and *E. dorrigoensis* could result and lead to economic losses in plantations. In contrast, *E. saligna*, *E. citriodora*, *E. paniculata*

and *E. microcorys* are resistant to damage by this weevil and would be most suitable for use in plantations or hybridization with susceptible species of *Eucalyptus* to obtain a resistant hybrid. South Africa has expertise in eucalyptus hybridization in commercial forestry, and *E. grandis* is the main species used to cross-breed with *E. urophylla, E. nitens* and *E. camaldulensis* (Morris, 2008), and in this regard the results of this study could assist in selection of appropriate breeding species. Even though *G. scutellatusF* is already present in South Africa, it is important to quarantine all eucalyptus material imported to the country, so as to avoid the introduction of another species of the *G. scutellatus* complex.

Recommendations

The larvae of *G. scutellatusF* showed in some test species a high rate of survival and feeding, while adult performance feeding and oviposition was not supported on these species as in *E. citriodora*. Further studies of host range and host preferences should measure larval head capsule width and pupal weight, to determine the growth rate (which also depends on the suitability of the host plant for foraging) and follow the survival rate until such time that either all immature stages are dead or will continue to complete the life cycle. I would also recommend multiple choice test in cages to be taken instead of paired choice test to determine and rank the host preferences, when the most preferred host (as in this case was assumed to be the *E. globulus* according to several literature reviews (Dungey and Pots, 2003; Hanks *et al*., 2000; Loch, 2008; Millar *et al*., 1998) is not well established since risk analysis is required to be calculated relative to the most preferred host plant. Host-specificity tests conducted on *G. scutellatus* should consider in identifying that this name refers to a species complex and identify the exact species before conducting the trials.

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Appendices

Appendix A: Quantification of the potential risk of *G. scutellatusF* to non-target species, calculated as the relative performance of the weevil on each test plant in sleeved-branch trials in comparison to *E. smithii*, the preferred host.

Test plants	Sleeved branch test				
	Larval feeding[y] (95 % CI)	Relative L. feeding preference (R^1)	Female oviposition (95 % CI)	Relative ovip. preferences (R^2)	Relative risk of attack (R^1*R^2)
E. smithii	3.3(2.8, 3.9)	1.00	1.6(0.9, 2.9)	1.00	1.00
E. urophylla	4.6(4.1, 5.2	1.39	2.5(1.5, 4.1)	1.56	2.17
E. viminalis	4.4(3.6, 5.5)	1.33	2.0(1.0, 4.1)	1.25	1.66
E. grandis	4.1(3.1, 5.3)	1.24	0.001[a]	0.0006	0.0007
E. tereticornis	5.0(5.0, 5.0)	1.51	1.5(0.8, 3.0)	0.94	1.43
E. camaldulensis	3.9(3.1, 5.0)	1.18	1.5(1.0, 2.6)	0.94	1.11
E. nitens	2.5(1.9, 3.1)	0.76	0.001[a]	0.0006	0.0005
E. dunnii	2.2(1.4, 3.4)	0.67	2.1(1.1, 4.1)	1.3	0.87
E. globulus	4.4(3.6, 5.5)	1.33	1.4(0.8, 2.3)	0.88	1.17
E. microcorys	3.7(2.9, 4.9)	1.12	0.001[a]	0.0006	0.0007
E. macarthurii	2.2(1.4, 3.4)	0.67	1.1(0.8, 1.5)	0.68	0.46
E. citriodora	4.8(4.4, 5.3)	1.46	1.1(0.8, 1.5)	0.69	1.01
E. paniculata	3.3(2.1, 5.1)	1.00	0.001[a]	0.0006	0.0006
E. saligna	0.001[a]	0.0003	0.001[a]	0.0006	$1.8*10^{-7}$
S. myrtifolia	0.001[a]	0.00005	0.001[ab]	0.0002	$0.1*10^{-7}$

[y] Feeding categories defined in chapter 2 section 2.2.1.

[a] Test plants with zero values are replaced by 0.001 for calculation purpose.

[b] Species not found during the survey were replaced by the respective results from the caged bouquet trial and divided by *E. smithii* in the same trial to calculate their relative suitability to *G. scutellatus*.

NB: The relative values R^3 and R^4 were calculated by dividing the value of each test plant for larval feeding and oviposition by the respective value of *E. smithii* in the same column.

Appendix B: Risk evaluation of *G. scutellatusF* to non-target species, calculated as the relative performance of the weevil on each test species in field surveys, in comparison to *E. smithii*, the preferred host.

Test plants	**Field survey**				
	Larval feeding[y] (95 % CI)	**Relative L. feeding preferences (R^3)**	**Female oviposition (95 % CI)**	**Relative oviposition (R^4)**	**Relative risk of attack R^3*R^4**
E. smithii	4(4, 4)	1.00	5.1(3.8, 6.8)	1.00	1.00
E. urophylla	4(4, 4)[b]	1.00	4.3(2.6, 7.1)	0.81[b]	1.00
E. viminalis	3.7(3.0, 4.5)	0.93	1.80(1.3, 2.6)	0.35	0.33
E. grandis	4(4, 4)[b]	1.00	5.0(3.8, 6.5)	0.98	0.98
E. tereticornis	2.2(1.5, 3.0)	0.55	1.2(0.8, 1.7)	0.24	0.13
E. camaldulensis	2.2(1.6, 3.1)	0.55	0.001[a]	0.0002	0.0001
E. nitens	0.001[a]	0.0003	1.9(1.4, 2.6)	0.37	0.0001
E. dunnii	4(4, 4)[b]	1.00	1.9(0.6, 6.7)	0.36[b]	1.00
E. globulus	4(4, 4)[b]	1.00	3.9(1.5, 10.1)	0.74[b]	1.00
E. microcorys	1.8(1.8, 1.8)	0.45	0.001	0.0002	$0.9*10^{-4}$
E. macarthurii	4(4, 4)[b]	1.00[b]	2.4(0.8, 7.0)	0.45[b]	1.00
E. citriodora	2.3(1.7, 3.1)	0.58	0.001[a]	0.0002	0.0001
E. paniculata	0.001[a]	0.0003	0.001[a]	0.0002	$0.6*10^{-7}$
E. saligna	1.1 (0.3, 0.3)	0.28	0.001[a]	0.0002	$5.6*10^{-5}$
S. myrtifolia	0.001[a]	0.0003	0.001[a]	0.0002	$0.6*10^{-7}$

[y] Feeding categories defined in chapter 2 section 2.2.1.
[a] Test plants with zero values are replaced by 0.001 for calculation purpose.
[b] Species not found during the survey were replaced by the respective results from the caged bouquet trial and divided by *E. smithii* in the same trial to calculate their relative suitability to *G. scutellatus*.
NB: The relative values R^3 and R^4 were calculated by dividing the value of each test plant for larval feeding and oviposition by the respective value of *E. smithii* in the same column.