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2012-01-31
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CONTRASTING GROWTH TRAITS AND INSECT INTERACTIONS OF TWO *TAMARIX* SPECIES AND A HYBRID (TAMARICACEAE) USED FOR MINE REHABILITATION IN SOUTH AFRICA

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

School of Animal, Plant and Environmental Sciences,

Johannesburg, October 2011

DECLARATION

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.

_____

Lael Edith Buckham

10th day of October 2011.

ABSTRACT

Both indigenous "*Tamarix usneoides*" and alien *T. ramosissima* co-occur in South Africa. *Tamarix usneoides* is potentially a metal and salt phytoremediation species. However, hybridisation is occurring between the two species and hybrids growth characteristics are deemed to be invasive. This study was undertaken at two sites on one Highveld gold mine, characterised by different soil types, where plant growth, reproductive potential and insect abundance and diversity were measured. This aims to establish the growth of *T. usneoides* in two site-species trials, and to identify differences between *Tamarix* taxa that are consistent across different environmental conditions and can be used for identification. This study also set out to establish if higher reproductive effort and enemy release are promoting the invasiveness of *T. ramosissima*. Trees in one site (a water logged, clayey "Glenrosa" soil) displayed significantly greater percentage survival, growth and reproductive effort than trees in the other site (a well drained, sandy "Hutton" soil). Extensive hybridisation between *T. usneoides* and *T. ramosissima* was observed with two hybrid states, morphologically similar to each parent species. The reproductive effort is consistently greater in *T. ramosissima* and *T. ramosissima* hybrid than *T. usneoides* and *T. usneoides* hybrid. After sampling 217 trees in this study, the three characteristics of (1) insertion of filaments into the nectar disc, (2) petal shape and (3) presence of salt glands on leaves most accurately distinguished between *T. usneoides* and *T. ramosissima* taxa. Three insect species have been identified from this study as potentially damaging agents on *Tamarix* spp. Insects can discern between the *Tamarix* tree taxa and *T. ramosissima* is being released from insect herbivore pressure, as very few insects and a lower diversity were recorded on *T. ramosissima* and its hybrids (*T. ramosissima* hybrids > *T. ramosissima*). To achieve phytoremediation objectives, careful tree identification and selection of genetically pure individuals for cloning is recommended. Additionally the removal of existing alien and hybrid plants, and further research to find a suitable host specific insect biological agent to control alien *Tamarix* is necessary.

DEDICATION

I dedicate this work to my family, particularly my father Norman Buckham who has always inspired and encouraged me, my mother Faith Buckham who has taught me to persevere and never give up, and my husband for being my source of fun and joy. I am very grateful for their support and financial assistance during this research. The patience and kindness of my supervisor is also greatly appreciated. I would also like to thank all the special people in my life that have encouraged me and made me believe I could finish this.

ACKNOWLEDGEMENTS

This study was conducted under the Mine Woodlands Project at the University of Witwatersrand and AngloGold Ashanti Ltd (AGA).

I am very grateful to my supervisors particularly Prof Marcus Byrne for his valuable time and assistance throughout this tough project, and Isabel Weiersbye for financial assistance and guidance. I thank AGA S.A. Region for enabling me to carry out my research and for financial support (NRF and DTI THRIP grant no. TP2009072900036 to Isabel Weiersbye and Ed Witkowski).

I thank all my fieldwork assistants and individuals involved in this project, particularly Benjamin Oageng, Mark Cooper, Lorato Letsholo, Jenny Botha and David Furniss.

I also thank Christien Bredenkamp for her valuable time and guidance throughout this project and for helping me unravel the mystery of plant hybridisation.

I appreciate the involvement of Glynis Goodman-Cron and thank her for assistance with the morphological identification of plant specimens.

I thank the specialists and Vivienne Uys at the Agricultural Research Council (ARC) for insect identification.

I appreciate the assistance given by Vivienne Williams with the diversity statistics.

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CHAPTER 1:
INTRODUCTION

1.1 PROJECT OVERVIEW

Tamarisk or Salt cedars comprise phreatophyte as well as riparian trees or shrubs of the Genus *Tamarix* (Family Tamaricaceae). In southern Africa there are three abundantly occurring species; *Tamarix usneoides* E. Mey. ex Bunge, *T. chinensis* Lour., and *T. ramosissima* Ledeb. (Henderson, 2001), with *T. parviflora* DC. also recorded (Germishuizen *et al.*, 2006).

Only *Tamarix usneoides* E. Mey. ex Bunge is native to southern Africa. *Tamarix ramosissima* Ledeb. is an alien invader (Henderson, 2001), native to North-Central China to Eastern Turkey. *Tamarix chinensis* Lour. is a native of East Asia particularly Mongolia, China and Japan. *Tamarix parviflora* DC. is native to South-Eastern Europe including Turkey, Greece, Crete and the Balkan States (Baum, 1978).

The damage caused by alien Tamarisk in riparian ecosystems is a well documented problem in the Western United States (USA) (De Loach *et al.*, 2003). In South Africa, alien Tamarisk is also highly invasive and has been declared as a category III invader of wetlands and watercourses (Henderson, 2001). The success of invasive Tamarisk in new areas is facilitated by characteristics such as (i) vegetative reproduction from the roots and crown if above-ground biomass is damaged, (ii) high seed production with adaptation for wind and water dispersal, (iii) tolerance to growth in extreme conditions and, (iv) release from natural enemies which control the spread of Tamarisk in their native home range (Gaskin, 2003; Glenn and Nagler, 2005).

With the goal of identifying suitable tolerant plant species for remediation of degraded areas, Weiersbye and Witkowski (2007) and Weiersbye *et al.*, (2006b)

identified plants including *Tamarix* spp. growing on Highveld gold mines. These species were tolerant of growth in the saline conditions.

Phytoremediation is the use of plant based technologies to mitigate pollution of soil, sediments and water (McGrath *et al.,* 1993; Chaney, 1983; Baker, 1981; Keller *et al.,* 2003). This technology relies on selecting plant species that are resistant to and/or have the ability to degrade contaminants, or accumulate high enough levels of pollutants to remediate soils (Bizly *et al.,* 2000; Lasat, 2002). As *Tamarix* spp. appear to tolerate growth on gold mine waste heaps or Tailings Storage Facilities (TSFs) in the Witwatersrand Basin, 'indigenous' *T. usneoides* has been included in a woodlands pilot study, initiated in 2003, which places plots of trees strategically at the base of TSFs where seepage occurs. These 'indigenous' *T. usneoides* individuals were cloned by the Department of Water Affairs and Forestry (DWAF), in the species native home range at Upington in the arid Northern Cape Province of South Africa.

Tamarix usneoides is easily distinguished from other *Tamarix* spp. by its pure white inflorescences which differ from the pink-purple colour of *T. ramosissima, T. chinensis* and *T. parviflora* (Christien Bredenkamp, South African National Botanical Institute (S.A.N.B.I) pers comm.). However, as the 'indigenous' *T. usneoides* clones, planted as part of the Mine Woodlands Project began producing inflorescences in 2008, trees displayed a variety of flower colours that ranged from white to white with pink stamens (Isabel Weiersbye, University of Witwatersrand, pers comm.). This prompted suspicion that some trees may be hybrids instead of 'pure' specimens and provided the rationale for this study.

Hybridization between *Tamarix* spp. is a well documented problem in the USA particularly between the two morphologically similar species *T. ramosissima* and *T. chinensis* (Gaskin and Schaal, 2002). Hybridisation of indigenous *T. usneoides* with alien *Tamarix* spp. has also been recorded in South Africa, as early as 1970s (Obermeyer, 1976) and has continued (Christien Bredenkemp, South African National Botanical Institute, pers comm.) with little intervention and control measures in place. Alien *Tamarix* spp. did not feature on the list of priority

invasive species until early in 2010, and currently there is still no registered herbicide that has been approved for the chemical control of alien *Tamarix* spp. in South Africa (Debbie Sharpe, Working for Water (WfW), pers comm.).

In order to confirm the identity of Tamarisk plants in this study, vouchers collected on the gold mine were submitted to scientists at the South African National Botanical Institute (S.A.N.B.I) in Pretoria for identification in 2009. These Tamarisk vouchers were more morphologically similar to *T. ramosissima* than other *Tamarix* spp., raising concern that alien *T. ramosissima* and its hybrids are becoming highly invasive in South Africa.

1.2 PROJECT AIMS, OBJECTIVES AND LIMITATIONS

This study took place at the Vaal River Mining Operations near Orkney, a gold mining operation owned by AngloGold Ashanti Ltd (AGA).

1. Initially the study aimed to compare the survival and growth of '*T. usneoides'* between two sites with different soil compositions. The results sought to confirm observations that '*T. usneoides*' performs better in water-logged 'poorer' soil conditions, making it a suitable phytoremediation species. However, '*T. ramosissima*' were also found among the samples.

2. As invasiveness is correlated with high reproductive potential, the flowering frequency (as a measure of reproductive effort) of '*T. usneoides*' in cultivated plots, and '*T. ramosissima*' in the wild population, were recorded during six sampling periods. This aimed to test the hypothesis that a higher reproductive output is promoting the invasiveness of '*T. ramosissima*'.

3. A release from natural enemies, a theory known as the Enemy Release Hypothesis (ERH) is proposed to facilitate the invasiveness of alien plants

like *T. ramosissima*. Therefore the insect abundance and diversity were derived and compared between '*T. usneoides*' and '*T. ramosissima*'. As insect sampling was carried out at both sites (in the cultivated plots), insects could also be associated with different soil conditions. Abundantly occurring insects were then identified to family, genus and species level where possible, to see if insects associated with *Tamarix* spp. may have an impact on the trees.

Extensive hybridisation became evident during field observations, and it was suspected that there was a mix up of *T. usneoides* tree labels or clones at DWAF Upington, or mis-identification of trees prior to cloning '*T. usneoides*' at the Upington nursery. It then became necessary to find the true species identity of sampled trees. This would enable useful and accurate conclusions from the growth, flowering and insect data. This study then undertook to achieve the following;

4. To review literature and identify morphological and vegetative characteristics which are stable under different environmental conditions and can be used to discern between *Tamarix* spp.

5. To test the validity of these characteristics through field observation and to identify the trees surviving on the Vaal River Mining Operations, in order to develop a morphological identification method that is accurate for future use.

6. To evaluate the extent of hybridization occurring between *T. usneoides* and *T. ramosissima*.

At present little is known about *Tamarix* spp. in southern Africa. As many plants are known to undergo morphological changes in response to environmental conditions, accurate morphological identification is necessary to discern between species. An understanding of plant reproduction and invasive biology is also necessary to find effective methods of control.

CHAPTER 2:
LITERATURE REVIEW

Many theories have been proposed to explain why some plants become invasive. In South Africa, as there are alien *Tamarix* spp. coexisting and/or competing with an indigenous species in the same area, two popular theories provide context to explain this problem. These theories are the Enemy Release Hypothesis and the Species Relatedness Hypothesis.

2.1 INVASIVE ALIEN PLANTS

The rates of biotic exchange across the earth's surface have increased over the past five centuries (Perrings *et al.*, 1992) facilitating the movement of plant species across the world. A non-indigenous plant that occurs out of its natural environment and home range, and invades previously pristine areas or ecological niches is regarded as an Invasive Alien Plant (IAP) (Bromilow, 2001). Over time IAPs become naturalised, invading in large enough numbers to become ecologically prominent, and dominate native communities (Crooks, 2002). These IAPs pose a substantial threat to biodiversity and to the maintenance of ecosystem services, as they affect the structure and function of ecosystems (Witkowski and Mitchell, 1987; Vitousek, 1990; Witkowski, 1991; Witkowski and Wilson, 2001) by lowering species diversity and causing local extinction of indigenous species. As natural predators which control the growth and spread of IAPs are absent in the new home range, an ecological imbalance is created as IAPs dominate plant communities resulting in a loss of plant diversity (Bromilow, 2001). Furthermore, the introduction of alien species into new areas where genetically similar species occur brings them into contact with genotypes from which they had previously been isolated. This contact may produce new prospects for hybridisation, and this is likely to create a genotype with hybrid vigour and high fitness that is better

equipped than the parent plants to deter herbivores and pathogens (Gaskin and Schaal, 2002).

Floodplains and riparian areas are amongst the most diverse ecosystems as they form the interface between the terrestrial and aquatic habitats in a landscape (Naiman *et al.,* 2005). These ecosystems are vulnerable and prone to IAP invasions, as rivers transport and disperse alien plant propagules (Thebaud and Debussche, 1991; Pyšek and Prach, 1993; Johansson *et al.,* 1996; Tickner *et al.,* 2001) and human and natural disturbances, particularly flooding events, allow IAPs to enter the ecosystem where bare ground is open for plant re-colonisation (Holmes *et al.,* 2005; Richardson *et al.,* 2007). As South Africa is an arid country with rapid development, erratic rainfall and low groundwater reserves, water scarcity is becoming a concerning prospect (Görgens and Van Wilgen, 2004; Van Wilgen *et al.,* 2001). IAPs are known to cause a decline in stream flow and water yield, and there is a need for their prevention and control (Binns *et al.,* 2001), particularly in riparian areas.

Pyšek and Richardson (2007) compared 64 IAPs and found that certain key traits are associated with invasiveness and give IAPs a competitive advantage over native plants. For alien Tamarisk, these traits include high water use, resistance to herbivory, larger and longer persisting seed banks, vegetative regeneration, greater resource allocation to reproduction and tolerance to fire (Brock, 1994; Gaskin, 2003; Pyšek and Richardson, 2007). Although extensive research has been conducted on the biology and ecology of invasive Tamarisk in the USA, to date no research has been carried out in South Africa comparing native and alien Tamarisk in the same area.

2.1.1 Enemy Release Hypothesis and IAPs

The Enemy Release Hypothesis (ERH) is often proposed to explain why IAPs become invasive. This is achieved by improved demographic performance due to

the absence of herbivores, parasites and pathogens which regulate their abundance in the native home range (Keane and Crawley, 2002; Dawson *et al.*, 2008; Strauss *et al.*, 2006; Proches *et al.*, 2008). Although ERH is controversial, with many studies supporting or disproving the hypothesis, it is still believed to be one of the underlying factors promoting invasions by IAPs (Keane and Crawley, 2002). This theory is popular in justifying the control of IAPs with introduced natural enemies from the plant's native home range, a method known as Biological Control (Callaway and Aschehoug, 2000).

2.1.2 Species Relatedness Hypothesis

The Species Relatedness Hypothesis is also popular and suggests that invaders which are more closely related to native species are likely to succeed in new environments, because of physiological similarities from a common ancestry (Strauss *et al.*, 2006). In New Zealand, there are reports of species that are more closely related to native plants establishing more rapidly, possibly due to shared traits that allow them to easily adapt to the new environment, although there has also been research showing that less related species are also more invasive (Strauss *et al.*, 2006).

Investigating the species relatedness hypothesis involves establishing similarities in morphology and/or genetics of invaders with native biota (relatives), and then comparing characteristics of native and alien species communities collectively to identify differences between species, which may account for invasiveness (Daehler, 2003). Additionally evaluating demographic processes such as survival, growth and fecundity will enable focussed control actions on the life stages of IAPs that will result in the largest reduction in their life history traits (Ramula *et al.*, 2008). This is especially relevant if proposing a biological control agent, to ensure that studies will focus on agents that will cause the most damage at the appropriate life stage of the plant.

2.2 GENUS *TAMARIX*

Genus *Tamarix* first radiated across China to Mongolia and Korea, and then into India, and across the Middle East to the Eastern Mediterranean areas where a second major centre of speciation developed. It also spread across Europe to Spain, as well as across Northern Africa to Senegal and Morocco, and through Eastern Africa to southern Africa (Baum, 1978; Liu and Zhang, 1987). The native distribution of Tamarisk worldwide is indicated in Figure 2.1. Its 54 species now occur naturally in the Mediterranean around Spain and Morocco, as well as in India, China, Mongolia and southern Africa (Baum, 1978).

Twelve native and alien species have been recorded growing throughout Sub-Saharan Africa (Klopper *et al.*, 2006). The distribution of Tamarisk trees is widespread and also occurs in the semi-arid parts of North Africa, Europe and Asia. *Tamarix* spp. are characterized as trees or shrubs with deep roots, herbaceous, small scale-like leaves and racemose inflorescences with bark colour varying according to species type and environmental conditions (Baum,1978). They are successful invaders as facultative phreatopytes (water loving plants) that can reproduce both sexually (via seed) or asexually (vegetatively), as well as being unpalatable to vertebrates because of a high tannin content.

These phreatophyte and riparian trees commonly known as Salt Cedars, obtain their name from their ability to utilize saline water by excreting excess salt through specialised glands in their leaves (Waisel, 1972; Gaskin, 2003). As *Tamarix* trees are stress tolerant plants, they can survive in highly saline, polluted environments.

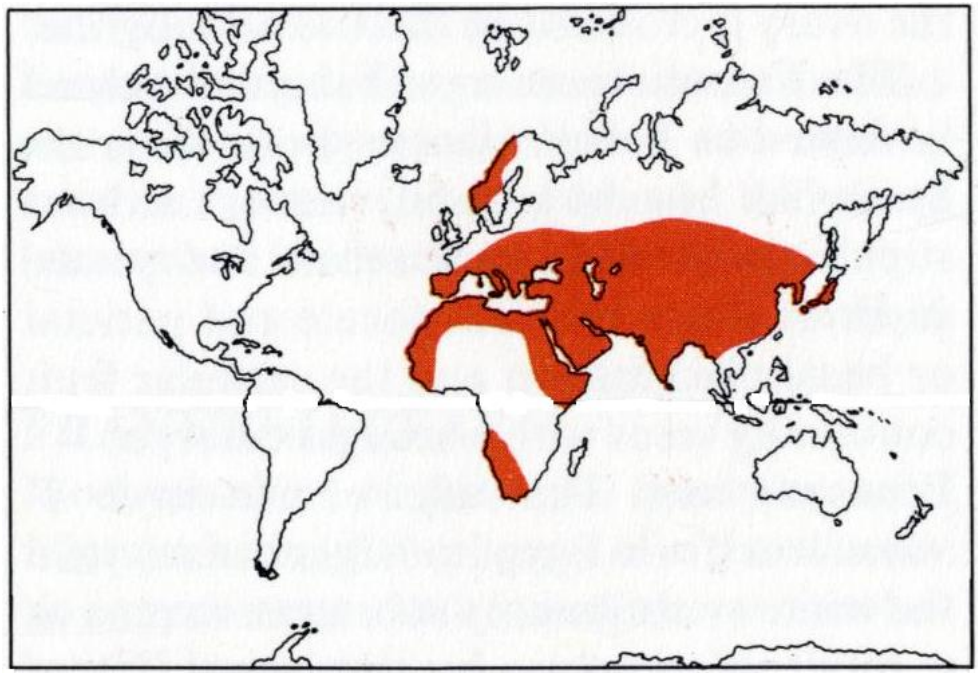


Figure 2.1, The native home range distribution of Genus *Tamarix* worldwide. (Heywood *et al*., 2007).

2.2.1 *Tamarix usneoides* (E. Mey ex. Bunge)

Tamarix usneoides E. Mey ex. Bunge is native to southern Africa. Plants previously known as *Tamarix angolensis* Nied., *T. austro-africana* Schinz and *T. engleri* Arendt are now regarded as one species; *T. usneoides* (Germishuizen *et al.* 2006; Klopper *et al.*, 2006). This evergreen tree has a congested growth form of branchlets with short internodes and displays a shrubby form with a brown-grey glabrous bark. Leaves are amplexicaul and vaginate, overlapping and completely covering young stems, and sometimes widening at the apex to form a cup-shaped rim. Salt glands are abundant across the leaf surface (Bredenkemp and Phepho, 2008). Inflorescences are very small, creamy white to whitish in colour (Fig 2.2) and appear in slender auxiliary racemes (Henderson, 2001; Van Wyk and Van Wyk, 1997; Bredenkemp and Phepho, 2008). The fruit appears as an ovate structure approximately 6mm in length, and seeds are tipped by a tuft of hairs and a feathery awn (Van Wyk and Van Wyk, 1997).



Figure 2.2, *Tamarix usneoides* in the site-species woodland trials at Vaal River Mining Operations with white flowers.

2.2.2 *Tamarix ramosissima* (Ledeb.)

Tamarix ramosissima Ledeb. is an abundantly occurring IAP, native to Eastern and Central Europe through to Eastern Asia, as well as in the Middle East (Henderson, 2001; Baum, 1978; Gaskin and Schaal, 2002; Klopper *et al.*, 2006). It has deep purple-pink flowers (Fig 2.3) and grows to between three and six meters in height (Henderson, 2001; Baum 1978). Individuals in this species are characterized by a prolific growth form of feathery candelabrum-like branchlets, with long internodes and non-amplexicaul, simple leaves with a reduced number or complete absence of salt glands. The bark is a reddish-brown colour (Bredenkemp and Phepho, 2008; Baum 1978), and leaves are simple and sessile with a narrow base between 1.5-3.5 mm in length. Inflorescences are aestivate and densely composed of racemes; 1.5-7cm in length and 3-4mm in width (Baum, 1978; Bredenkemp and Phepho, 2008; Henderson, 2001).



Figure 2.3, Alien *Tamarix ramosissima* growing in the wild population at Vaal River Mining Operations with pink-purple flowers.

2.2.3 Hybridisation between *Tamarix usneoides* and *Tamarix ramosissima*

Hybridisation is the formation of offspring between closely related parents of different species. This often presents new genetic combinations (hybrids) that may be better suited than parent plants to new environments (Raven *et al.,* 1986). As hybrids possess characteristics inherited from the alien parent plant, they are likely to interact with the biotic and abiotic environments in different ways from native species (Kiaer *et al.,* 2007). This raises the concerns of; (i) potential impacts of gene flow from alien species to native relatives, potentially leading to increased invasiveness by hybrid plants and competitive exclusion of other plant species in the community, (ii) the evolution of resistance to plant pests by hybrid plants, and (iii) genetic swamping in small wild populations which could lead to a decrease in population size and local extinction of the native species (Kiaer *et al.,* 2007).

Hybridisation first discovered in the 1970s (Obermeyer, 1976) between indigenous *T. usneoides* and alien *T. ramosissima* is suspected to be an on-going problem in South Africa. Recent invasions have been confirmed in the Free State and North West Provinces as well as in the Eastern Cape Province of South Africa (Christien Bredenkemp, South African National Botanical Institute, pers comm.), particularly in riparian areas and along water courses. The hybridisation between *T. usneoides* and *T. ramosissima* has the potential to create an aggressive invader through genetic assimilation of genes from both parents. As documented in the USA between *T. ramosissima* and *T. chinensis*, this may lead to increased hybrid vigour and plant yield creating hybrid swarms and monospecific stands (Hwa and Yang, 2008; Huxel, 1999; Gaskin and Schaal, 2002; Brotherson and Field, 1987). These hybrid swarms alter the population structures of species and displace native plants.

2.3 CONTROL OF WOODY INVASIVE ALIEN PLANTS (IAPS) LIKE *TAMARIX*

There are three key methods of IAP control; (i) Mechanical control, (ii) Chemical control, and (iii) Biological control as well as an integrated approach which may involve a combination of two or more methods (Wyatt, 1997; Hobbs and Humphries, 1995).

Mechanical control involves manual actions for plant removal such as hand pulling or uprooting plants, cutting or slashing followed by the application of a foliar spray, ring-barking and tree felling, which are slow labour intensive processes that often lead to soil disturbance and cause soil erosion or further infestations (Wyatt, 1997).

Chemical methods involve the use of registered herbicides to control IAPs (Wyatt, 1997) and are favourable in instances where plants occur on steep slopes that are prone to erosion. Herbicides are applied as a foliar application to the leaf area, as

basal bark treatment, through cut and stump treatment and as soil applied herbicides. Chemical methods are unsuitable in instances where they persist in the environment long after application and where they have high toxicity and pose danger to other plants and animals (Wyatt, 1997).

Biological control is favoured for the success it has achieved with limited impacts to the environment (Wyatt, 1997; Muller-Scharer and Schaffner, 2008). The biological control method uses biological agents to reduce the abundance of IAPs in their new home range below an ecological threshold, and correct the ecological imbalance (Muller-Scharer and Schaffner, 2008; Wyatt, 1997; Van Driesche and Bellows, 1996). A biological control programme involves identifying agents in the IAP's native land which infest and damage the plant. This process is followed by a rigorous quarantine, screening and testing process before an agent is selected for release (Wyatt, 1997; Muller-Scharer and Schaffner, 2008).

There is need for control of alien *Tamarix* spp. introduced into South Africa as horticultural plants (Obermeyer, 1976), as their high seed outputs and persistence in the environment facilitate rapid reproduction. A single mature *Tamarix* plant produces as many as 500,000 to one million seeds in a growing season (Tomanek and Ziegler, 1962). The seeds have awns for effective wind and water dispersal, and seed germination can take place within one day of contact with moisture. Seeds can lay dormant for up to one year if conditions are appropriate (Everitt, 1980; Warren and Turner, 1975).

Alien Tamarisk has become naturalised in the USA, Mexico and Australia, and research efforts into its control have increased since the 1990s (Duncan and McDaniel, 1998). Mechanical and chemical control, fire, flooding and inundation, and biological control have all been attempted with varying results (Brock, 1994; DeLoach, 1991; DeLoach *et al.*, 1996; Graf, 1978, 1979, 1982). In the USA, successful control has been achieved with chemicals, using Imazapyr and Glyphosate, and biological control using the leaf beetle, *Diorhabda elongata* Brullé. (Coleoptera, Chrysomelidae) and the mealybug, *Trabutina mannipara* Hemprich and Ehrenberg (Homoptera, Pseudococcidae). Most accounts of control

have taken advantage of local conditions to achieve success, with mechanical control proving successful where there was adequate shade from existing vegetation to prevent resprouting Tamarisk (Busch and Smith, 1995). Single chemical treatments proved unsuccessful in controlling and eliminating mature plants (Duncan and McDaniel, 1998), while fire stimulated rapid plant growth if roots were undamaged. *Tamarix* also has the ability to re-sprout vegetatively from root cuttings providing environmental conditions are warm and moist (Horton *et al.,* 1960, Duncan and McDaniel, 1998).

In South Africa tree-felling and stump poisoning to control pink and purple-flowered *Tamarix* on TSFs has been practiced for many years by AngloGold Ashanti at the West Wits Operation near Carletonville, although this resulted in vigorous suckering of the plants (Isabel Weiersbye, University of Witwatersrand, pers comm.). The control methods currently in use involve cut and stump poison with Imazapyr, or excavation of the entire plant and root system. Imazapyr is a broad-spectrum imidazolinone herbicide manufactured in the USA (Cox, 1996). Its toxicity to humans, animals and plants increases with herbicide concentration (Cox, 1996), and there is particular concern for impacts to soil, non-target plants and water sources (Cox, 1996; Washington State Department of Transport, 2006), particularly notable for aquatic phytotoxicity (Roshon *et al*, 1999). The use of Imazapyr is not advisable in sensitive areas such as in riparian zones, wetlands or near water bodies, as there is potential for application drift from residue leaching and rainfall runoff, and toxicity and environmental persistence is expected (Cox, 1996; Washington State Department of Transport, 2006). An environmentally acceptable method like biological control, could be used to prevent the spread of invasive *T. ramosissima*, as well as the *T. usneoides* × *T. ramosissima* hybrids in sensitive areas.

South Africa is one of the five most active countries in biological control (Crutwell McFadyen, 1998) with legislation and procedures that govern the investigation and release of agents. South Africa has had an impressive record, with success in controlling the alien cactus species (i) *Opuntia ficus-indica* and (ii) *Opuntia aurantiaca* (Zimmermann and Moran, 1991; Moran and

Zimmermann, 1991). There was also successful control of the weedy species *Hypericum perforatum* (St. John's wort) in the 1970s with the introduction of the leaf-feeding beetle *Chrysolina quadrigemina* (Coleoptera, Chrysomelidae) and the bud-galling midge *Zeuxidiplosis giardi* (Diptera, Cecidomyiidae) from Australia (Gordon and Kluge, 1991), where chemical methods had failed or proved too costly.

2.4 PHYTOREMEDIATION

As stringent legislative requirements such as the Conservation of Agricultural Resources Act (CARA) No. 43 of 1983, govern the use and control of alien species, and indigenous flora is always preferred for rehabilitation and re-vegetation purposes; *T. usneoides* presents an exciting opportunity for phytoremediation of polluted groundwater and soils on gold and uranium mines in South Africa.

Phytoremediation describes the passive *in situ* use of plants to remediate soil, water and sediment contamination (Cunningham and Ow, 1996; Dietz and Schnoor, 2001). Uncontained pollution is of concern because of the potential for metabolite and contaminant introductions into the food chain with the intake of polluted water, causing health problems (Dietz and Schnoor, 2001). Various mechanical, biological and chemical methods which include land filling, incineration, use of radionuclides and low and high thermal treatments have been practised to achieve remediation, although these are often costly and limited by the volume and composition of the contaminant, site characteristics, time constraints and cost implications (Cunningham *et al.*, 1995; USEPA, 2000). Some phytoremediation technologies, such as constructed wetlands, floating plant systems and reedbeds have long been used to achieve remediation of water and sediments. As soil remediation is a complex endeavour (Cunningham *et al.*, 1995), research efforts have looked into phytoremediation to achieve this. The natural ability of plants to restore mechanically and chemically altered sites is

evident, from plant succession that forms communities along road verges, to the colonisation of an impacted site like Mount St. Helens after a volcanic eruption (Cunningham and Ow, 1996). Certain plants have shown the capacity to tolerate relatively high concentrations of chemicals without lethal effects and declines in growth (Weiersbye and Witkowski, 2007; Dietz and Schnoor, 2001). These plants directly or indirectly absorb, sequester and degrade contaminants over time, and the remedial effect is evident as the chemical and physical nature of the contaminant within the soil is no longer at levels that pose health and environmental risks (Cunningham and Ow, 1996). However, although these tolerant plants may continue to grow, overtime reproduction is negatively impacted and they are unable to persist.

As there are high costs involved in the removal of heavy metals from soils, the problem has often been ignored by industry. Phytoremediation is now favoured for its low maintenance costs and because of its applicability to a wide range of contaminants, particularly heavy metals and chlorinated solvents. This approach relies on the use of indigenous plants that are tolerant (Weiersbye *et al.*, 2006b), can regenerate under prevailing conditions (Weiersbye and Witkowski, 2007), and have the ability to sequester and immobilize harmful pollutant ions in the rooting zone, or even extract and concentrate pollutants such as sulphates, chlorides, metals and radionuclides from polluted groundwater and soils in above-ground biomass (Weiersbye, 2008).

2.4.1 The Mine Woodlands Research Project

Anglo Gold Ashanti Ltd, a global gold mining company, entered into a partnership with the University of Witwatersrand in 1995 to form the ‘Ecological Engineering and Phytotechnology Programme’ (EEPP). The overall aim of the programme is to quantify the effectiveness of strategic plantings on and around TSFs for pollution control. For example, the Mine Woodlands Research Project is testing the ability of tree species to reduce the impacts of acid mine drainage on

groundwater and rivers (Dye and Weiersbye, 2010).

Tamarix usneoides has been included in site species trials at three gold and uranium mining operations on the Highveld, including Vaal River Operations near Orkney in the North West Province.

Research into *Tamarix* spp. which are generally halophytic, salt-includer plants has revealed an ability to tolerate growth in harsh saline environments. This is facilitated by the controlled uptake of ions and the synthesis of compatible compounds, often using salt glands in the leaves (Flowers and Colymer, 2008). Some halophytes have salt excretion organs such as salt bladders, trichomes or salt glands, in the case of some *Tamarix* spp., which regulate plant tissue ion concentration (Manousaki and Kalogerakis, 2010). Studies carried out on *Tamarix smyrnensis* Bunge. and *T. aphylla* L. have shown that these trees are able to extract sodium and chloride ions, cadmium, zinc, copper and lead from the soil and excrete them through salt glands in their leaves as a detoxification mechanism (Manousaki *et al.*, 2008; Hegemeyer and Waisel, 1988).

Tamarix usneoides is also seen as a useful plant to address other environmental issues faced by mines including; (i) dust prevention by acting as a wind break on tailings, (ii) seepage extraction through high water intake which is useful in wet dams to reduce the instability of high volumes of wet slime, and (iii) hydraulic control provided by high evapotranspiration rates which retain groundwater seepage from TSFs (Duncan, 1997) by drawing out large quantities of groundwater, often a constraint for shallow rooted or slow growing plants.

The hybridisation between indigenous *T. usneoides* and alien *T. ramosissima* poses a serious threat to a phytoremediation approach. The *T. usneoides* x *T. ramosissima* hybrids are expected to be ineffective in reclaiming Acid Mine Drainage (AMD) due to an absence or low numbers of salt glands on the leaves. A study carried out by Dennis (2008) which tested the ability of *T. usneoides* trees on gold mines to accumulate pollutants from AMD, found '*T. usneoides*' leaves to hyper accumulate calcium (Ca), sulphur (S), chlorides (Cl-) and sodium (Na), and to accumulate some metals and excrete minerals through the foliar salt glands,

although the study does not mention the abilities of the hybrid plants. Morphological identification of *T. ramosissima* trees indicated that these specialised salt glands, if present, are in low numbers or entirely absent in *T. ramosissima* and *T. ramosissima* x *T. usneoides* hybrids, possibly making them less effective accumulators (Christien Bredenkemp, South African National Botanical Institute, pers comm.). Based on this morphological difference in the presence and abundance of salt glands, successful phytoremediation may be compromised if *T. usneoides* x *T. ramosissima* hybrids and alien *T. ramosissima* are present in the "cultivated" woodland plots.

2.4.2 Origin of *T. usneoides* and putative hybrids in the Mine Woodlands Project (MWP) site species trials

Tamarix usneoides were cloned by Working for Water (WfW) and the Department of Water Affairs and Forestry (DWAF) in Upington. All trees were georeferenced and labelled with numbered metal tags, and vouchers collected by DWAF were sent to the South African National Botanical Institute (S.A.N.B.I) in Pretoria for identification over the course of three years. Trees thought to be pure *T. usneoides* were planted in 2003 and 2004 at two site-species trials at the Vaal River Mining Operations. Six Upington clones were planted per trial. However, in 2008, some of these trees produced pink or white and pink inflorescences, and are suspected to be hybrid *Tamarix* plants. It is thought that either the tree tags or vouchers, or identifications were mixed-up, resulting in hybrid trees being cloned in error and mixed into the delivery.

Alien species planting without special approval is prohibited by legislation for certain species deemed to be problematic, particularly by the Conservation of Agricultural Resources Act (CARA) Act 43 of 1983. If IAPs are present, all landowners are legally obliged to remove and control such plants. The University and AngloGold Ashanti therefore commissioned this study, and molecular research in order to be able to replace hybrids with pure indigenous trees.

2.5 WASTE PRODUCTION FROM GOLD MINING

Gold has been mined on the Witwatersrand Basin for over a century and is a major contributor to South Africa's economy, although with serious negative impacts to the environment (Weiersbye *et al.,* 2006b; Bailie, 2009).

During the gold mining process in South Africa, underground ore is brought to surface where it is subjected to a milling, distilling and extraction process using the MacArthur-Forrest method (using cyanide) for deeper un-oxidized ores containing pyrite (FeS_2) (Naicker *et al.,* 2002). The waste is toxic, composed of silicates, potentially toxic metals and metalloids (such as Cu, Ni, Pb, Zn, Fe, Al, Mn, U and As), and sulphides which create acidic soils, dust pollution and brackish water conditions. The wastes are stored in slimes dumps (Mendez and Maier, 2008; Johnson and Hallberg, 2005) known as Tailings Storage Facilities (TSFs) (Welch, 1995). The major source of Acid Mine Drainage (AMD) is the accelerated oxidation of iron pyrite (FeS_2) and other sulphidic minerals resulting from exposure to oxygen and water. Poor storage practices in unlined TSFs can then result in leaching into groundwater and streams (Allanson *et al.,* 1990; Sutton *et al.,* 2006).

Although the TSFs are localized, their zone of influence is larger due to contaminant transportation via wind and water, including AMD (Weiersbye *et al.,* 2006a). The main environmental problems arising from TSFs are; (i) steady increase of dissolved solid loads in the waters surrounding TSFs, (ii) rainwater percolating through TSFs which create plumes beneath the dumps, that emerge at the surface as polluted streams and hence create AMD (Blight, 1991; Van As *et al.,* 1992; Mizelle *et al.,*1995; Jones *et al.,* 1988), (iii) highly acidic topsoil which is contaminated with Co, Ni and Zn, most of which will become biologically available over time and will leach into the groundwater creating groundwater pollution, and (iv) wind borne dust clouds which arise from the exposed slimes dams and are carried into the surrounding landscape (Blight, 1991; Van As *et al.,*1992; Mizelle *et al.,*1995). This oxidation of sulphides becomes visible

overtime as colour changes materialize in soils and water bodies long distances away from the original source (Fig 2.4).

Substantial literature is dedicated to the detrimental impacts of AMD on freshwater ecosystem functions, particularly through the introduction of suspended sediments into rivers and wetlands leading to increased acidity in water and lower pH, as well as salinisation of water courses through the introduction of heavy metals which persist in water and soil (Coetzee, 2010; Allanson *et al.*, 1990; Rogers and Coetzee, 1991). The TSFs on the Witwatersrand Basin contain high concentrations of pyrite, between 0.2%-3% (Witkowski and Weiersbye, 1999).

Over time as tailings from decommissioned mines lie abandoned, they are exposed to oxygenated rainwater and the action of bacteria, which results in the oxidation of pyrite and other materials in the outer layers of the mine dump (Johnson and Hallberg, 2005). This causes more aggressive AMD than TSFs with lower pyrite concentrations. This waste often flows undeterred into surface water streams (Fig 2.4) or enters the groundwater table beneath the dumps (Jones *et al.*, 1988). This poses a long-term environmental problem that extends beyond the mine's life. Evidence of this seepage into the water table has been documented in the Witwatersrand Basin, particularly in the South-Eastern parts of Johannesburg as well as in and around the suburbs of Germiston (Jones *et al.*, 1988).

In other parts of the world, losses to surrounding land as a result of contamination have been reported in Santa Maria de La Paz, Mexico, as Castro-Larragoitia *et al.*, (1997) noted tailings deposits in nearby communities and agricultural fields, as well as in Cornwell, United Kingdom where Neal *et al.* (2005) noted water quality degradation and fish kills in surrounding rivers and estuaries as a result of AMD from the Wheal Jane tin mine.

Legislation has also improved since the beginning of gold mining when early industry regulation was composed primarily of recommendations and statutes. Mines have been plagued by criticisms of environmental atrocities and now strict environmental legislation is in place to ensure that industry takes responsibility to

prevent environmental damage (Sutton and Weiersbye, 2007)



Figure 2.4, TSFs scattered across the landscape, and located in close proximity to water courses in the Welkom region, Witwatersrand Basin of South Africa. TSFs are open to wind and water action as they lie devoid of plant life. Arrow indicates the change in water colour as a result of AMD from tailings spilled into the watercourse from surrounding TSFs.

2.6 LEGISLATION PERTAINING TO GOLD MINING

Legislation pertaining to mining activities has become more stringent with the ratification of the Minerals and Petroleum Resource Development Act (MPRDA), Act No. 28 of 2002 that makes it necessary for mines to comply with an acceptable environmental management programme. The Act clearly stipulates "development and use of resources in an ecologically sustainable manner", ensuring activities will not result in unacceptable pollution, ecological degradation or damage to the environment. The MPRDA concurs with the National Environmental Management Act (NEMA), Act No. 107 of 1998 which outlines certain environmental principles. These principles, which refer to mining operation permit holders include; (i) the management of environmental impacts in accordance with an approved environmental management programme, (ii)

rehabilitation of the environment affected by prospecting or mining operations to its natural and predetermined state, or to a land use that is acceptable to sustainable development, and (iii) responsibility for environmental damage, pollution or ecological degradation which arises inside or outside the boundaries of the permitted mining area or operation (Government Gazette, 2002).

As mines pose pollution risks to above and below ground water, and create impacts on land that leave it barren and dusty, they also have to comply with other legislation. The National Water Act (NWA) No. 36 of 1998 provides for the protection of water sources. Under this act it is an offence to pollute public and private water including seawater, rainwater and subterranean water. Landowners are also required to take responsible measures to prevent pollution from occurring and recurring. This places pressure on mines to establish a mine closure programme that is cost effective and continues beyond the mine's life.

CHAPTER 3:

MATERIALS AND METHODS USED TO STUDY TREE GROWTH, REPRODUCTIVE POTENTIAL AND INSECT OCCURENCE ON *TAMARIX* SPECIES

3.1 INTRODUCTION TO EXPERIMENTAL TECHNIQUES

This study was carried out at AngloGold Ashanti (Ltd) Vaal River Mining Operations in Orkney, South Africa. The mining operation spans an area of approximately 12000 ha (Herbert, 2008). Two field sites at the base of the Mispah and West Complex TSFs were selected. For the purposes of this study, the sites are symbolized by the acronyms "M" and "WC" and are known as the "good soil site" or "poor soil site" respectively. The "good" and "poor" soil descriptions are in relation to the soil conditions at the sites. Plant growth and the number of flowering individuals (as a measure of reproductive effort) were measured in the cultivated plots and compared between the two sites as a means of establishing differences between the two soil types. The presence, diversity and abundance of insects were also recorded at both sites and compared between the two soil types.

3.1.1 Mispah (M) Good Soil Site

The smaller Mispah site was characterised by deep soils derived from highly weathered dolomite (Herbert, 2008). *Tamarix* had poor survivorship under these "good" soil conditions. Four replicate plots of *Tamarix* had been planted with 63 trees in each plot, within the 4-6 year age class. The plots were composed of individuals of *T. usneoides* and *T. usneoides* hybrid. Survival and growth data were collected over a six year period, by field assistants working with the MWP, between 2003 and 2008. Data for 2009 were collected during this study by L. Buckham (author) of the University of Witwatersrand.

Tree survival was low in the plots (N=4, Mean 7.75 ± 6.13).

3.1.2 West Complex (WC) Poor Soil Site

The larger West Complex site, located in the centre of the AMD seepage plume, varies in lithology between poorly-weathered and chert-rich dolomite, with smaller inclusions of modestly weathered dolomite without chert (hard rock) (Herbert, 2008). Seepage from the dams are sometimes visible and appear (temporarily) at the surface in places. Parts of the site suffer from alternating periods of over-saturation and drought, as well as extreme salt accumulation within the topsoil (Herbert, 2008). Overall the soils are poorly-drained. Flat boulders constitute between 5-15% of the top and subsoil profile. The *Tamarix* appeared to survive and grow well under these "poor" soil conditions (Herbert, 2008). Four plots were selected for sampling within the 4-6 year age class containing individuals of *T. usneoides* and *T. usneoides* hybrid. Tree survival was high at the WC site (N=4, Mean 62.25 ± 0.95). Growth data were collected over a five year period between 2004 and 2008 and data for 2009 was collected during this study by L. Buckham (author) of the University of Witwatersrand. A younger plot of 2-year old trees, planted with 80 individuals in the same arrangement, was also sampled for insects. This plot was included in the study to enable comparison with the similar aged "wild population" of invasive *Tamarix* plants at the WC site. The schematic layout (with tree numbers not accurately represented) of the WC site is indicated in Figure 3.1.

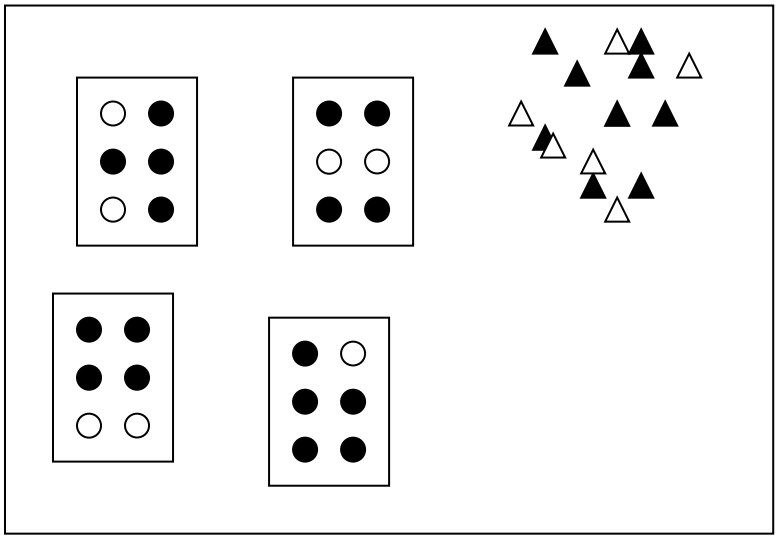


Figure 3.1, The relative placement of different *Tamarix* populations sampled in cultivated plots and in a wild population at the West Complex (WC) site, Vaal River Mining Operations. The number of trees is not accurate, and is only used to illustrate the layout of the trees on the site. *T. usneoides* ○, *T. usneoides* hybrid ●, *T. ramosissima* Δ, *T. ramosissima* hybrid ▲.

3.2 PHYSICAL DESCRIPTION OF STUDY AREA

The Vaal River Mining Operations are located along the Vaal River within the Grassland Biome. This biome extends across the central part of South Africa. These temperate grasslands experience strong summer rainfall and dry winters. Rainfall varies across the biome from 400-2500 mm per annum. The flowering period of grassland flora (forbs and grasses) is in February, March and April. Summer temperatures are high, between 22°C and 34°C and frost is common in winter months (Mucina and Rutherford, 2006).

Within the Grassland Biome, the two study sites lie in the sandy *Cymbopogon-Themeda* veld of Acocks (1953), and the Dry Sandy Highveld Grassland of Low and Rebelo (1996). According to the Mucina and Rutherford (2006) vegetation classification system, the vegetation is Vaal-Vet Sandy Grassland which is an endangered (highly threatened) vegetation type. It is situated only within the North-West and Free-State Provinces. The landscape is flat with scattered hills and the Vaal River runs through the middle of the Vaal River Mining Operations.

The mean annual precipitation (MAP) for the Vaal-Vet Sandy Grassland is approximately 560 mm per annum and mean annual temperature (MAT) is around 17°C.

The geology within the Vaal River Mining Operations comprises chert-rich and chert-poor dolomite. Gold mines are highly concentrated in this area and mining forms the greatest threat to vegetation in this region (Mucina and Rutherford, 2006; Weiersbye and Witkowski, 2007).

3.3 SITE LAYOUT

Planting of propagated *Tamarix* cuttings was undertaken at M in February 2003 and at WC in February 2004, one year apart. This difference in planting was the result of differences in rainfall between the two sites (Herbert, 2008). As a result of drought conditions, lower than average rainfall was experienced at the Vaal River Mining Operations area in 2003. Rainfall was significantly lower at the WC site where high clay and saline soils result in lower water availability to plants (Herbert, 2008). Planting had to be delayed until 2004 when there had been sufficient rainfall for growth.

Trials of trees known as "site species trials", have been planted at the M and WC sites with the purpose of screening tree species for their performance under representative conditions, and developing suitable sivicultural strategies for sustainable woodland management (Herbert, 2008). The trials are each composed of twenty plant species with four randomly placed plots of each tree species, including "indigenous" *T. usneoides*. Each plot covers an area of 0.04725 ha and all four plots occupy an area of 0.189ha at the base of the TSF. The entire "site species trials" are matched in size and species composition at each of the two sites, with an approximate size of 5 hectares (ha), with 2 ha composed of alien trees and 3 ha containing indigenous trees (including "indigenous" *Tamarix usneoides*). In each plot, 63 trees (9 trees planted in 7 rows) were planted with 3m

row spacing and 2.5m spacing between each individual tree.

The "*T. usneoides*" trees planted at The Vaal River Mining Operations were supplied by the Department of Water Affairs and Forestry (DWAF) at Upington in 2003 and 2004. The *Tamarix* trees were cloned from six trees at Upington in the Northern Cape Province (Herbert, 2008). These "*T. usneoides*" trees were planted as indigenous species in the site species trials. At the initiation of this study, the identity of these trees as *T. usneoides* was thought to be certain, however as this study progressed, extensive hybridisation was discovered and it emerged that due to human error, the exact identity of the parent plants were unknown and inconsistent within the sites and plots.

Tamarix ramosissima was introduced to Highveld gold mines and is very common on gold TSFs and saline soils in the Witwatersrand Basin (Weiersbye *et al.*, 2006b, Weiersbye and Witkowski, 2007). At WC, a population of wild invasive *Tamarix* trees had colonised an area adjacent the site species trials. Some of these trees were included in this study to enable comparison between indigenous and alien *Tamarix*. For the purposes of this study; the "site species trials" of *Tamarix* trees will be referred to as the "cultivated plots" at both M and WC sites, and the invasive *Tamarix* population at the WC site will be referred to as the "wild population".

3.4 EXPERIMENTAL PROCEDURES

3.4.1 *Tamarix* Identification

Tamarix is a taxonomically challenging genus among the angiosperms. As plants undergo morphological changes in response to their environment (Baum, 1978; Gaskin, 2003), this study set out to find characters that are consistent across different environmental conditions. The presence and density of salt glands

(Dennis, 2008; Bredenkamp and Phepho, 2008), the petal shape (Baum, 1978) and the insertion of filaments into the nectar disc (Baum, 1978) most accurately distinguish between *Tamarix* species, including *T. ramosissima* and *T. chinensis* which are morphologically similar. These characters were used in the final identification of *Tamarix* plants.

A voucher specimen was collected from each plant during this study to identify each sampled tree by means of these stable morphological characteristics. The identity of vouchers was confirmed by taxonomists at both S.A.N.B.I and the C.E Moss Herbarium at the University of Witwatersrand. This was necessary as the true identity of trees was unknown prior to cloning since tree tags, vouchers or identifications were mixed-up, and extensive hybridisation between *T. usneoides* and *T. ramosissima* was suspected. Accurate tree identification was necessary as hybridisation is known to create a gradient of characters ranging from being morphologically similar to one parent or to the other. Trees were georeferenced and vouchers are lodged with the C.E Moss Herbarium.

The identity of georeferenced and tagged *Tamarix* plants was confirmed using morphological characters, after fieldwork had been completed. This was undertaken in the laboratory using a binocular dissecting microscope (Nikon SMZ 1500 stereo microscope). A voucher specimen composed of bark, leaves, and inflorescences was collected from each tree and lodged at the C.E. Moss Herbarium at the University of Witwatersrand. Six morphological characteristics were selected that could distinguish between species (Table 3.1).

Each plant voucher was labelled and placed in a plant press. The plant press was placed in an oven for three days to remove moisture, and then in a freezer for another three days to kill any fungi, insects or pathogens, to enable voucher preservation.

Plant samples were then examined and their character traits recorded. The data were entered into an Excel spreadsheet and classified according to a subjective method which categorized plant vouchers based on all six characters listed in Table 3.2 (Conclusion 1) as either *T. usneoides*, *T. usneoides* hybrid, *T.*

ramosissima hybrid or *T. ramosissima*. Results were then compared to an objective method (Conclusion 2) using only the first three characters (Table 3.1). The objective method is more easily replicated and is likely to be most accurate.

Table 3.1, Six morphological characteristics of *T. usneoides* and *T. ramosissima* plants used for species identification of *Tamarix* vouchers collected at the Vaal River Mining Operations.

Characteristic	***T. usneoides*** **(Literature References)**	***T. ramosissima*** **(Literature References)**
Insertion of filaments	peridiscal (1)	hypodiscal (1)
Petal shape	ovate-elliptic (1)	obovate-elliptic (1,2)
Presence of salt glands on leaves	present, abundant (3)	absent (3)
Petal colour	white (2)	pink-purple (2)
Orientation of leaves	vaginate, overlapping (1,2,4)	sessile, not overlapping (1,2)
Orientation of bracts	vaginate (1)	sessile (3)

Peridiscal: the filaments are inserted beside the nectar disc. Hypodiscal: the filaments lie below the nectar disc. Ovate-elliptic is egg or oval shaped with a tapering point. Obovate-elliptic is teardrop shaped with stem attached to the tapering point. Numbers correspond to literature references as follows: (1) Baum (1978); (2) Henderson (2001); (3) Bredenkamp and Phepho (2008); (4) Obermeyer (1976).

As the first three characters (insertion of filaments, petal shape, presence of salt glands on leaves) are very objective and can only be classified into two categories, identifying these characters is very simple, accurate and easily undertaken by different individuals. However, the last three characters of Table 3.1 (petal colour, orientation of leaves and bracts) are not easily distinguished and are based on the researcher's interpretation. For this reason, they are considered less accurate characters for voucher classification. Therefore, in the instances where plant identity was uncertain, the decision of Conclusion 2 was accepted.

3.4.2 Tree Growth

Tree growth measurements were derived through allometric relationships. This enabled the canopy volume, canopy area and wood volume values to be calculated from simple measures of tree dimensions (Witkowski and Wilson, 2001; Netshiluvhi and Scholes, 2001). This aimed to establish if site conditions affect *Tamarix* tree growth and size. The latter could influence attractiveness to insects.

Tree size (an indicator of growth over the whole trial period) was derived from easily measured variables (plant height, stem number and individual stem basal circumference, canopy diameter, stem circumference at 60cm and stem circumference at 120cm) over 48 months to establish allometric relationships between measured variables and other growth parameters. For this study tree size measurements were only conducted in cultivated plots at Mispah and West Complex on similar aged trees (4-6 year age class), where planting conditions were controlled. When the woodlands plots were established; trees were planted one year apart at Mispah and West complex sites. Linear regressions were plotted for canopy volume, canopy area, wood volume and height to derive regression equations with which growth over time could be predicted, as a measure of tree performance, as well as establish if site conditions were having an impact on tree growth.

Five trees in each cultivated plot (N =20) have been selected and measured for growth since planting took place, avoiding the outer rows to minimise edge effects. Where possible the same tree was measured annually or biannually to enable growth rates to be established. However, if a tree had experienced damage suspected to affect normal growth, or had died, the adjacent tree was measured. Measurements were undertaken as follows (Fig 3.2):

- Tree height (h) using a measuring pole.

- Widest canopy diameter (D1) and diameter at right angles to D1 (D2).

- Stem basal circumference (C0) using a measuring tape.
- Stem circumference at a height of 60cm (C1) using a measuring tape.
- Stem circumference at a height of 120cm (C2) using a measuring tape.
- Where trees were multi-stemmed, the number of main plant stems (stems arising from the ground) were counted, and in these instances, C1, C2 and C3 had to be measured individually. Stem circumference was measured to derive a stem basal diameter according to equation 1.

$$\text{Diameter} = \sum (C_1 + C_2 .... C_n)/\pi \qquad \text{[Equation 1]}$$

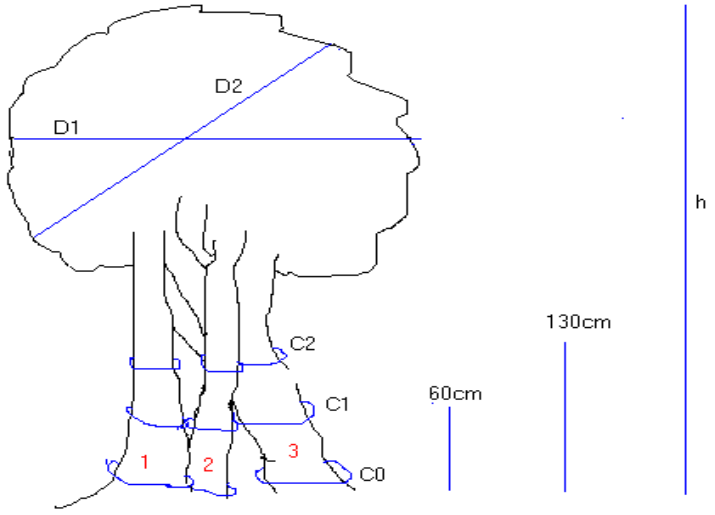


Figure 3.2, The dimensions measured for each tree at Mispah (M) and West Complex (WC) "site species trials" between 2005 and 2009 at the Vaal River Mining Operations (extracted from Wanenge, 2009).

The data collected through non-destructive measurements was input into equations 2, 3 and 4 to derive plant growth values for canopy volume, canopy area and tree wood volume that could be used to determine the growth rates of *Tamarix* trees.

Tree Canopy volume	=	$4/3\ \pi\ [(D_1/2) \times (D_2/2) \times (h/2)]$	[Equation 2]
Tree Canopy area	=	$\pi\ (D_1/2) \times (D_2/2)$	[Equation 3]
Tree wood volume	=	$[(C_0 + C_1 \text{or } C_2] \times h$	[Equation 4]

Linear regression analysis, R^2 values and *p*-values were computed for trees in the cultivated plots at M and WC sites.

3.4.3 Flowering Frequency

There are many methods to measure the sexual reproductive effort of plants. The frequency of flowering was recorded in *Tamarix* trees at both sites to establish if soil type and site conditions impacted on the flowering and reproductive potential of *Tamarix* spp. The occurrence of flowering was recorded as present if buds, inflorescences or fruit were recorded on the trees. Flowering was recorded in the field and verified in the laboratory (where possible) using voucher specimens collected from trees during fieldwork.

The occurrence of flowering was recorded as presence or absence readings during 2009, in January, March, May, September and November. Flowering was also recorded in January 2010 although the "wild plant population" had been chemically and mechanically removed in December 2010, so flowering could only be recorded in the "cultivated plots". No sampling took place in seven of the 13 months, in February, April, June, July, August, October and December 2009. Sample sizes are indicted in Table 4.2.

3.4.4 Insect Identification

Morphospecies are used as a technique of separation between insect species, as a surrogate for taxonomic species (Derraik *et al.,* 2010). It is used as an alternative

technique for invertebrate studies where there are time and financial constraints, a lack of experts, as well as to help overcome difficulties associated with invertebrate studies (Derraik *et al.,* 2010). In order to determine the insect diversity of an area, usually a tree, quadrat or trap, a series of samples are taken to measure the number and type of occurring insects. The rate at which new morphospecies are added to the catalogue supplies important clues about the species richness, density and species abundance distribution of the sampling area. Species accumulation curves, also known as rarefaction curves, are a method of estimating insect (morpho) species richness, plotted as the number of species recorded (S) as a function of the sampling effort or number of samples (N) (Magurran, 2004).

3.4.5 Insect Sampling and Data Analysis

Four methods were initially tested to sample insects on trees in the cultivated plots and in the wild population. These consisted of tree fogging, tree beating, foliage collection for emergence boxes and tree sweeping. Foliage collection and tree sweeping yielded the largest numbers of insects and were therefore used for insect sampling in this study. The sampling design initially made provision for equal sample sizes in the cultivated plots at both sites, and in the wild population at the WC site. However, with the discovery of extensive hybridisation after insect sampling had been completed; sample sizes for each tree species and their hybrids were then subsequently inconsistent. Sample sizes are indicated in Table 3.2. The collection of flowering foliage for the emergence box method was not undertaken at the M site, due to the low occurrence of flowering in trees at this site.

3.4.5.1 Tree sweeping (TSW) method

Insects were sampled on trees in cultivated plots at both sites using a handheld net of 35cm diameter on a 30cm handle swept over the foliage on four sides (N, S, W, and E) of the tree, concentrating on areas of green vegetation and flowers for one minute (Fig 3.3). The net contents were then placed in an emergence box for 30 minutes. Emergence boxes were then emptied in to 70% alcohol. The trees sampled were randomly selected in the cultivated plots at each site. Insects were pinned and sorted into morphospecies.

Table 3.2, Insect Sampling methods used to sample 217 trees during January, March and May 2009 at the Mispah (M) and West Complex (WC) sites, at the Vaal River Mining Operations. Trees were composed of *T. usneoides* (T.u), *T. usneoides* hybrid (T.u.h), *T. ramosissima* (T.r) and *T. ramosissima* hybrid (T.r.h) individuals.

Site		**Sampling Method**								
		EMB	**N**			**TSW**	**N**			**Total**
M	**Tree Species**		**Jan**	**Mar**	**May**		**Jan**	**Mar**	**May**	
	T. usneoides					√	4	7	0	11
	T. usneoides hybrid					√	12	25	0	37
WC	**Tree Species**		**Jan**	**Mar**	**May**		**Jan**	**Mar**	**May**	
	T. usneoides	√	6	10	9	√	11	4	11	51
	T. usneoides hybrid	√	4	29	6	√	23	20	6	88
	T. ramosissima	√		7						7
	T. ramosissima hybrid	√		23						23
Total			10	69	15		50	56	17	

N=number of trees sampled. EMB = Emergence Box Method, TSW= Tree Sweep Method. *T. ramosissima* and *T. ramosissima* hybrids are absent at M site. √ indicates the sampling method used on each tree type. N is blank where no sampling took place.



Figure 3.3, Insect sampling using the Tree Sweeping Method (TSW) in cultivated plots at Vaal River Mining Operations.



Figure 3.4, Insect sampling using the Emergence Box, taken during fieldwork at Vaal River Mining Operations.

3.4.5.2 Emergence box method (EMB)

Insects were sampled in the" wild population" and in the "cultivated plots" at the WC site, by collecting a 15cm branch of flowering foliage which was placed in an emergence box, similar to the one indicated in Figure 3.4, for a three week period in the laboratory. The flowering foliage was collected from each of the four sides of the tree (N, S, W, and E). Emerging insects were collected and placed in 70% alcohol. Insects were pinned and sorted into morphospecies.

3.4.5.3 Species accumulation curves

The rate of insect morphospecies accumulation in *T. usneoides, T. ramosissima*, and their respective hybrids (four plant types) was computed. Data from the two sites and the three sampling months (January, March, May) was pooled to achieve comparable sample sizes, and plotted to depict the number of insect morphospecies recorded (S) as a function of the number of trees sampled (N) during the entire study. The insect morphospecies data were sorted using the programme Statistical Estimation of Species Richness and Shared Species from Samples (Estimate S), version 7.5.2 (Colwell, 2004), to produce a smooth curve for each plant species and their respective hybrid (Colwell, 2000).

This was done to indicate how insects were concentrated on the trees, and to establish if there was overlap in the Confidence Intervals of tree types, which would indicate that the morphospecies recorded were similar. The graphs are based on the number of trees sampled (N); *T. usneoides* (N=52), *T. usneoides* hybrid (N= 125), *T. ramosissima* (N=14) and *T. ramosissima* hybrid (N=26).

3.4.5.4 Insect identification

Insect morphospecies which yielded five or more individuals were sent for further

identification to the Agricultural Research Council (ARC), Biosystematics Division. Drs M. Mansel, M. Stiller, I.M Millar, C. Eardley, M. Kruger and E. Grobbelar identified insects to Family, Genus and Species level where possible.

3.4.6 Statistical Analysis

Data were analysed (with STATISTICA six sigma (Statsoft Release 7, 2006)) to test for a normal distribution. Growth data were then analysed by a simple linear regression to establish the relationship between canopy volume, wood volume, canopy area, height and growth period.

$$Y = mx + c \qquad \text{[Equation 4]}$$

m is the slope of the regression line

The significance of the regression was calculated to see if there was a correlation between growth and age, as well as to compare how *Tamarix* grows on different soil types. A two sample T-test was conducted on growth data from trees aged 67 months at M and 66 months at WC to test for growth differences between the two sites. The occurrence of flowering was recorded at both sites, but not tested due to small numbers of flowering trees in some plots.

The number of insects (abundance) and the number of morphospecies (diversity) were compared between plant types using parametric T-tests, where only *T. usneoides* and *T. usneoides* hybrids were sampled, and Analysis of Variance (ANOVA) tests where *T. usneoides*, *T. usneoides* hybrids, *T. ramosissima, T. ramosissima* hybrids were compared. Further post-hoc analysis, Least Square differences (LSD), were computed to establish where there are differences in insect abundance and diversity. The number of morphospecies was then plotted using Estimate S (version 7.5.2) (Colwell, 2004) to display the rate of accumulation of new (morpho) species and to measure insect diversity on each tree type.

CHAPTER 4:

RESULTS OF TREE IDENTITY, GROWTH AND INSECT INTERACTIONS IN *TAMARIX* SPECIES

4.1 INTRODUCTION

Results are presented in four sections focusing on *Tamarix* identification, plant growth and flowering, insect abundance and diversity and insect identification.

4.2 *TAMARIX* IDENTIFICATION

Hybrid *Tamarix* trees have been inadvertently cultivated in site species trials at Vaal River Mining Operations. Hybrids are the majority of individuals in the population comprising 74% to 81% of individuals in the cultivated plots at WC and M respectively, and 60% of individuals in the wild population. Pure *T. usneoides* make up only 19% to 26% of individuals in these "cultivated plots" (Table 4.1).

4.3 PLANT GROWTH

Even though the trees are a mix of *T. usneoides* and *T. usneoides* hybrid, they are in similar proportions at the Mispah (M) and West Complex (WC) sites (Table 4.1) enabling the comparison of growth between the sites.

Table 4.1, Tree identities for *Tamarix* plants using two morphological methods (The Subjective and the Objective Methods) collected at the Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

			Number of trees identified by each method		**Trees changing identity**		
Site	**Tree location (N)**	**Plant Identity**	**Subjective**	**Objective**	**Subjective - Objective Change**	**Number of changing trees**	**% Plant Type (objective method)**
WC (N=168)	Cultivated (128)	*T.usneoides* (T.u)	37	33	T.u→T.u.h	4	26
		T.usneoides hybrid (T.u.h)	91	95		0	74
	Wild (40)	*T.ramosissima* (T.r)	14	16		0	40
		T.ramosissima hybrid (T.r.h)	26	24	T.r.h→T.r	2	60
M (N=48)	Cultivated (48)	*T.usneoides* (T.u)	9	9		0	19
		T.usneoides hybrid (T.u.h)	39	39		0	81
	Wild (0)	*T.ramosissima* (T.r)	0	0		0	0
		T.ramosissima hybrid (T.r.h)	0	0		0	0

N indicates the number of trees sampled. T.u and T.u.h have been planted in cultivated plots at M and WC. T.r and T.r.h grow invasively in a wild population. T.r and T.r.h are absent in the cultivated plots at the M site.

The four size parameters, canopy volume, canopy area, wood volume and height were measured to confirm or disprove observations that trees grow better at the WC site. The canopy volume, canopy area and wood volume were greater (over the whole trial period) at the M site, increasing at a faster rate than the WC site (Fig 4.1, 4.2, 4.3). However, the WC site recorded consistently taller trees than the M site with growth in terms of tree height increasing at a faster rate at the WC site (Fig 4.4). Fire had only occurred at the WC site in the year prior to this study.

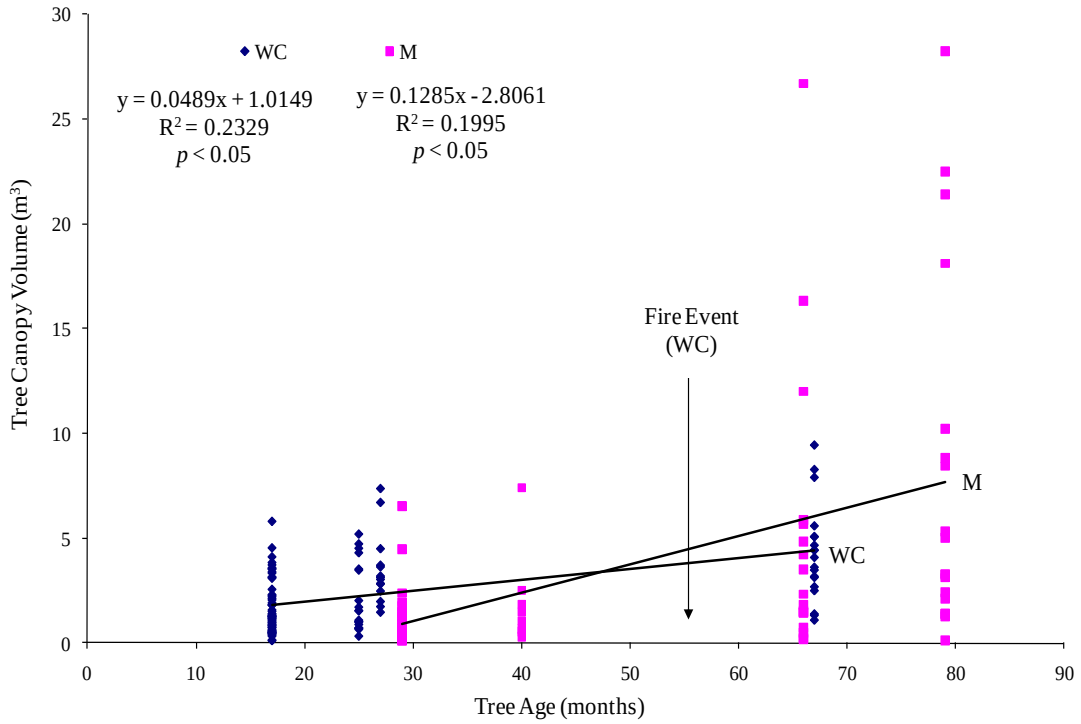


Figure 4.1, Increase over time in tree canopy volumes of *T. usneoides* and *T. usneoides* hybrid trees in cultivated plots at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

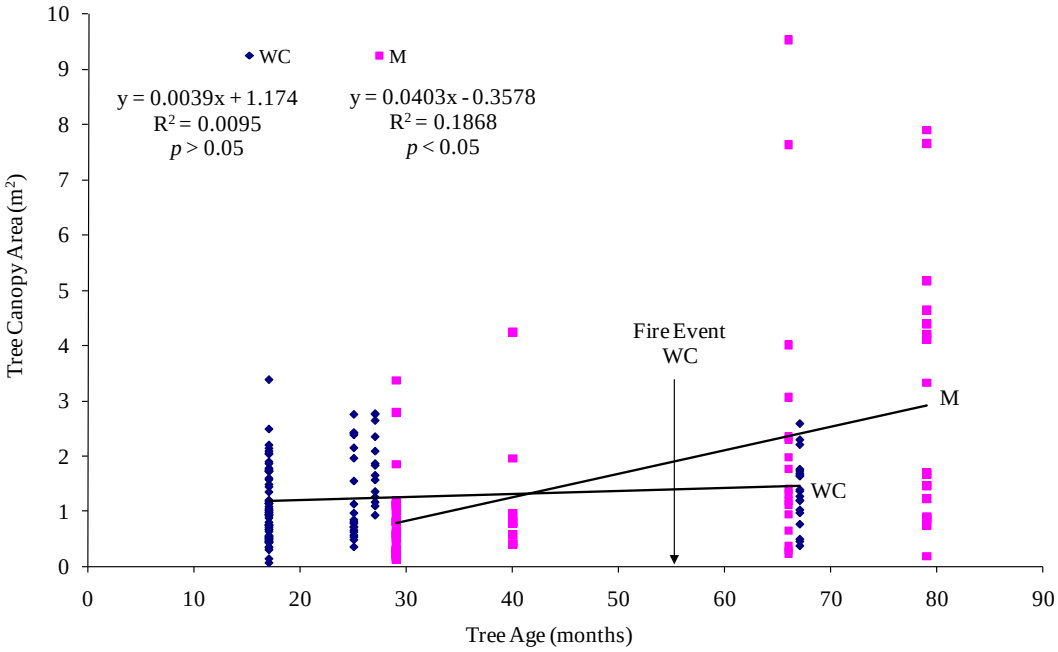


Figure 4.2, Increase over time in tree canopy area of *T. usneoides* and *T. usneoides* hybrid trees in cultivated plots at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

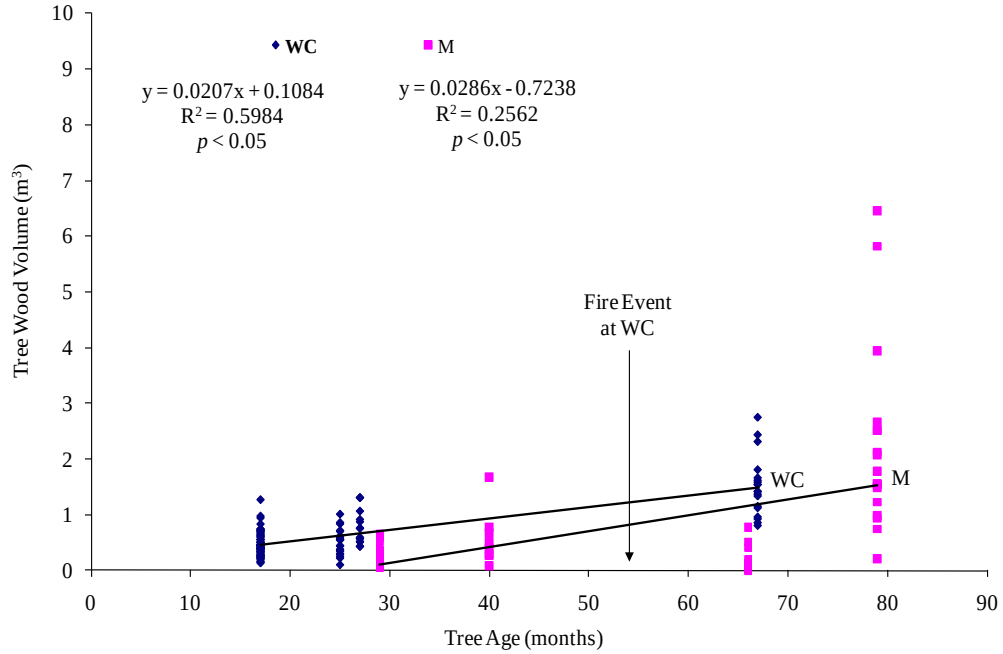


Figure 4.3, Increase over time in wood volumes of *T. usneoides* and *T. usneoides* hybrid trees in cultivated plots at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

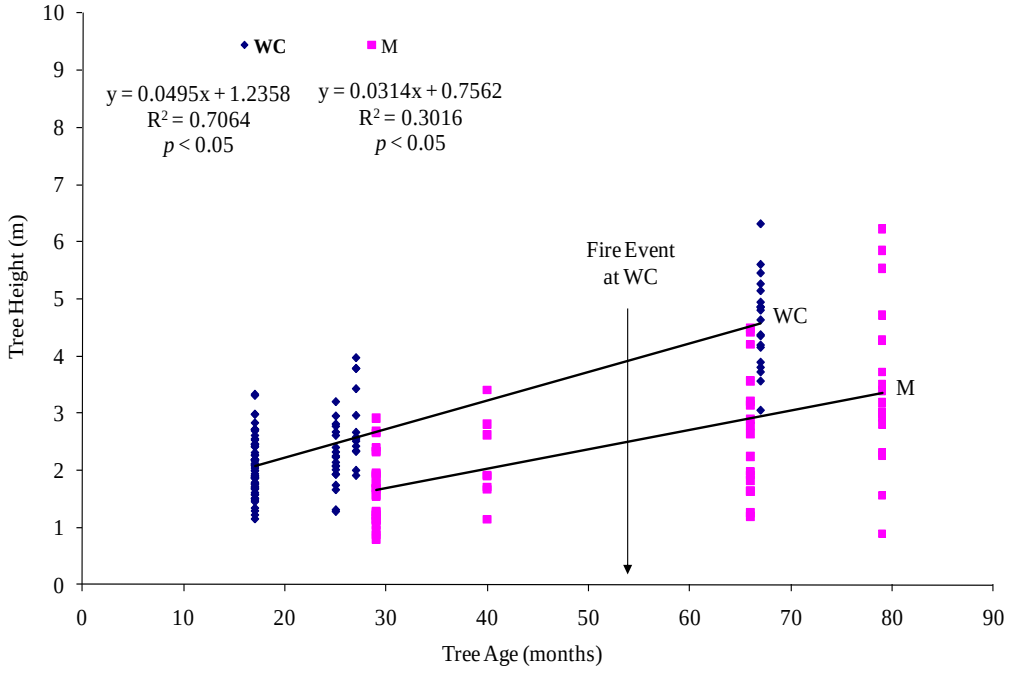


Figure 4.4, Increase over time in height of *T. usneoides* and *T. usneoides* hybrid trees in cultivated plots at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

Tree canopy volume displayed a steeper growth curve at the M site than WC site, indicating that the rate of increase in tree canopy volume is faster at the M site. There was a weak correlation between canopy volume and tree age at the WC site and the M site. The relationship between canopy volume and age was significant at both sites (Fig 4.1).

Tree canopy area displayed a steeper growth curve at the M site than the WC site, indicating that the rate of increase in tree canopy area is faster at the M site. There was no correlation between canopy area and tree age at WC and a weak correlation at the M site. The relationship between canopy area and age was significant at the M site and not significant at the WC site (Fig 4.2).

Tree wood volume at M site increased faster than the WC site with a steeper curve displayed. The wood volume at the WC site also increased, but at a slower rate over time. There is a strong correlation between tree wood volume and tree age at the WC site, and a weak correlation at M site. The relationship between tree wood

volume and age is significant at both sites (Fig 4.3).

Tree height increased faster over time at the WC site compared to the M site with a steeper curve at WC. There was a strong correlation between height and tree age at the WC site and a weaker correlation at M site. The relationship between plant height and age was significant at both sites (Fig 4.4).

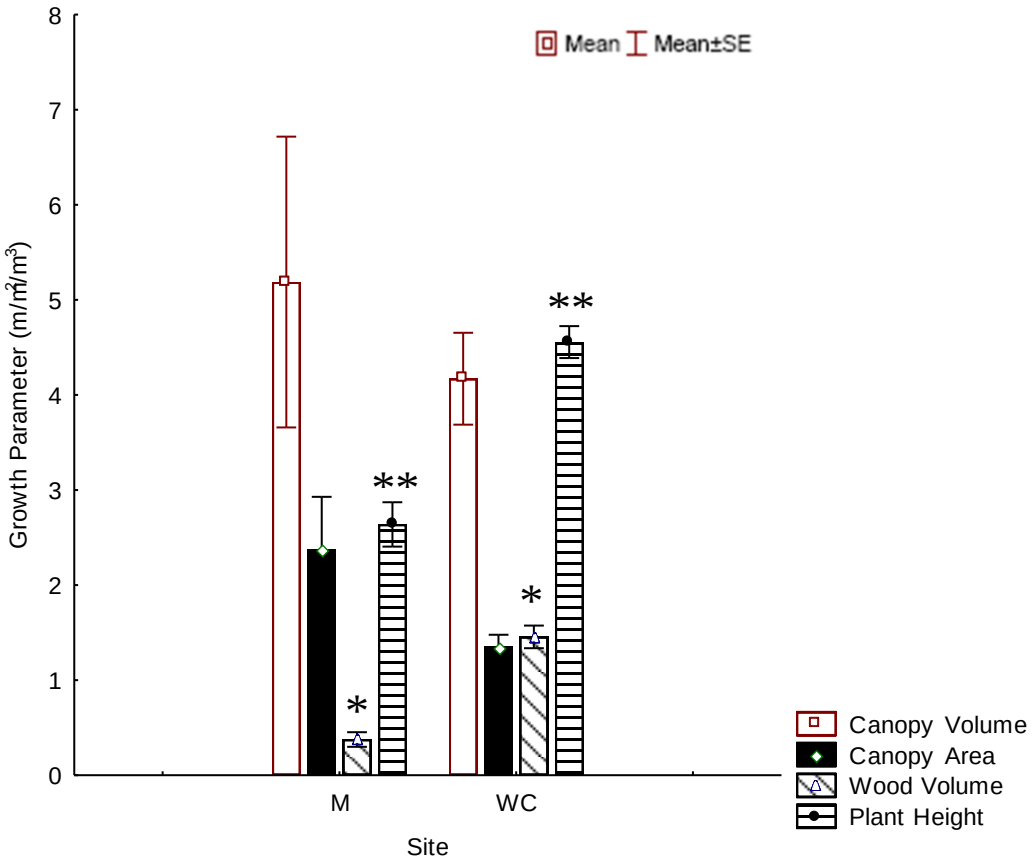


Figure 4.5, Comparison of tree dimensions (canopy volume, canopy area, wood volume, and height) at one stage of growth in *T. usneoides* and *T. usneoides* hybrid trees aged 66 months at Mispah (M) site and 67 months at West Complex (WC) site, at Vaal River Mining Operations.* indicates significant difference in growth parameters between sites at $p < 0.05$.

Tree dimensions were compared (at one stage of growth) between the M and WC sites for similarly aged trees (4-6 year age class). Trees at the M site had larger canopy areas and canopy volumes. However, the differences between sites were not significant for canopy volume (t=0.63,*p*=0.5332, df=36) and canopy area (t=1.77,*p*=0.0840, df=36). There were significant differences in wood volume (t=-

7.05,p<0.01, df = 36) and tree height (t=-6.44,p<0.01, df = 36) between the two sites with taller trees and larger wood volumes at the WC site (Fig 4.5).

4.4 PLANT FLOWERING

The occurrence of flowering was measured as presence or absence of flowers on six occasions, in January, March, May, September and November 2009 as well as in January 2010, and recorded in Table 4.2. No sampling took place in seven of the 13 months, in February, April, June, July, August, October and December 2009.

Table 4.2, Comparison of flowering in *T. usneoides* (T.u) and *T. ramosissima* (T.r) and their hybrids (T.u.h and T.r.h) as a measure of their reproductive potential at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations.

		Jan-09	Mar-09	May-09	Sep-09	Nov-09	Jan-10
Site	Tree Species	% Flowering (N)	% Flowering (N)	% Flowering (N)	% Flowering (N)	% Flowering (N)	% Flowering (N)
M	T.u	50(2)	50(2)	0(5)	0(5)	0(5)	60(5)
	T.u.h	38(8)	11(9)	0(22)	0(22)	0(22)	5(22)
WC	T.u	91(11)	87(15)	87(23)	0(39)	0(40)	56(39)
	T.u.h	73(11)	63(46)	27(45)	0(69)	0(70)	11(66)
	T.r	100(8)	100(15)	0(15)	0(15)	73(11)	Removed
	T.r.h	100(2)	100(25)	0(25)	0(25)	75(4)	Removed

N is the total number of trees measured for flowering.

4.4.1 Results for flowering in *Tamarix usneoides* (T.u) and *T. usneoides* hybrid (T.u.h)

The presence of flowering was greater in T.u and T.u.h plants at the WC site than at the M site. A greater number of flowering individuals were recorded in trees sampled at the WC site in January 2009, than at the M site, for both T.u and T.u.h.

Flowering was more abundant in T.u than in T.u.h trees in the months of January,

March and May of 2009 at WC site and in January and March 2009 at the M site.

At the M site T.u had a greater proportion of flowering individuals in January 2010 than in January 2009 (Table 4.2).

4.4.2 Results for flowering in *Tamarix ramosissima* (T.r) and *T. ramosissima* hybrid (T.u.h)

In November 2009, which is early in the flowering season, flowering was lower (73%) for T.r and T.r.h (75%), compared to later in the season. In January and March 2009, 100% of the trees sampled were flowering for T.r and T.r.h. No flowering took place in May and September 2009 (Table 4.2).

At WC, site conditions of rocky waterlogged and highly polluted soils are similar in the cultivated and wild populations, yet the incidence of flowering was greater in T.r and T.r.h, found in the wild population, than in T.u and T.u.h found in the cultivated plots, even in January when their flowering periods overlapped.

4.4.3 Site Differences in the occurrence of flowering

Flowering overlapped at M and WC sites for three months of the year, in January, February and March 2009 (Table 4.2). The number of flowering trees in the cultivated plots was significantly different between the M and WC sites in March ($t=3.225$, $df=70$, $p<0.001$) as well as in May, when no flowering was recorded at the M site ($t=5.025$, $df=95$, $p<0.000$). There were a significantly greater number of flowering individuals (over all three months) at the WC site. Sample sizes for plant taxa (*T. usneoides*, *T. usneoides* hybrids, *T. ramosissima, T. ramosissima* hybrids) were very small, and data could not be pooled for the sites to attain adequate sample sizes for statistical comparison between the plant taxa. As such, further testing for species differences in flowering could not take place.

No flowering in *Tamarix* spp. took place in the winter months from June to September. At the study site, the flowering period for T.r and T.r.h is between November and March (5 months), and for T.u and T.u.h between January and May (5 months), with difficulty finding flowers during May. Both (pure) *T. usneoides* and *T. ramosissima* flowered for five months of the year (Table 4.2).

4.5 INSECT ABUNDANCE AND DIVERSITY

4.5.1 Insect abundance in *Tamarix* trees

A T-test was used to compare the total numbers of insects on *T. usneoides* (T.u) and *T. usneoides* hybrid (T.u.h) in January, March and May 2009. N indicates the number of trees sampled.

At the M site, no significant differences were recorded in the number of insects collected on T.u (N=4) and T.u.h (N=12) (t=0.977, df=14, p=0.345) in January. Significant differences were recorded between T.u (N=7) and T.u.h (N=25) in March (t=2.570, df=30, p=0.015), with a greater number of insects recorded on T.u.

A T-test was used to compare the total numbers of insects on *T. usneoides* (T.u) and *T. usneoides* hybrid (T.u.h) at the WC site, collected in January, March and May 2009 using the TSW method. In January, T.u had a small sample size of trees (N=1) and could not be statistically compared to T.u.h (N=23). In March, there were no significant differences (p=0.608) in the number of insects collected on T.u (N=4) and T.u.h (N=29). In May, there were no significant differences (p=0.125) in the number of insects collected on T.u (N=11) and T.u.h (N=6).

An ANOVA was used to compare the total numbers of insects on *T. usneoides*

(T.u), *T. usneoides* hybrid (T.u.h), *T. ramosissima* (T.r) and *T. ramosissima* hybrid (T.r.h) in January and March at the WC site. In January, there were significant differences (p=0.00) in the number of insects, between T.u (N=6) and T.r (N=7), with a greater number recorded on T.u, as well as between T.r and T.r.h (N=3) (p=0.015), with a greater number recorded on T.r.h. In March, there were no significant differences (p=0.44) between trees (Fig 4.6)

A T-test was used to compare the total numbers of insects on *T. usneoides* (N=9, 24.444±21.70) and *T. usneoides* hybrid (N=6, 40.166±20.79) at the WC site in May, sampled with the EMB method. No significant differences in the total number of insects collected were recorded (t=1.39, df=13, p=0.186) (Fig 4.6).

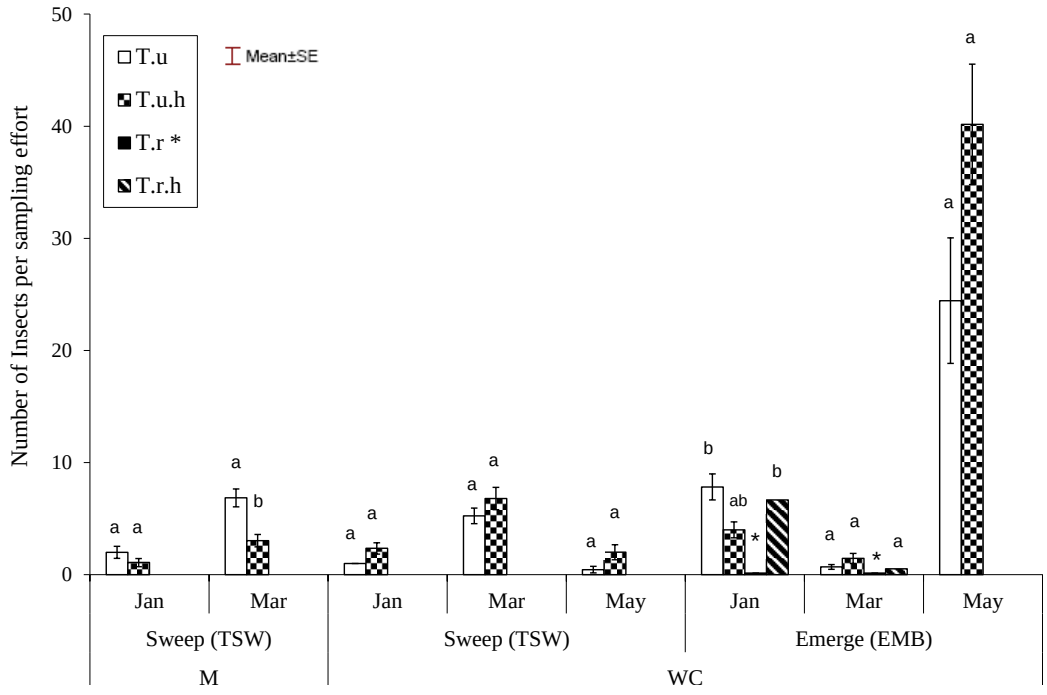


Figure 4.6, Insect abundance (Number of Insects) recorded per sampling effort for *T. usneoides* (T.u), *T. ramosissima* (T.r) and their respective hybrids at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations, during three sampling months. * Indicates the presence of T.r where insect numbers were low. Letters on graph denote significant differences between species. EMB is the Emergence Box Method and TSW is the Tree Sweep Method. A T-test was used to compare insect abundance between T.u and T.u.h. An ANOVA compared insect abundance between the four plant types (T.u, T.u.h, T.r and T.r.h). T.r and T.r.h do not occur at the Mispah site.

Overall there were differences in insect abundance between the months, particularly in January and March, at both M and WC sites. Insect abundance was highest in March. *Tamarix usneoides* at the M site recorded more insects than T.u at the WC site in both months. Insect collections on T.u.h recorded a greater number of individuals at WC site than at M site in January and March. Large insect numbers were recorded in May at the WC site. The trees did not flower in May at the M site (Fig 4.6).

4.5.2 Insect diversity in *Tamarix* trees

A T-test was used to compare the number of morphospecies collected on *T. usneoides* (T.u) and *T. usneoides* hybrid (T.u.h) at the M site during January and March 2009 using the TSW method. There were no significant differences recorded in the number of insect morphospecies on T.u (N=4) and T.u.h (N=12) (t=0.767, df=14, p=0.455) in January. Significant differences were recorded between T.u (N=7) and T.u.h (N=25) in March (t=2.315, df=30, p=0.028), with a greater insect diversity on T.u (Fig 4.7). N indicates the number of trees sampled.

A T-test was used to compare the number of insect morphospecies collected on *T. usneoides* (T.u) and *T. usneoides* hybrid (T.u.h) at the WC site in January, March and May 2009. These data were collected using the TSW method. In January, T.u had a small sample size (N=1) and could not be statistically compared to T.u.h (N=23). In March, when sample sizes were larger and adequate for statistical comparison; there were no significant differences (t= -0.918, df=31, p=0.365) in the number of insect morphospecies on T.u (N=4) and T.u.h (N=29). In May, there were no significant differences (t=1.097, df=15, p=0.290) in the number of insect morphospecies on T.u (N=11) and T.u.h (N=6) at the WC site (Fig 4.7). N indicates the number of trees sampled.

An ANOVA was used to compare the number of insect morphospecies on *T. usneoides* (T.u), *T. usneoides* hybrid (T.u.h), *T. ramosissima* (T.r) and *T.*

ramosissima hybrid (T.r.h) in January and March 2009 using the EMB method. In January, there were significant differences (p=0.00) in the number of insect morphospecies on T.u (N=6) and T.r (N=7), on T.r and T.r.h (N=3) (p=0.00), on T.r and T.u.h (N=4) (p=0.00) and on T.u and T.u.h (p=0.004) with a greater insect diversity on T.u, T.u.h and T.r.h than on T.r. In March, there were no significant differences between trees (Fig 4.7).

A T-test was used to compare the number of insect morphospecies on *T. usneoides* (T.u) and *T. usneoides* hybrid (T.u.h) at the WC site in May. No significant differences in insect diversity were recorded (t=0.594, df=13, p=0.563).

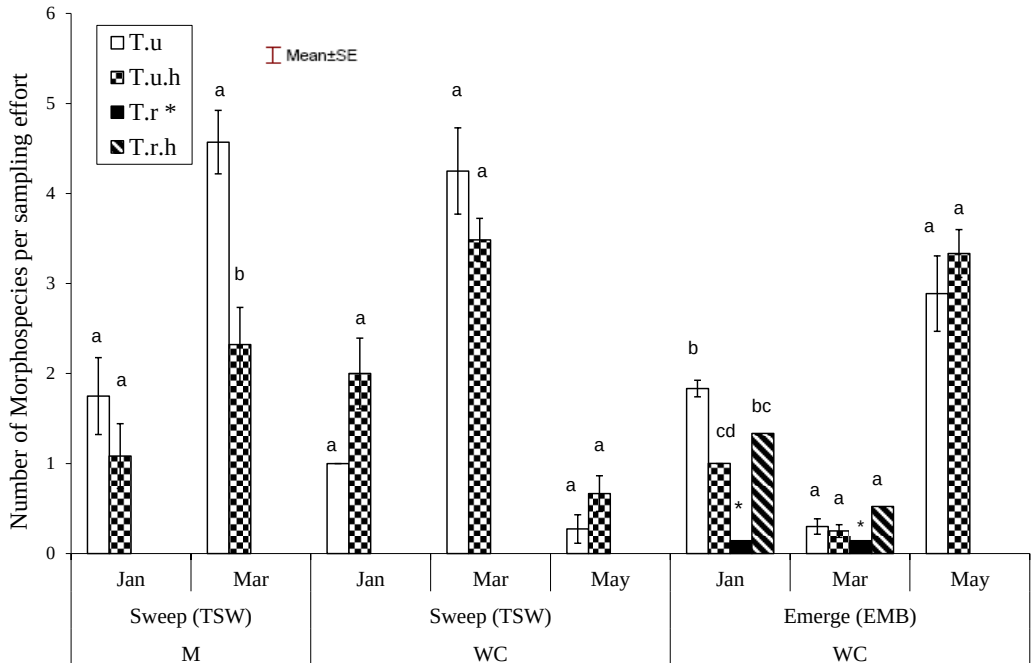


Figure 4.7, Insect diversity (Number of Morphospecies) per sampling effort was recorded for *T. usneoides* (T.u), *T. ramosissima* (T.r) and their respective hybrids at Mispah (M) and West Complex (WC) sites, Vaal River Mining Operations, during three sampling months. * Indicates the presence of T.r where insect numbers were low. Letters on graph denote significant differences between species. EMB is the Emergence Box Method and TSW is the Tree Sweep Method. A T-test was used to compare insect diversity between T.u and T.u.h. An ANOVA compared insect diversity between the four plant types (T.u, T.u.h, T.r and T.r.h). T.r and T.r.h do not occur at the Mispah site.

Overall there are differences in the number of insect morphospecies between M and WC sites in January and March 2009, with a greater number of insect morphospecies (diversity) at the WC site. However, T.u at the M site recorded a greater number of insect morphospecies than T.u at WC in January and in March. The number of insect morphospecies on T.u.h was larger at the WC site than at M site in January and in March. A large number of insect morphospecies were recorded in May at the WC site (Fig 4.7).

4.5.3 Comparison of insect abundance and diversity at Mispah (M) and West Complex (WC) sites in January and March only

The number of insects (abundance) and number of morphospecies (diversity) were statistically compared between M and WC in January (summer) and March (autumn) when tree flowering overlapped, using an ANOVA to establish if there were site and seasonal differences in insect occurrence.

The number of insects ($F=8.697$, $df=3$, $p=0.00$) and the number of morphospecies ($F=6.42$, $df=3$, $p=0.00$) were greater at the WC site than at the M site in January and March. LSD results indicated significant site and seasonal differences in the number of insects. There were seasonal differences between January and March at the M site ($p=0.037$), and between January and March at the WC site ($p=0.00$), as well as significant differences (over both months) between the M and WC sites ($p=0.00$) at 95% CI. There were significant differences in the number of morphospecies between January and March at the M site ($p=0.01$), and at the WC site ($p=0.002$), and significant differences for both months (combined) between the M and WC sites ($p=0.00$) (Fig 4.8).

The insect abundance and diversity was consistently greater at the WC site than at the M site, with greater insect abundance and diversity in March than in January at both sites.

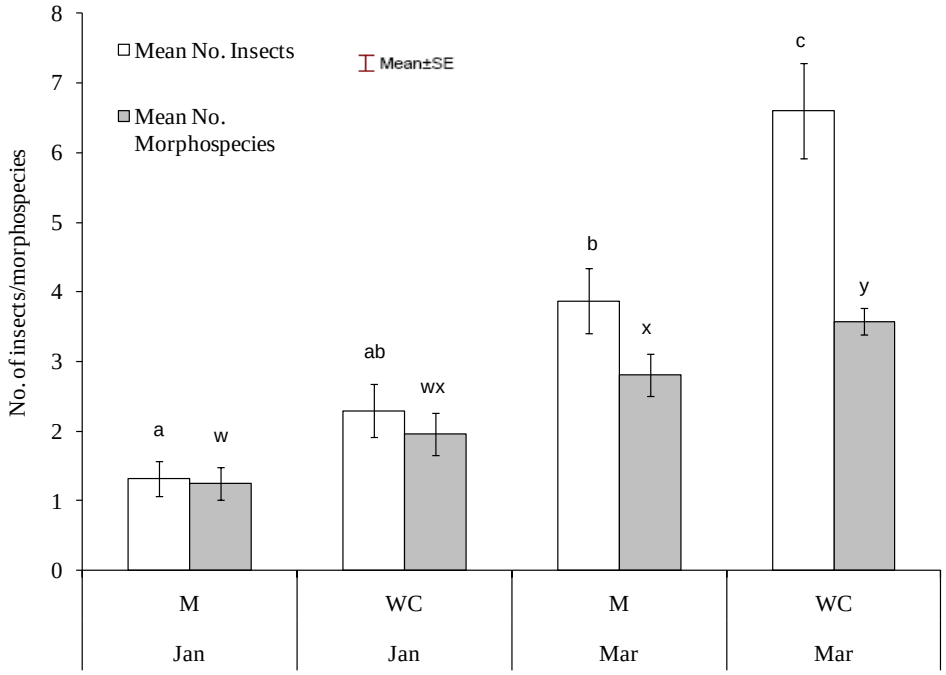


Figure 4.8, Comparison of the mean insect abundance (Number of Insects) and diversity (Number of Morphospecies) (of all plant taxa combined) at Mispah (M) and West Complex (WC) in January and March, in cultivated plots at Vaal River Mining Operations. The letters (a,b,c, w,x,y) indicate significant differences in the number of insects and morphospecies across all sites and months.

Species accumulation curves were plotted for *T. usneoides*, *T. ramosissima* and their hybrids to compare insect richness, diversity and the rate of species accumulation, in order to reveal the plant-insect interactions on the different plant taxa. Saturation was not reached on any of the curves indicating that trees were undersampled (Fig 4.9).

As the Confidence Intervals (CI) overlap for T.u and T.u.h, the insect morphospecies collected on these tree taxa are similar. However, these CI do not overlap with T.r and T.r.h indicating that the insect morphospecies on T.u and T.u.h are significantly different from T.r and T.r.h (Fig 4.9).

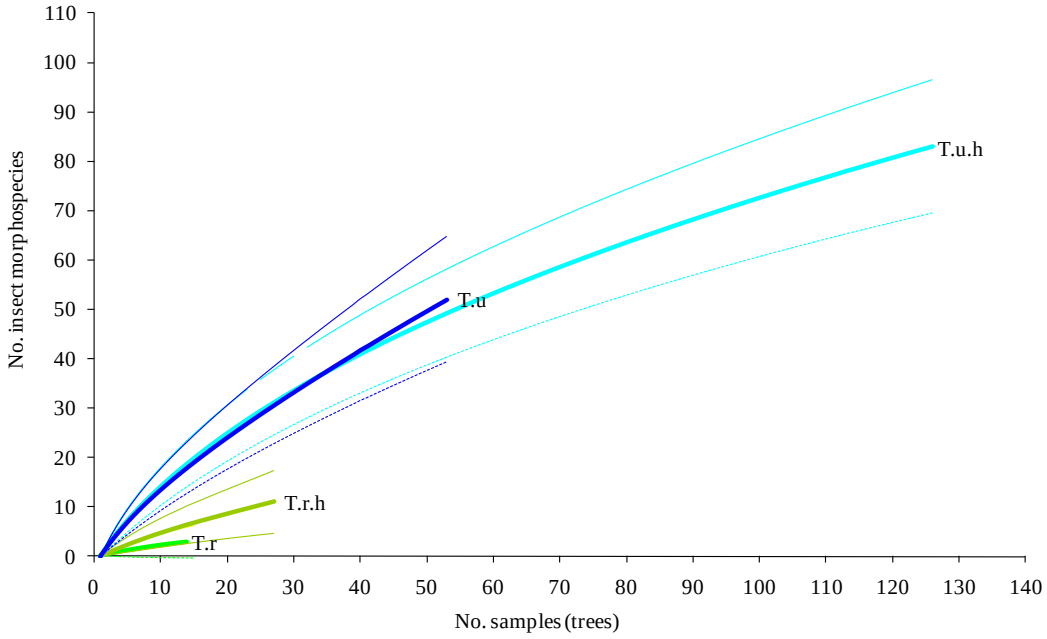


Figure 4.9, Species accumulation curve comparing the number of morphospecies on tree taxa sampled at the West Complex (WC) and Mispah (M) sites (data combined) at Vaal River Mining Operations, on *T. usneoides* (T.u), *T. ramosissima* (T.r) and their hybrids (T.u.h and T.r.h). Thick solid lines indicate the plant types (plotted with real data), with upper solid lines indicating 95% Confidence Interval and lower dashed lines indicating 95% Confidence Interval.

The number of morphospecies (diversity) is greater on T. u and T.u.h than on T.r and T.r.h. After 10 trees had been sampled for each tree type, the insects recorded were as follows T.u.h (14) > T.u (13) > T.r.h (5) > T.r (2), with T.u.h and T.u yielding considerably more morphospecies than T.r and T.r.h. The curve for T.u.h starts to stabilise at around 120 samples whilst the curve for T.u is still increasing at around 50 samples. This indicates that further sampling probably would yield new insect morphospecies for T.u and possibly T.u.h. The curve for T.r.h is still increasing but is reaching equilibrium, whilst the curve for T.r has already levelled indicating that further sampling may yield a few new individuals for T.r.h, but is unlikely to yield many new individuals on T.r (Fig 4.9).

4.5.4 Identity of insects collected from *T. usneoides, T. ramosissima* and their respective hybrids

A total of five Orders, two Sub-Orders, eight Families and five Species were recorded during sampling.

Of the insects identified only *Gullifera* sp. (Hemiptera, Cicadellidae) and *Anoplolepis custodiens* Smith. (Hymenoptera, Formicidae) yielded less than ten individuals (Table 4.3). Identification of Miridae beyond family was impossible as there is no local specialist in South Africa. Insects identified to family, genus and species levels were ranked in terms of their abundance in the field. All insects that appear in Table 4.3 were recorded at the WC site, as all insect morphospecies at the M site yielded less than five individuals.

Of the insects identified to species level, one insect *Opsius stactogalus* Fieber (Hemiptera, Cicadellidae) is a specialist herbivore associated specifically with Genus *Tamarix* (Watts et al., 1977; Harding, 1930; Brock, 1994).

Table 4.3, Insect identities and abundance (>5 individuals) that were collected during January, March and May 2009 at the West Complex site (WC), Vaal River Mining Operations. None of the listed insects were collected at the Mispah (M) site.

Order	Sub Order	Family	Species	Common Name (Abundance Ranking)	Information and Economic Importance (References)
Hemiptera (bugs)	Homoptera	Cicadellidae	*Opsius stactogalus* (Fieber)	Leafhopper (5)	Cosmopolitan species usually associated with *Tamarix* (A)
		Cicadellidae	*Gullifer sp.*	Leafhopper (10)	Phytophagous insect. Wide distribution. A pest of economic significance in agriculture (potatoes, maize) (A)
Hemiptera (bugs)	Heteroptera	Miridae (1)		(2)	No local specialist for this family in South Africa. Miridae occurs on many plants.
		Miridae (2)		(8)	
		Miridae (3)		(1)	
		Miridae (4)		(7)	
		Miridae (5)		(4)	
Hymenoptera (ants)		Formicidae	*Anoplolepis custodiens* (Smith)	Pugnacious Ant (10)	Diverse habitats, seed dispersers (B)
Coleoptera (beetles)		Melyridae: Melyrinae	*Astylus atromaculatus* (Blanchard)	Spotted Maize Beetle (6)	Adults eat pollen. Found clustering on pollen. Introduced from South America. Now an agricultural pest.
Lepidoptera (moths)		Nolidae: Sarrothripinae	*Characoma submediana* (Wiltshire)	(3)	Small to medium sized moths. Inadequately studied (C)
Diptera (flies)		Chironomidae		(12)	Abundant in all habitats (B)
		Cecidomyiidae		(9)	Cause galls in host plants, widespread distribution (B)

NB. 1-Most abundant, 12-least abundant (A) Agricultural Research Council, 2010. (B) Picker *et al.* 2002, (C) Yshistjakov, 2008.

CHAPTER 5:

DISCUSSION OF TREE GROWTH, REPRODUCTIVE POTENTIAL AND INSECT OCCURENCE ON *TAMARIX* SPECIES

5.1 INTRODUCTION

Tree growth is reliant on several site and environmental factors such as soil moisture, microclimatic conditions and soil texture which affect radial and vertical growth in trees (Robertson, 1992). Size of tree (in an unburnt plot) is an indicator of growth, and also relates to tree reproductive capacity. This research investigated different aspects related to the biology of *Tamarix* growing on a Highveld gold mine (including the effect of site conditions on tree growth and reproductive potential, morphological identification of *Tamarix* trees, hybridisation, plant-insect interactions and the identity of insects associated with *Tamarix*).

The aims were:

(1) to establish if site conditions have an effect on *Tamarix* tree growth and incidence of flowering (as a surrogate measure of reproductive effort), in order to deduce on which site types *Tamarix* has potential as a phytoremediation plant, (2) to investigate if the release from natural enemies is potentially increasing the invasiveness of *T. ramosissima* in support for a biological control approach for alien *Tamarix*, (3) to investigate if the flowering frequency differed between *Tamarix* species as a means of discerning between them, (4) to find a method of morphological identification between *Tamarix* species and hybrids, and (5) to identify *Tamarix* trees to species, growing on a Highveld gold mine for removal purposes.

5.2 *TAMARIX* TREE IDENTIFICATION AND HYBRIDISATION

Controlling the spread of *Tamarix* as an IAP, conserving biodiversity and minimising the impacts on water resources all rely on accurate identification of *Tamarix* trees at the species level (Gaskin, 2003). Conversely, a successful and effective phytoremediation programme using *Tamarix usneoides* also requires accurate species identification. This is necessary for two reasons; firstly an unskilled labour force is used to mechanically and chemically remove IAPs, through the government Working for Water (WfW) initiative. These teams are in need of an easy morphological method of plant identification. Secondly, other means of identification, such as genetic methods are costly, time consuming and impractical where quick tree identification is needed.

This research investigated the morphological characters of *Tamarix* spp. using relevant literature to find characteristics that are consistent in local *Tamarix* trees at a species level. After sampling 217 trees in this study, the three characteristics of, (1) insertion of filaments into the nectar disc, (2) petal shape and (3) presence and abundance of salt glands on leaves most accurately distinguished between *T. usneoides* and *T. ramosissima* individuals, and these results coincided with insect abundance and diversity data.

Tamarix chinensis and *T. ramosissima*, both widespread in South Africa, have a very similar morphology which makes it difficult to distinguish between them (Gaskin and Schaal, 2002; Brock, 1994). This raises concern that plants sampled in this study are *T. chinensis* individuals instead of *T. ramosissima* individuals (Lesley Henderson, South African National Botanical Institute (S.A.N.B.I), pers. comm.). To address this concern, examples of morphological differences between *T. chinensis* and *T. ramosissima* were sought. The key species differences are in; (1) the halophilous nature of *T. ramosissima* which is not evident in *T. chinensis*, (2) the shape of filaments inserted into the nectar disc of inflorescences, and (3) the petal shape (*T. chinensis* is ovate and *T. ramosissima* is obovate) (Gaskin and

Schaal, 2002). *Tamarix chinensis* was not recorded in the study site.

Trees were identified as *T. usneoides* and *T. usneoides* hybrid in the cultivated plots and as *T. ramosissima* and *T. ramosissima* hybrids in the wild population, with the majority of individuals identified as hybrids in the "wild" population and in the "cultivated" plots at both sites. Hybridisation is extensive (>50%), although greater in the "cultivated plots" than in the "wild population". As cultivated plots had similar proportions of hybrid (± 80%) and pure (± 20%) trees at the M and WC sites, tree growth could be compared between the two sites to see if it varied with soil conditions.

Tamarix ramosissima and *T. ramosissima* hybrids are absent in the "cultivated plots" (were not planted). This is because trees that resembled *T. ramosissima* at Upington were actively avoided for cloning (Isabel Weiersbye, University of Witwatersrand, pers comm.). The cultivated trees are F_1 (first generation) clones from selected white flowered "*T. usneoides*" trees from Upington, within the native home range of *T. usneoides* in southern Africa. This study found that *T. usneoides* and *T. usneoides* hybrids are similarly absent in the "wild population" at the WC site, probably because *T. usneoides* has only recently started flowering (in 2008), and hybridisation between the cultivated and wild plants has not yet occurred. Barriers to hybridisation could result from different flowering periods between the species as *T. usneoides, T. ramosissima* and their hybrids flower during specific months. The flowering time of *T. usneoides* and their hybrids may not have coincided with sampling time in the "wild population", increasing the likelihood that *T. ramosissima* and *T. ramosissima* hybrids were randomly selected from flowering plants. *Tamarix ramosissima* and *T. ramosissima* hybrids also reproduce vegetatively (Gaskin and Schaal, 2002; Brotherson and Field, 1987) and vigorous suckering has been observed from plant fragments on Highveld gold mines (Isabel Weiersbye, University of Witwatersrand pers comm.). This increases the likelihood that individuals in the "wild population" are *T. ramosissima* or *T. ramosissima* hybrid through vegetative reproduction.

There are implications for transgressing legislation, as *Tamarix* in the "cultivated

plots" was regarded to be indigenous *T. usneoides*, and not listed as *T. usneoides* hybrid in the permit granted to the Mine Woodlands Project for the planting of alien trees. It is recommended that the *T. usneoides* hybrids identified through this study are now felled and replaced with "pure" *Tamarix usneoides.*

The negative biodiversity impacts of alien species introductions on native populations, is a well documented problem (Gaskin, 2003; Catford *et al.*, 2009). The exclusion of native vegetation by changing soil properties, occupying niche space, and allelopathy are some of the strategies that IAPs use to dominate an area (Pyšek and Richardson, 2007). These threats are increased by hybridisation which brings previously isolated genotypes into contact with each other, increasing their potential to mate and dilute or genetically assimilate native genotypes, and drive the extinction of "pure" native plants (Huxel, 1999).

Through hybridisation, there may be continuous selection for non-native alleles leading to more rapid displacement of native alleles and loss of genetic diversity. There are a number of examples where native species (and their alleles) have diminished or become extinct due to hybridization. In the USA, *Spartina alterniflora* (Poaceae) an alien plant species is hybridising with native *Spartina foliosa*, creating an invader with higher pollen output and greater male fitness than the native species. These hybrids now completely occupy lower intertidal habitats in the San Francisco Bay (Anttila *et al.*, 1998).

Other examples of (potential) extinction caused by hybridization include the endemic shrub *Cercocarpus traskaei* through hybridisation with the widespread *Cercocarpus betuloides,* and the endangered Lotus *scoparius traskiae* which is being threatened by hybridisation with the Lotus *argophyllus* o*rnithopus*. Rare species, with small populations are most in danger of extinction from hybridisation (Levin *et al.*, 1996).

A similar fate may befall *Tamarix usneoides* in South Africa if "pure" genotypes are not identified and protected, particularly as *T. ramosissima* and hybrids are also present in *T. usneoides* native home range at Upington (Obermeyer, 1976), and hybrids are being spread around the Highveld on gold mines. This study

recommends that only "pure" *Tamarix usneoides* (which now comprise 20% of the individuals in cultivated plots) are selected for future cloning exercises, and research effort is dedicated to biological control agents.

5.3 *TAMARIX* PLANT GROWTH

Plant development is influenced by a complex variety of factors. Most plants rely on a balance of resources such as water, energy, and mineral nutrients which is very rarely achieved across landscapes that differ in resource availability (Chapin *et al.*, 1987). Consequently living organisms have to regulate their activities in accordance with resource availability in their surroundings, and in plants this is achieved by changing growth patterns (Raven *et al.*, 1986).

It is also known that plants respond to resource imbalances by allocating new biomass to organs that acquire the most limiting resources (Chapin *et al.*, 1987). Although the *Tamarix* trees are cultivated and planted equal distances apart (2.5m x 3.0m), the trees at the WC site are generally taller, ranging between 3m and 7m, than trees at the M site, which ranged between 0.5m and 7m in 2009 (Fig 4.4). The trees at the WC site are all similar in height and tree percentage survival has been greater than at the M site, meaning more trees are present in the cultivated plots at the WC site. In the four plots sampled per site, average tree percentage survival to date at the M site was 23%, whereas average tree survival at the WC site was 99%. As there are more trees at the WC site, the total wood volume (recorded in 2009) of 78.50m^3 (N=18 trees) for this site is greater than the total wood volume at the M site, 53.67 m^3(N=18 trees).

Space and shade intolerance may be factors that limit tree canopy area and volume in plots at the WC site, as tree percentage survival and density is greater. In terms of competition for space, a logical expectation is that an increase in height is consistent with an increase in canopy area, canopy volume and wood volume, provided no resources are limited. As trees at the WC site are growing at a similar

rate in each plot, they are increasing in height, growing towards the light, as well as laterally in their canopy size. The trees are therefore very tall with sizeable canopies. Above ground, trees primarily compete for space and light (Shirley, 1945), and horizontal growth can take place until a tree is competitively limited by an adjacent tree for crown space (Shirley, 1945). A tree will then be forced to grow upwards where there is no competition for space. This is known as self thinning and occurs in communities where tree density is great (Long and Smith, 1983). At the M site, where space and shading effects are probably not limiting plant growth, due to the higher percentage mortality of trees at an early age, greater resources can be allocated to canopy area and canopy volume. In turn the trees have a shrubbier canopy appearance, unlike the tall tapering "christmas" trees observed at the WC site over the growth period.

In a study conducted on shade tolerance of Box Elder and Tamarisk trees (particularly *Tamarix ramosissima, T. chinensis, T. gallica* and hybrids) in the USA, Tamarisk was found to be intolerant of shading effects which led to increased plant mortality and a reduction in growth, particularly in plant canopy area (Dewine and Cooper, 2008). These shading effects may account for the smaller canopy area and canopy volume of trees at the WC site.

Also, the planting difference of one year between sites may have given *Tamarix* trees at the M site a growth advantage, as results for similar aged trees indicate that canopy area and canopy volume are greater at the M site compared to the WC site (Fig 4.5). To address the difference in planting time, canopy area, canopy volume, wood volume and height growth of similar aged *Tamarix* trees were compared (at one stage of growth). There were significant differences between sites in wood volume and plant height, with both being greater at the WC site. This is expected as a greater wood cross-sectional area and volume is anticipated for taller trees to provide mechanical support. As *Tamarix* species are halophytes and known to be adapted to saline environments (Di Tomaso, 1998), it is expected that growth would be better at the WC site which is more polluted and in constant receipt of saline seepage from the West Extension and West Complex TSFs. The M site also has constant subterranean saline seepage from the Mispah TSF, but the

soils have better drainage and are therefore much drier (Herbert, 2008). Based on these findings and the use of *Tamarix usneoides* to remove salts through canopy biomass, future planting should focus on cloning trees with sizeable canopies (high leaf area indices) that are tolerant to the harsh conditions at the WC site.

The fire at WC site in August 2008 may have also influenced the canopy area and canopy volume data collected in 2009, as the tree canopies were damaged, and trees were only starting to recover in 2009. Fire is often regarded as a stabilising factor affecting the aerial parts of trees, preventing growth and controlling tree density (Gillon, 1983; Dereix and N'Guessan, 1976; Brookman-Amissah *et al.*, 1980; Olla-Adams and Adegbola, 1982). When above ground shoots are subjected to fire damage, vegetation structure is maintained by bringing individuals back to a previous stage of growth and restricting trees at a certain height. However, trees that grow tall enough are able to escape damage from fire (Trollope, 1984). In Guinea, Menaut *et al.* (1990) found that fire may change growth parameters like the size of the individuals and density of the community, but does not affect survival, tree distribution and community structure if trees are tall.

Vegetation responses of *Pinus* and *Larix* spp. in disturbed mined sites in South Wales, United Kingdom, varied in response to site differences (particularly differences in soil type and levels of soil pollution) with large tree sizes and canopies on some sites and poor, stunted growth in other sites (Bending and Moffat, 1999). In that study, the researchers concluded that water logging, anaerobic soil conditions, landform design, soil forming materials and spoil placement techniques affected tree growth. Low soil pH and extractable magnesium negatively impacted growth, and high nitrogen and phosphorus concentration increased plant growth (Bending and Moffat, 1999). Site conditions are therefore expected to impact *Tamarix* plant growth at the Vaal River Mining Operations. Although specific site conditions were not measured in this study, Herbert (2008) described conditions as ranging from marginal to harsh on gold mines due to low precipitation, high temperatures, periodic frost and hail, steep slopes and polluted, saline water seepage. While plant requirements for establishment and growth vary between species, factors like phosphorus

availability, aluminium toxicity, drainage, water-holding capacity, and availability of potassium, calcium, and magnesium most likely influence species composition and plant growth, although drainage and water requirements may also affect the establishment of individual species (Sollins, 1998).

As *T. usneoides* and the hybrid are halophytes and paradoxically survive and grow well at the WC site, salt concentration and moisture appear to be the resources that favour plant growth. It is therefore confirmed that saline, moist sites are suitable for *T. usneoides* planting on gold mines.

5.4 FLOWERING IN *TAMARIX* TREES

Plants can reproduce sexually or asexually (vegetatively). Reproduction is usually measured as sexual reproduction in plants, as asexual reproduction is difficult to measure without reproductive structures (Harper, 1977). Botanists have used different methods to determine the reproductive effort of plants. However, the flowering output, which is the production of floral parts in a plant, is an easy and accurate method of recording the total reproductive effort of a plant (Thompson and Stewart, 1981). *Tamarix*, an angiosperm, bears flowers which aggregate to form inflorescences and produce a determinate shoot that bears sporophylls (seeds) (Raven *et al.,* 1986).

Both *T. usneoides* and *T. ramosissima* flowered for a five month period with *T. ramosissima* flowering earlier in the growing season. The flowering period is between January and May for *T. usneoides* and *T. usneoides* hybrid, and between November and March for *T. ramosissima* and *T. ramosissima* hybrid. In its native home range, in southern Africa (Northern Cape and Namibia), flowering on *T. usneoides* occurs in February and March with rare flowering events occurring from September to January and April to June (Curtis and Mannheimer, 2005). Baum (1978) recorded flowering in *T. usneoides* to take place between July and October and occasionally in January, February and May. This suggests that it can

flower at any time of the year. Little information has been recorded on *T. ramosissima* flowering in southern Africa, although it is recorded to flower from May to October in Eastern Europe, Middle East and Asia (Baum, 1978), which is during the summer and autumn months in southern Africa.

The reproductive effort (flowering frequency) is consistently greater in *T. ramosissima* and *T. ramosissima* hybrid than *T. usneoides* and *T. usneoides* hybrid at the WC site (where all plant types occur). As IAPs have different strategies which confer a competitive advantage in their new home range, it is expected that the earlier flowering time (starting in November) and longer period of flowering (100% from January to March) in *T. ramosissima* facilitates high seed output. Richardson and Cowling (1992) found that species with high seed outputs are more invasive. This is potentially troublesome for the spread of *T. ramosissima* in South Africa, as in the USA Forcella (1985) found that *T. ramosissima* is a prolific seed producer and this has contributed to making it an aggressive invader, and the second most important IAP in the USA. Seeds can lay dormant for up to one year if conditions are appropriate, and germinate quickly if they encounter moisture (Everitt, 1980; Warren and Turner, 1975).

As reproductive effort was lower at the M site (for *T. usneoides* and *T. usneoides* hybrid) than the WC site, this indicates that site conditions may be having an effect on flowering. The flowering period is shorter at the M site lasting only three months compared to five months at WC site. Ahlgren (1960) found a correlation between plant reproduction by seed or spores and plant location on high moisture soils, indicating that moisture may be a requirement for flowering and seed production. As there are several factors such as light, moisture, temperature and soil nutrients affecting reproduction and growth in plants, differences in site conditions (soil type, moisture content and pollution levels) may be supporting flowering at WC site and hindering flowering at M site. As Tamarisk in the USA has demonstrated flowering in spring and summer following a rainfall event, and seeds germinate with 24 hours of imbibition (Brock, 1994; Horton, 1960), it appears that the presence of moisture is a requirement for reproduction or for flower development.

As sexual reproduction in out-breeding populations increases genetic diversity, it is recommended that pure *T. usneoides* which is capable of flowering should be cloned instead of other successful trees. This will ensure abundant seed production by *T. usneoides* trees which can be dispersed into new areas, allowing the species to proliferate on gold mines and in surrounding areas. Recruitment in *T. usneoides* also relies on adequate visitation by pollinators. *Tamarix usneoides* recorded a high number of insects although none of the abundant insects are specific pollinators of *T. usneoides.* Native bees are amongst the most important pollinators of native plants. Shmida (1991) and Free (1993) found that if bees are lured away from native vegetation by alien species, natives experience declines in fruit and seed production and finally in their recruitment (Totland et al. 2006). This further motivates the removal of alien *T. ramosissima* and hybrids, to promote insect pollination in *T. usneoides*, and facilitate the proliferation of this species. This will likely assist to address the problem of diminishing *T. usneoides* alleles in populations and curb the potential extinction of *T. usneoides.*

5.5 INSECT ABUNDANCE AND DIVERSITY IN *TAMARIX* TREES

The Insect Abundance (number of insects) and Diversity (number of morphospecies) were recorded and compared between the M and WC sites, to establish if the presence of insects was similar between the sites. Insect abundance and diversity between *T. usneoides*, *T. ramosissima* and their respective hybrids was also investigated to test if the release from natural enemies could be promoting the invasiveness of *T. ramosissima*. Abundant insects were identified with the aim of finding insect species that potentially inflict damage on *Tamarix* trees and impact growth and/or reproduction.

The insect abundance and diversity was consistently higher at the WC site than at the M site (Fig 4.8). During January and March when only *T. usneoides* and *T. usneoides* hybrids were sampled using the same collection method, insect

abundance and diversity was greater at WC site than at M site in January, and significantly greater in March. This finding indicates that insects prefer trees at the WC site to trees at the M site, possibly because there is a better percentage tree survival and bigger trees at the WC site, which are more attractive to insects and supports higher population numbers.

This difference in insect abundance and diversity may also be attributed to the lengthier flowering period at the WC site (January to May) compared to the M site (January to March). As the number of pollinators increases throughout the flowering season, abundance will be higher on late-flowering plants (Hegland and Totland, 2005). McCall and Primack (1992) found that late flowering species had higher insect visitation frequencies than early-flowering species. The WC site recorded a larger proportion of flowering plants with a longer flowering period than M site which would theoretically support a larger insect abundance. Rice *et al.*, (1984) have found that tree density determines faunal (bird and insect) abundance and diversity, with higher numbers where tree density is greater. This supports the results of higher insect diversity and abundance at the WC site as tree survival, density and height is greater at this site. Samways *et al.* (1996) also found that tall indigenous vegetation had significantly more insect species than shorter indigenous vegetation, supporting this finding.

There were also distinct seasonal differences in insect presence, with a greater number of insects and morphospecies (abundance and diversity) recorded in March than in January at both sites. This may be the result of increasing insect numbers throughout the spring/summer season (Hegland and Totland, 2005). This suggests that any further insect sampling exercises on *Tamarix* spp. should concentrate on dense communities of flowering trees, later in the spring/summer season, to acquire the most accurate account of plant-insect interactions.

Using natural enemies to contain the spread of IAPs has been practised in South Africa to control detrimental weeds like *Lantana camara* L. (Baars and Neser, 1999). At times insect biological control agents occur naturally with the weed, through simultaneous introduction into the new home range. In the case of *L.*

camara, two biological agents; *Epinotia lantana* (Busck) and *Lantanophaga pusillidactyla* (Walker) are thought to have been introduced with the weed into South Africa (Baars and Neser, 1999). However, at other times, biological agents need to be researched and collected from the native home range of a species.

Three individual species have been identified from this study as potentially damaging agents on *Tamarix* spp. *Characoma submediana* Wiltshire. (Lepidoptera, Nolidae) was recorded emerging from *Tamarix* inflorescences in the "cultivated plots" at the WC site, which are composed of *T. usneoides* and *T. usneoides* hybrid trees, with a few individuals emerging from the *T. ramosissima* hybrids in the "wild population". *Characoma submediana* is a generalist feeder, predating at least two plant species in South Africa. The larvae of the same *C. submediana* were found to feed on the seeds, fruit and flowers of *L. camara*, with extensive feeding causing the flowers to abort, thereby inhibiting reproduction (Baars, 2003). High numbers of *Opsius stactogalus* Fieber. (Hemiptera, Cicadellidae) were also discovered on *Tamarix* spp. This leaf hopper which is thought to be native to Europe, appears to be specific to *Tamarix* spp. in the USA (Watts *et al.*, 1977; Harding, 1930; Brock, 1994), particularly on *T. gallica* and *T. africanus* (now *T. usneoides*), as well as on *T. ramosissima* (Durst *et al.*, 2008). Different observations have been made regarding its impact on *Tamarix* spp., with Harding (1930) recording no damage to the trees, yet in growth trials conducted in New Mexico, Brock (1994) noted that uninfected plants showed 75% more growth than trees infected with the leafhopper. Field observations by Watts *et al.*, (1977) indicated that damage from *O. stactogalus* seems to be rare in the South-Western parts of the USA.

Species of gall midges in the Cecidomyiidae were also found on *Tamarix* trees in this study. In another study carried out on native *T. aphylla*, *T. tetragyna*, *T. nilotica* and *T. jordanis.* (in Israel, Turkey and India) to establish the identity and impact of gall forming arthropods, *Arthrocnodax vitis* Rubsaamen. (Hymenoptera, Cecidomyiidae) was found to form bud galls on *Tamarix* trees, causing the flower buds to develop a scale-like covering and eventually die off (Gerling *et al.*, 2010).

Biological control relies on an agent to affect or arrest the growth and/or reproduction of IAPs in order to minimise their spread and impact. As these three insects have emerged as phytophagus insects associated with *Tamarix* spp. elsewhere in the world, and now here in South Africa, they appear to have the potential to impact *T. ramosissima* and hybrids on gold mines. Even more encouraging is that the fauna is not shared between *T. usneoides* and *T. ramosissima*, and their respective hybrids, increasing the potential for biological control which relies on using a host specific agent to achieve plant control. It is recommended that further research be undertaken into the impacts inflicted on *Tamarix* spp., particularly *T. ramosissima* by the three insects identified in this study, to establish if they may have an effect on growth or reproduction of plants.

5.6 ENEMY RELEASE

Alien invading plants are thought to succeed largely because they have escaped natural enemies (Callaway and Aschehoug, 2000). This is known as the enemy release hypothesis. In studies conducted in South Africa, Samways *et al.,* (1996) found that species richness and diversity were lower in alien vegetation, compared to indigenous vegetation, although not significantly so. In a study carried out on Australian woody species in the Western Cape of South Africa, Proches *et al.,* (2008) also found less herbivorous insects associated with alien plants compared to indigenous plants. In this study, the very low abundance and diversity of insects on "pure" *T. ramosissima* compared to *T. usneoides*, *T. usneoides* hybrid and *T. ramosissima* hybrid indicates that insects favour these plants over *T. ramosissima*. As insects perceive differences between the trees, they select indigenous *T. usneoides* or a hybrid of *T. usneoides* above *T. ramosissima* or its hybrid.

Insects potentially inflict damage to plants through herbivory and predation in the form of seed predation, leaf mining and inflorescence predation. Several studies have shown that the richness and abundance of insects in the introduced range of a

plant is lower resulting in lower herbivory of IAPs (Strong *et al.*, 1984 and references therein; Fenner and Lee 2001; Hierro *et al.*, 2005; Cripps *et al.*, 2006). IAPs escape this predation in their new home range as they have not evolved with natural predators. They have also been established for a short period of time; not enough to allow a predator host shift towards them. The results of this study indicate that lack of natural predators and the early commencement of flowering which does not coincide with periods of high insect abundance and activity, is likely contributing to the spread and success of *T. ramosissima*.

This study suggests that enemy release, in the form of lower insect abundance and diversity is occurring on *T. ramosissima*. At the Vaal River Mining Operations there are a number of reasons that can be proposed for this occurrence including, (i) a flowering period that does not overlap with periods of high insect activity, and (ii) vegetative reproduction in the "wild" population, with continuous selection for characteristics which deter insects. Based on these findings, it is recommended that a biological agent in the form of a natural enemy is necessary for *T. ramosissima*, to curb growth and reproduction and maintain the population at a low level. Further research into local agents, including the insects identified in this study, as well as other agents is recommended.

CHAPTER 6:
CONCLUSIONS

Hybridisation produces two hybrid states, more morphologically similar to either parent plant, *T. usneoides* and *T. ramosissima*. Accurate morphological identification between these species and their hybrids for IAP management relies on using three characters; (1) petal shape, (2) insertion of filaments into nectary disc and (3) presence and abundance of salt glands on the leaves. However, the applicability of this method (to utilise all three traits) is still limited to the flowering period of the plants and is difficult to use during other periods of the year. The presence of salt glands on the leaves can be identified at any time of the year, but the accuracy of plant identification is enhanced by using all three characters simultaneously.

Soil conditions at one site (M) appear to be hindering the growth of *T. usneoides* and *T. usneoides* hybrids in the "cultivated plots". Taller trees with larger wood volumes and better percentage survival (to date) are evident in trees at the WC site, and poorer tree percentage survival, stunted growth and shrubbier tree canopies are evident in trees at the M site. Competition for canopy space may be the constraint limiting the size of tree canopies at the WC site. This tolerance to prevailing soil conditions at the WC site where soils are shallow and waterlogged with high pollution (salinity) levels, concurs with other research where *Tamarix* is reported to tolerate growth in saline environments (Di Tomaso, 1998). The low insect numbers on *T. usneoides* and *T. usneoides* hybrids at the M site are possibly due to the poor performance of trees, with shorter flowering periods and fewer flowering trees.

Tamarix is a useful plant for phytoremediation purposes as it thrives in the saline, shallow and water logged soils at the WC site, over deep, well drained, less polluted soils. To enhance the effectiveness of the woodlands programme for phytoremediation of polluted soils and groundwater, it is necessary to identify "pure", resilient *T. usneoides* individuals from existing "cultivated plots" for

cloning, and remove *T. usneoides* hybrid trees that have been planted.

The onset of earlier flowering in *T. ramosissima* compared to *T. usneoides* increases the competitive ability of *T. ramosissima* to set seed and spread into new areas, as seeds are dispersed at a time when insect activity is lower, and seeds may avoid predation. As *T. ramosissima* and *T. chinensis* were not listed as priority species for control by WfW until early in 2010, there is currently no comprehensive method of species identification and no control for these alien plants.

Considering the traits of invaders and invaded communities by comparing between natural and alien vegetation is an effective mechanism to understand invasiveness (Strauss *et al.,* 2006). It is now confirmed that hybridization is occurring between indigenous *T. usneoides* and alien *T. ramosissima* on gold mines. This can be prevented by early, timeous identification of *Tamarix* using the flowering period and the key morphological characteristics to remove hybrid and alien trees, and to curb the opportunities for plant hybridization

This study has also confirmed that the hybridisation of *T. usneoides* x *T. ramosissima,* first recorded in the 1970s by Obermeyer (1976) in the arid-semi arid regions of the Northern Cape, is present in the North-West and Free State provinces of South Africa where this study was conducted. In this study, hybridisation was extensive in the "wild population" with hybrids making up 60% of individuals. Conversely hybrids comprised ±80% of the individuals in the "cultivated plots" which were reared as clones of six "*T. usneoides*" trees from Upington. This suggests that either there was human error in selecting and cloning "*T. usneoides*" for the site species trial, or hybridisation is already so extensive in Upington, the native home range of *T. usneoides* that "pure" *T. usneoides* now occur in low numbers or are difficult to find at Upington. This is concerning for biodiversity as *T. usneoides* is in danger of extinction from a loss of native "*T usneoides*" alleles through hybridisation.

The cultivation of *T. usneoides* hybrid clones from Upington in the site species trial was replicated at West Wits and FreeGold Mining Operations (Isabel

Weiersbye, University of Witwatersrand, pers comm.). It is therefore recommended that *Tamarix* clones from the Upington trees must be identified for hybrid plant removal. Failure to do so could place the University of Witwatersrand, DWAF Upington and AngloGold Ashanti Ltd as the responsible parties for the spread of hybrid *Tamarix* on the Highveld, a particularly controversial issue in aquatic systems, and for the loss of a valuable phytoremediation species in *T. usneoides*.

RECOMMENDATIONS

As the results of hybridization between *T. usneoides* x *T. ramosissima* presented in this study are established from only one site where *Tamarix* was cultivated, it is recommended that studies are carried out in natural populations of *T. usneoides* in southern Africa, to establish the extent of hybridization in natural areas. This will inform management strategies to preserve *T. usneoides* and combat the spread of alien and hybrid *Tamarix.*

Accurate species identification is necessary prior to cloning, and results from this study, as well as data from other sites (where possible) should be used to identify pure, resilient *T. usneoides* trees, that grow well and have the capacity to reproduce, for cloning purposes. This will ensure that the integrity of the phytoremediation approach is maintained.

The finding of this research, that insects indeed perceive differences between trees, and that *T. ramosissima* is being released from insect pressures, provides support for biological control as a cost effective method of alien *Tamarix* control.

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