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SALT EXCRETION IN ALIEN AND NATIVE *TAMARIX* SPECIES IN SOUTH AFRICA AND THE EFFECTS OF SALT ON THE *TAMARIX* LEAFHOPPER (*OPSIUS STACTOGALUS*)

Stawm Allem

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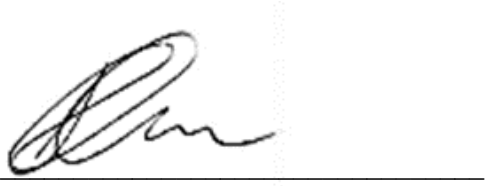
A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science.

School of Animal, Plant and Environmental Sciences, Johannesburg,

May 2018

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.



Stawm Megan Allem

17th day of May 2018

ABSTRACT

Phytoremediation using *Tamarix* on contaminated mine sites in South Africa has been common practice for over a century. The use of exotic *Tamarix* species for phytoremediation has resulted in the spread of *Tamarix* as an alien invasive. In this study, the ability of different *Tamarix* species to take up and excrete salt has been investigated. The study included one indigenous species (*Tamarix usneoides*), two exotic species (*Tamarix ramosissima* and *Tamarix chinensis*) and two hybrids (*T. chinensis* x *T. ramosissima* and *T. chinensis* x *T. usneoides).* In addition, the effects of salt on the herbivory of the *Tamarix* Leafhopper (*Opsius stactogalus*) on the selected *Tamarix* species was investigated. A pot experiment with the aforementioned *Tamarix* species was conducted with each species exposed to salt at a concentration of 3% (w/w) (180mM) for a 3 week period. Subsequently, the salt treated plants were exposed to *O. stactogalus* to investigate the effects of salt on the herbivory of the insects for a further three week period. Phase 1 of the experiment involved the salt treatment and Phase 2 involved the insect treatment, where ten replicates were used for *T. usneoides*, *T. ramosissima* and *T. usneoides x T. chinensis* and five replicates were used for *T. chinensis* and *T. ramosissima x T. chinensis* in both phases of the experiment for the treatments and the controls. The experiment took place in an open air area exposed to natural light and sheltered from wind and rain at ambient temperature and humidly ranging from 22.1°C to 42.9°C (average 32.5°C) and from 21% to 97% (average of 59%), respectively. The specific aims of this work were to investigate whether the indigenous *Tamarix* species excretes more salt than the exotic species and the hybrids and to investigate the effects of salt on the herbivory of *O. stactogalus* on the selected *Tamarix* species. Measurements of chlorophyll content, plant vigour, stomatal conductance, chlorophyll fluorescence, water pressure and electroconductivity were used to evaluate the potential of salt excretion by each of the *Tamarix* plants and their effects on the plants as well as the herbivory of *O. stactogalus*. The experimental data suggest that the exotic species, *T. chinensis*, excreted significantly more salt than the other *Tamarix* taxa (including the indigenous *T. usneoides*) and that salt had no significant effect on the herbivory of *O. stactogalus* or the plant vigour of the selected *Tamarix* taxa. These findings suggest that the exotic species (*T. chinensis*) may be the most effective species for salt extraction from soils, but that the indigenous species excretes the same amount of salt as *T. ramosissima* and the hybrids (*T. chinensis* x *T. ramosissima* and *T. chinensis* x *T. usneoides).* Although *T. usneoides* excretes less salt than *T. chinensis*, other traits, such as superior

plant growth under saline conditions may give mining companies an incentive to plant the native *T. usneoides* rather than the exotic invasive species.

DEDICATION

I dedicate this work to my family, to my mother who has always showed unwavering faith in my ability to accomplish anything, my father who has taught me that the unconventional can be just as effective as the conventional (just far less boring). To my husband who has endured my stress and complaints and comforted and consoled me when I needed it most, and to my aunt who is always waiting in the wings to come to my rescue. In particular, I would like to dedicate this work to my grandmother who so desperately wanted me to finish this work, and always knew that I would. I would like to thank my supervisor for keeping me on track and pointing me in the right direction whenever I got lost and to my friends for their support and willing ears.

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CHAPTER 1

1 INTRODUCTION

Invasive alien *Tamarix* are a threat to native ecosystems in South Africa. Many mining companies use *Tamarix* for phytoremediation of contaminated soils. Mayonde et al. (2015) found the exotic *Tamarix* species, *T, chinensis* and *T. ramosissima*, with a large number of putative hybrids, to be the most dominant invasive *Tamarix* species in South Africa. *Tamarix* species have been used for the phytoremediation of tailings storage facilities at gold mines since the early 1930s, however the choice of *Tamarix* species for this purpose has mostly been arbitrary. Due to the negative effects of using invasive *Tamarix* species for phytoremediation, propagation and distribution of the indigenous species' rather than the exotic species and hybrids is preferable. If indigenous *Tamarix* (*T. usneoides*) is capable of excreting more salt than alien *Tamarix* species and hybrids, *T. usneoides* is a more suitable candidate for phytoremediation. This would give mining companies more of an incentive to plant indigenous *Tamarix* species and reduce the threat posed by invasive alien *Tamarix* species since the mining companies would be less inclined to plant the exotic *Tamarix* taxa if they are less effective phytoremediators.

The effects of high salt concentration on the *Tamarix* Leafhopper has also been investigated because a high concentration of salt or metals in plant tissues is known to deter herbivory in some insects (Coleman et al., 2005; Boyd, 2010; Davis et al., 2015). Such negative interaction between herbivorous insects and the salt concentration in *Tamarix* leaf tissues will be investigated using the *Tamarix* Leafhopper (*Opsius stactogalus)* as a proxy to assess whether high salt concentration may impede the ability of *O. stactogalus* to feed on *Tamarix* and thereby reduce their invasiveness and whether salt could affect the host specificity of insects used as biocontrol agents.

1.1 PROJECT AIMS, OBJECTIVES AND LIMITATIONS

1.1.1 Research Questions

- Is the indigenous *Tamarix usneoides* capable of excreting more salt than the exotic *T. chinensis* and *T. ramosissima* and their hybrids?

- What is the impact of high soil salt concentration on *Tamarix* taxa plant vigour?
- What impact does high salt concentration have on the feeding of the *Tamarix* leafhopper, *Opsius stactogalus*, and on the selected *Tamarix* taxa?

1.2 Research Aims

The main aims of this study are to determine:

1. Whether the indigenous *T. usneoides* is capable of excreting more salt than the invasive alien *T. chinensis and T. ramosissima* and their hybrids *T. usneoides* x *T. chinensis* and *T. ramosissima* x *T. chinensis*.
    - Salt excretion in each plant was determined by immersing a branch from each plant in a test tube containing a known volume of distilled water for two minutes, an electroconductivity meter was then used to determine the electroconductivity of the solution once the branch was removed. The electroconductivity was then used as a proxy to determine the amount of salt excreted by the plant.
2. The effects of increased soil salt concentration on plant vigour of the different *Tamarix* species and their hybrids (namely *T. usneoides*, *T. chinensis*, and *T. ramosissima*, *and the hybrids T. usneoides* X *T. chinensis,* and *T. ramosissima* X *T. chinensis).*
    - The effects of high soil salt concentration on the plant vigour of *Tamarix* spp. was determined by measuring plant parameters such as the number of buds grown per week, length of the leading branch and the length of the shortest branch in each of the *Tamarix* seedlings. Instruments were used to measure physiological plant parameters such as chlorophyll fluorescence, stomatal conductance and leaf water potential as a proxy to determine plant vigour and the effects of salt stress on the *Tamarix* taxa.
3. The effects of the salt treated *Tamarix* plants on the feeding of *O. stactogalus*.

Instruments were used to measure physiological plant parameters such as chlorophyll fluorescence, stomatal conductance and leaf water potential, which were used as a proxy to determine plant vigour and the effects of insect stress on the *Tamarix* taxa. If herbivory was unaffected by salt one would expect the insects to have a more pronounced stress on the plants and *vice versa*.

CHAPTER 2

2 LITERATURE REVIEW

2.1 The GENUS *TAMARIX*

The *Tamarix* genus consists of approximately 55 species (Fay, 2007), although it is thought to contain as many as 90 species (Villar et al., 2014). *Tamarix* species are morphologically very similar and are extremely difficult to distinguish if the plant bears no fruit or flowers. Hybridization of species also adds to the difficulty of taxonomic distinction of *Tamarix* (DiTomaso, 1998).

According to Baum (1978) *Tamarix* shows two main centers of speciation; in the Middle East and the Indo-Turanian region in central Asia. The genus then migrated south and west towards Africa and Europe as well the Pacific coast of Asia (Villar et al., 2014). Due to its exceptional biological characteristics and adaptive capabilities, *Tamarix* has now spread and established itself in 44 countries worldwide (Virla et al., 2010). This invasive spread has largely been aided by human activities such as global trade and tourism. The density of the *Tamarix* genus worldwide illustrating the geographic prevalence of *Tamarix* is shown in Figure 2.1.

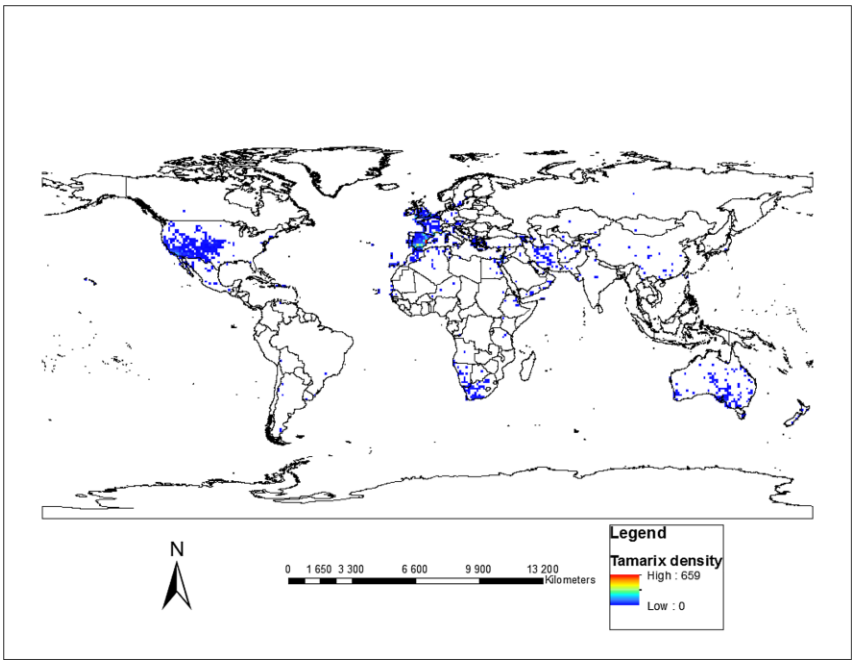


Figure 2.1: Density of the genus *Tamarix* shown on the world map. Data were derived from point co-ordinates from herbarium records (GBIF, 2016) and field surveys (Mayonde et al., 2015).

Tamarix, commonly known as the saltcedar or Tamarisk, is a vigorous woody tree or shrub with slender branches. *Tamarix* is a facultative phreatophyte, which invades pastures and riparian habitats (Brotherson & Field, 1987). The needle-like leaves of the plant are able to excrete salt through external multicellular salt glands (Wilson et al., 2017).

As an invasive alien plant, *Tamarix* is infamously known for causing ecosystem management problems. They spread rapidly and dominate many riparian habitats by replacing the native riparian species, which is often attributed to their high rate of seed production and effective means of dissemination (Warren and Turner, 1975). A mature *Tamarix* tree is able to produce up to 500 000 seeds per season, which are highly viable and well equipped with long hairs that facilitate their wind dispersion; the seeds may also be dispersed by water along rivers (Brotherson and Field, 1987).

Mature *Tamarix* plants reproduce vegetatively by adventitious roots or sexually by producing seeds (Brotherson and Field, 1987), and are highly competitive with other vegetation for space and water. The plant is easily capable of out-competing other vegetation as it has an extensive root system which is capable of growing deep into the water table, a characteristic feature that categorize them as phreatophytes. *Tamarix* is also capable of growing where no ground water is accessible (DiTomaso, 1998), adding to the plants resilient and competitive nature.

There are a number of factors that contribute to the extreme resilience and invasibility of the *Tamarix* species in areas of introduction which often causes reduced biodiversity (DiTomaso, 1998). *Tamarix* plants excrete and drip salt onto the soil beneath its canopy, which inhibits the growth of its competitors and the *Tamarix* seeds are resistant to inundation (Brotherson and Field, 1987). For instance, Warren and Turner (1975) found that the seeds of *T. chinensis* were able to survive inundation for up to 70 days.

Once *Tamarix* matures, it is extremely resistant to mechanical injury resulting from grazing, cutting or burning. It is also very resilient to a host of unfavourable environmental conditions such as heat, cold, and drought (Brotherson and Field, 1987). Although *Tamarix* has one of the highest evapotranspiration rates of any phreatophyte, it is capable of surviving drought by

shedding its leaves under drought conditions (DiTomaso, 1998). *Tamarix* is a facultative halophyte (DiTomaso, 1998). Halophytes constitute plants that are able to survive in salt concentrations of 200mM NaCl or more. Some halophytes show optimal growth in saline conditions while others grow optimally in non-saline soils, all halophytes however, rely on the controlled uptake and compartamentilisation of Na^+, K^+ and Cl^- (Flowers and Colmer, 2008), *Tamarix* prefer saline soils (DiTomaso, 1998).

2.2 *TAMARIX* IN SOUTH AFRICA

The *Tamarix* species found in South Africa include: *T. usneoides*, *T. ramosissima* Ledeb and *T. chinensis* Lour, of which *T. usneoides* is the only indigenous species (Mayonde et al., 2015; Weiersbye et al., 2006) . In South Africa *Tamarix* is distributed in the southwestern regions in semi-desert climates where it occurs along riverbeds and in areas containing brackish water, and this includes the Northern, Western and Eastern Cape provinces (Pretoria National Herbarium, 2012; Mayonde et al., 2015). *Tamarix ramosissima* and *T. chinensis* have a very high invasive potential in South Africa, in addition to this the morphological confusion between these two alien invasive species make them the focal exotic *Tamarix* species in South Africa (Gaskin, 2003; Mayonde et al., 2015).

2.3 PHYTOREMEDIATION

Phytoremediation involves using green plants to remove pollutants from the environment (Manousaki et al., 2009; Newete and Byrne, 2016). Phytoremediation is categorized into three main subgroups: rhizofiltration, phytoextraction and phytostabilisation (Nouri et al., 2011).

Rhizofiltration uses the roots of plants to remove contaminants by absorption, adsorption or precipitation where contaminants are accumulated in or on the plant roots (Newete and Byrne, 2016), the whole plants are later harvested, which results in the reduction of heavy metal contamination. Phytoextraction involves uptake and accumulation of heavy metals in the harvestable plant parts (shoots) and subsequent harvest and safe disposal (Nouri et al., 2011). Phytostabilisation involves the use of metal-tolerant plants to reduce the mobility of metals which results in stabilization of metals from the substrate (Abdel-Ghani et al., 2007; Nouri et al., 2011).

Phytoremediation is a promising technology, which allows for an environmentally friendly and cost-effective solution for the remediation of polluted soil (Yanai et al., 2006; Manousaki et al., 2008). It has been found that plants with a high salt tolerance, such as *Tamarix* may have a greater potential to absorb heavy metals from soil. Manousaki et al., (2009) found that an increase in salinity enabled more efficient absorption of cadmium by *Tamarix*, because salinity changes the metal availability in sediments and is a key factor in the transportation of metals from roots to the upper regions of the plant which enhances the phytoremediation processes, which indicates that the presence of salt could potentially improve phytoremediation in *Tamarix*, making it an ideal candidate for phytoremediation in saline soils.

The ability of *Tamarix* to tolerate high concentrations of salinity makes them one of the most common trees used for phytoremediation on mine sites in South Africa (Weiersbye et al., 2006). Using molecular evidence, Mayonde (2013) found that most *Tamarix* species planted before 2005 in South Africa on mine sites for phytoremediation were *T. ramosissima* and *T. chinensis*. This has resulted in these two exotic species being the common invasive *Tamarix* species in South Africa, particularly in mining areas (Mayonde et al., 2015). *Tamarix usneoides* is considered to be a suitable candidate to use for phytoremediation as it is able to tolerate a wide range of pH and has deep roots which are able to access pollutants plumes within the soil (Wilson, 2010). However, *T. chinensis* and *T. ramosissima* were used for phytoremediation on mine sites more extensively than the native species due to a lack of regulation and restrictions and because of morphological similarity between species making it difficult to identify between the *Tamarix* species. This has resulted in the inadvertent spread of unwanted alien invasive *Tamarix* species due to the ease of hybridization between the exotic *Tamarix* species (*T. ramosissima* and *T. chinensis*) and the native *Tamarix* species (Mayonde, 2013).

2.4 SALT EXCRETION IN *TAMARIX*

Tamarix has the ability to extract salt from the soil and excrete it in harvestable plant tissues in the leaves (Kleinkopf and Wallace, 1974). The salt glands of *Tamarix* are found on the adaxial and abaxial leaf surfaces, as well as the surfaces of young stems (DiTomaso, 1998). The salt glands are made up of eight radially arranged epidermal cells, of which the six outer cells are

secretory cells and the two inner cells are referred to as collecting cells as they are highly vacuolated; these vacuolated cells enable the collection and segregation of salt from the plant tissues (Bosabalidis and Thomson, 1984). Bosabalidis (1992) found that the salt glands of *Tamarix* are not permanent, but differentiate throughout leaf expansion allowing them to adapt to environmental conditions.

Tamarix transport salt from the soil to their leaves through the xylem tissue (Campbell et al., 1974). The majority of studies carried out on the secreted salts of the *Tamarix* found the salt glands to be non-selective and the excretions to be dependent on the salt composition of the soil (Kadukova et al., 2008; Manousaki et al., 2009). Many salts and minerals and even trace elements are excreted by the salt glands of *Tamarix*. Some of these include: sodium, manganese, potassium, calcium, nitrate, copper, sulphur, aluminium, silica, barium and lithium (Di Tomaso, 1998). Such excretions of various elements of salt indicate that the salt glands are not selective, and allow the *Tamarix* to survive in a wide variety of saline soil types. Nevertheless studies found that high external salt concentrations have a negative effect on *Tamarix* causing impaired growth and plant mortality (Manousaki et al., 2009; Kadukova et al., 2008; Imada et al., 2015). This could be due to increased levels of energy expenditure used to transport salt and maintain normal water relations (Kleinkopf and Wallace, 1974; Shimony and Fahn, 1988). Kleinkopf and Wallace (1974) also found that stem and root growth was inhibited by far lower salt concentrations than the leaves. This is because the salt glands in the leaves of *Tamarix* concentrate the salt and then excrete salt crystals on the leaf surface, which alleviates the leaf from high salt stresses, allowing the leaf to grow (Kleinkopf and Wallace, 1974).

Kleinkopf and Wallace (1974) exposed *T. ramosissima* to a variety of salt concentrations and found that a significant impact of salt concentration on *Tamarix* was not reached until using a concentration of 100mequiv./l NaCl. At this salt concentration it was found that yields of leaves, stems and roots in the plant decreased significantly. Carter (2011) showed that *Tamarix* were able to acclimatize to extreme salt concentrations (40 000 ppm NaCl or $40g.l^{-1}$ of NaCl) over a relatively short period of time of 35 days. Salt tolerance in *Tamarix* may negatively impact the herbivory of insects used as biocontrol agents to control *Tamarix.* High concentration of salt or metals in plant tissues is known to deter herbivory in some insects (Davis et al., 2015; Coleman

et al., 2005; Boyd, 2010; Newete et al., 2014). Such negative interaction between herbivorous insects and the salt concentration in *Tamarix* leaf tissues will be investigated using the *Tamarix* Leafhopper, since the *Diorhabda* beetle is still under investigation for host specificity in quarantine at the University of the Witwatersrand in South Africa.

2.5 BIOLOGICAL CONTROL OF *TAMARIX*

DeLoach et al. (2000) argue that the invasion by *Tamarix* in the western riparian ecosystems of the United States is one of the worst ecological disasters ever to befall the region. In the United States the infestation of *Tamarix* in riparian zones has been a major problem as *Tamarix* degrades wildlife habitat, replaces indigenous plant populations, reduces biodiversity, uses large quantities of groundwater, increases the prevalence of wildfires, alters stream channels and has caused the decline of many wildlife and fish species (DeLoach et al., 2000). The level of *Tamarix* invasion in the western United States is immense, it is estimated that 0.4 to 0.6 million ha are invaded by *Tamarix* (Brotherson and Field, 1987).

Alien invasive *Tamarix* has many competitive advantages over native plants due to changes in riparian zones caused by humans as well as the intrinsic biological characteristics of *Tamarix* (DeLoach et al., 2000). This has made biological control of the saltcedar critical to preserving ecosystems and controlling the rapid spread of invasive *Tamarix.* DeLoach et al. (2000) identified classical bilogical control as the method of choice to control *Tamarix*. Müller-Schärer et al. (2004) defined classical weed biological control as “the deliberate relase of natural enemies from the alien plants’ native range to decrease its abundance in the introduced range below and econoligcal or economic threshhold".

The taxonomic isolation of *Tamarix* has allowed many insect species to coevolve with it in its origin of speciation in Asia, and therefore these insects are unlikely to attack other plants in other parts of the world (DeLoach et al., 2000). Kovalev (1995) identified 25 insect species that have coevolved with *Tamarix*, and as a result, they are either completely or mostly specific to the *Tamarix* genus. These insects are all potential biological agents for the control of invasive alien *Tamarix* species. Biological control is a proven and effective method for controlling alien invasive weeds. However, it requires careful host-specificity testing to prevent potential damage

to non-target plants and the presumption that biological control can entirely eradicate the *Tamarix* weed is incorrect (Lewis et al., 2003).

Attempts to eradicate the invading *Tamarix* spp. have included herbicidal and mechanical controls as well as manual removal; these methods have shown limited success as they are expensive, labour intensive and require recurrent treatments (DeLoach et al., 2000). In response to the limited success of these methods a biological control program was established by the U.S. Department of Agriculture (USDA), which involved the introduction of host-specific insects from the native range of *Tamarix* spp. in Eurasia (DeLoach et al., 2000). In 1994, after many years of quarantine testing to ensure host specificity and effectiveness; the saltcedar leaf beetle (*Diorhabda elongata*) from Fukang (China) and Chilik (Kazakhstan) as well as the middle-eastern mealy bug (*Trabutina manipara*) were approved for release in the USA for biological control of *Tamarix* (Dudley and Kazmer, 2005).

Unfortunately, the middle-eastern mealy bug was later removed from consideration as a biocontrol agent because its appropriate habitat was off-limits due to the occurrence of the endangered south-western willow flycatcher, which has been found to nest on *Tamarix* plants (Dudley and Kazmer, 2005). *Diorhanda elongata* on the other hand is still considered a viable agent. In the spring of 2001 open release of *D. elongata* was carried out at seven *Tamarix* infestation sites and moderate to good establishment of the beetle has been observed (DeLoach et al., 2004). *Diorhabda elongata* overwintered successfully at five sites in the USA (Nevada, Wyoming, Utah, California and Colorado) and in the summer of 2002 DeLoach et al. (2004) observed dramatic defoliation of *Tamarix* at the sites located further north but no defoliation was observed in the sites located further south. This is because *D. elongata* requires longer day lengths, which are found in the northern areas but not in the areas located further south. *Diorhabda* beetles from Turpan, Greece, Uzbekistan and Tunisia are adapted to shorter day lengths and therefore are promising agents for control in the southern regions (DeLoach et al., 2004). *Diorhabda elongata* released in Texas in 2004 to 2005, however, defoliated six hectares of *Tamarix* located in a riparian habitat; it also defoliated dense stands of *Tamarix* amounting to about 200 ha in north-central California (Carruthers et al., 2008). *Diorhabda carinata*, *D. elongata* and *D. sublineata* found in Uzbekistan, Greece and Tunisia respectively are compatible

with day lengths in areas south of 38°N latitude (Milbrath et al., 2007; Tracy and Robbins, 2009). Of these species, modelling results suggest that D. *carinata* and *D. sublineata* are most likely to establish in areas located further south (Texas and northern Mexico) due to the day length and climatic conditions in these areas (Tracy and Robbins, 2009). The *Diorhabda* beetle has been imported into South Africa for use as a bio-control agent for invasive alien *Tamarix* and is currently under investigation for host-specificity in quarantine.

2.6 THE *TAMARIX* LEAFHOPPER (*OPSIUS STACTOGALUS*)

The *Tamarix* leafhopper, *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae) is found exclusively on *Tamarix* plants (Virla et al., 2010). It is a small insect (0.81-4.5mm long) and originates from Europe (Wiesenborn and Wlesenborn, 2015). Harding (1930) found three generations of *O. stactogalus* a year in North America, and the adults live for one month. The eggs of *O. stactogalus* are oviposited underneath thin layers of bark on *Tamarix* and overwinter in this stage (Louden, 2010). Eggs usually hatch in early May and the leafhopper grows from neonate to adult in five stages in approximately one month.

Opsius stactogalus has been known to reduce the growth of *Tamarix* plants due to the cumulative feeding undertaken by large populations (Virla et al., 2010). Wiesenborn (2004) studied the mouth of *O. stactogalus* and confirmed that the insect feeds mainly on phloem which causes internal cell damage in *Tamarix*, resulting in chlorosis (yellow or white stippling) of the foliage and reduced stem growth (Louden, 2010).

Although *O. stactogalus* is often found in great numbers on *Tamarix*, the damage caused by the leafhopper is generally considered insignificant according to Virla et al. (2010) in Argentina and DeLoach et al. (2004) in North America. These findings imply that *O. stactogalus* is not a viable bio-control agent for invasive *Tamarix* populations as the leafhopper is incapable of inflicting significant damage to the plant. However, the leafhoppers' relationship with *Tamarix* is important, because it may influence biological control agents introduced to these plants by competing for resources with them.

CHAPTER 3

3 METHODS AND MATERIALS

Three *Tamarix* species and their hybrids were grown from cuttings in a greenhouse at the University of the Witwatersrand, Johannesburg, where they were allowed to grow for five months. The plants were housed in a greenhouse that received natural light and sheltered the plants from rain. The average maximum and minimum temperatures were recorded as 42.9°C and 22.1°C, respectively (32.5°C average) and the maximum and minimum humidity was recorded as 96.6% and 21.0%, respectively (58.8% average).

The experiment consisted of two phases. Phase 1 involved the introduction of salt to the selected *Tamarix* species to record the effects of salt on the plants. Phase 2 involved the introduction of *Tamarix* leafhoppers (*Opsius stactogalus*) to the plants to observe how salt affected the herbivory by these insects. The experiment was undertaken over a period of six weeks, with each phase lasting three weeks (Figure 3.1).

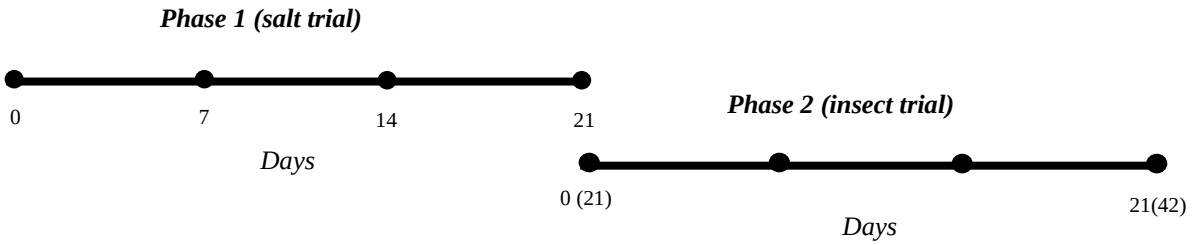


Figure 3.1: Experimental time line showing the start and end days and duration of Phase 1 (salt trail) and Phase 2 (insect trial).

3.1 Experimental design

3.1.1 *Tamarix* propagation

The plants used in the experiment were propagated in an open greenhouse using cuttings from established *Tamarix* plants harvested in the field from wild populations. Samples were collected from the Western Cape, Eastern Cape and Northern Cape Provinces of South Africa. The *Tamarix* harvested from the field were stripped of all leaves and branches and stems were cut into 10 cm cuttings to be used for propagation. The cuttings were initially placed in river sand in large plastic tubs with drainage holes in the bottom (Figure 3.2a). During this period the plants were irrigated twice a day and kept under shade netting in a greenhouse. Once the cuttings

showed root growth they were potted individually in potting soil in one litre plastic pots (Figure 3.2b). The pots were colour coded according to taxon to ensure the *Tamarix* were identified correctly. The pot locations were randomized to minimize bias due to environmental effects, such as exposure to sunlight.

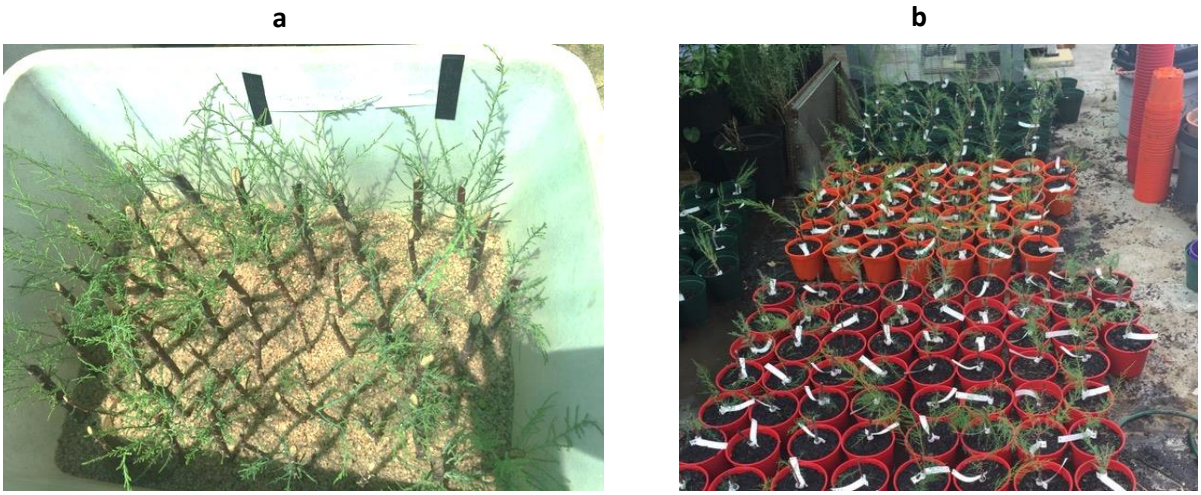


Figure 3.2: illustrates *Tamarix* cutting in river sand trays and pots **(a)** Ten cm *Tamarix* cuttings dipped in root growth hormone and planted in river sand until plant establishment, and **(b)** established seedlings from cuttings developed in the river sand trays transplanted in pots with potting soil, each *Tamarix* taxa placed a designated colour to avoid confusion with the species identities.

The individually potted *Tamarix* were kept in the greenhouse where they were irrigated twice a day and allowed to mature for a total of five months before the experiment commenced.

3.1.2 *Tamarix* taxa and replicates

The taxa chosen for the experiment included one indigenous species (*T. usneoides*), two alien species (*T. chinensis and T. ramosissima*) and two hybrids (*T. usneoides x T. chinensis* and *T. ramosissima x T. chinensis*). The selected species were used because of their availability and ease of propagation (Table 3.1). Ten plants were randomly chosen from each taxon and assigned as controls (no salt or insects), and 10 as treatment (either salt or insect). The salt plants in Phase 1 of the experiment were the same plants used for the insect/salt treatment in Phase 2 (Table 3.1), where the already salt treated plants were inoculated with insects. Less replicates were used for *T. chinensis* and *T. chinensis x T. ramosissima (*five replicates each) due to cutting mortality before the start of the experiment.

Table 3.1: The five *Tamarix* taxa, with their respective number of replicates, used in this study to determine the impact of salt on plant growth and insect parameters such as feeding, oviposition and survival rates. N.B. the number of replicates for Tc and Tr x Tc were smaller than the others due to seedling mortality before the start of the experiment.

***Tamarix* Taxa**	**Replicates**	**Control**	**Phase 1**	**Phase 2**	**Total**
			Salt	**Insect**	
T. usneoides (Tu)	10	10	10	10	**30**
T. ramosissima (Tr)	10	10	10	10	**30**
T. chinensis (Tc)	5	5	5	5	**15**
T. usneoides x T. chinensis (Tu x Tc)	10	10	10	10	**30**
T. ramosissima x T. chinensis (Tr x Tc)	5	5	5	5	**15**

3.2 Phase 1: Salt trial

3.2.1 Salt treatment

The salt treatment method was adapted from Carter (2011); Manousaki et al. (2008); Manousaki and Kalogerakis (2009) and Kadukova et al. (2008). The salt water was prepared using table salt and tap water in a ratio that allowed for a salt concentration of 3% (w/w). To formulate the 3% (w/w) concentration, 480 g of table salt was added to 16 liters of tap water. A volume of 400 ml of salt water was added to each pot at a concentration of 3% (w/w) i.e. 180 $mmol^{-1}$ over a four day period (100 ml per day) to reduce plant physiological shock due to salt stress.

3.2.2 Measurements and sampling

3.2.2.1 Salt stress

Measurements of chlorophyll fluorescence, stomatal conductance and leaf water potential were taken weekly over a three week period, with baseline measurements taken before salt treatment at the beginning of the experiment. The instruments used to take the measurements included the following:

- Decagon SC-1 Porometer (Decagon Devices, Inc. Pullman WA 99163)
- OS1p Chlorophyll Fluorometer (Opti-Sciences, Hudson NH 03051)
- Schonlander Pressure Chamber, 1505D (PMS Instrument Company, Albany, OR 97322)

3.2.2.2 Plant vigour

The effects of salt on the rate of plant growth were recorded weekly for a period of three weeks by measuring the following plant parameters:

- Length of the longest branch;
- Number of dead branches; and
- Number of new buds.

3.2.2.3 Salt content

Electroconductivity was used as a proxy for salt content measurement in each of the taxa. In order to assess the amount of salt present in the salt glands of each *Tamarix* taxa, the following method was used:

- Approximately (0.200g) of leaf mass was harvested from the leading branch of each plant at the end of Phase 1 of the experiment (day 21);
- The harvested biomass was then placed in a test tube containing 10 ml deionised water and swirled for 1 minute;
- The electroconductivity was recorded in microsiemens per centimetre (uS/cm) and later converted to microsiemens per centimetre per gram per millilitre (µS/cm/g/ml).

3.2.2.4 Chlorophyll content

Leaves from each of the *Tamarix* plants were removed before the salt treatment during Phase 1 of the experiment and processed to measure leaf chlorophyll content. This process was repeated after week three using fresh leaf samples to assess the potential effects of salt on leaf chlorophyll content.

- 0.03g of fresh leaf samples were cut from each plant and subsequently bagged and labelled;
- Each sample was individually added to a pestle, to which liquid nitrogen (N_2) was then added and the leaf sample was crushed into a fine powder;
- The leaf powder was poured into a labelled test tube and 10 ml of acetone (80% v/v) was pipetted on to the crushed leaf (Figure 3.3);
- The test tubes were stored at 5°C overnight;

- Each test tube was centrifuged for 2 minutes at 3500 RPM, and the resulting supernatant was then poured into a spectrophotometer cuvette which was placed into a spectrophotometer;
- Absorbance was measured and recorded at wavelengths of 645 and 663 nm respectively for each sample using the spectrophotometer;
- Arnon's (1949) equations for chlorophyll extracted in acetone were used to calculate total chlorophyll content as follows:
    - Chla (g l-1) = 0.0127 A663 – 0.00269 A645
    - Chlb (g l-1) = 0.0029 A663 – 0.00468 A645
    - Total Chl (g l-1) = 0.0202 A663 + 0.00802 A645.

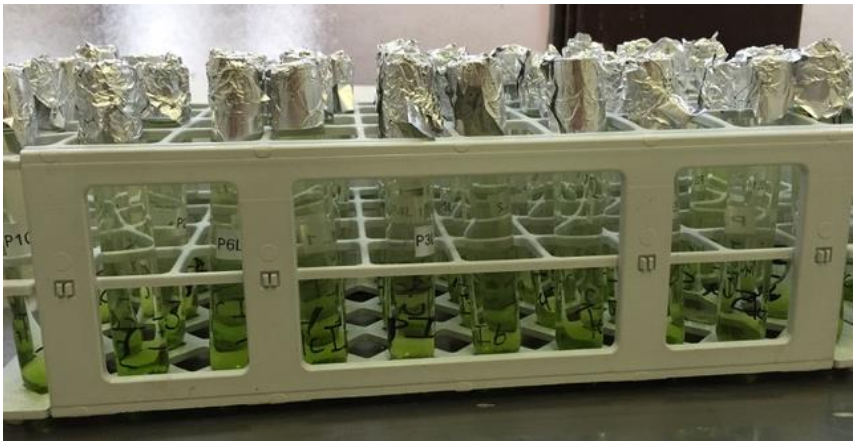


Figure 3.3: Test tubes containing crushed leaf powder and 10 ml acetone (80% v/v) in method used for chlorophyll extraction.

3.3 Phase 2: Insect trial

3.3.1 Insect inoculation

Tamarix leafhoppers were collected with sweep nets from a stand of *T. ramosissima* in Germiston, Johannesburg, at the following coordinates: 26°12'37.80"S; 28° 2'12.06"E in October 2016 (Figure 3.4a). *Tamarix* branches were inserted into the sweep nets and shaken thoroughly to dislodge the leafhoppers, which were then transferred into plastic containers, sealed with a mesh lid. The leafhoppers were pootered into small net draw-bags (Figure 3.4b), which were then tied onto the longest branch of each *Tamarix* plant. The plants were then left for a period of three weeks to allow leafhopper feeding and reproduction.

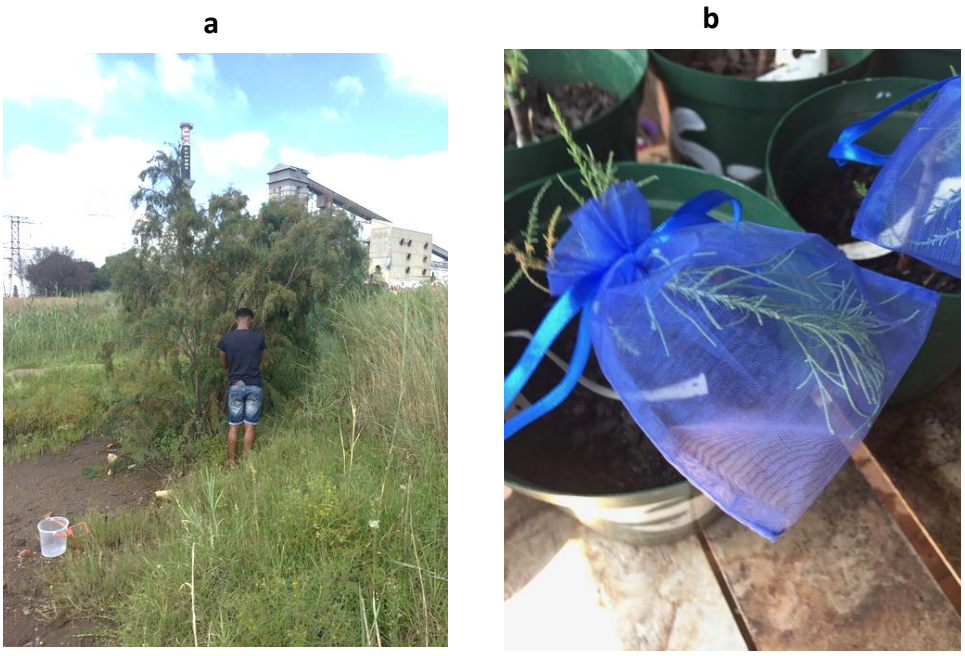


Figure 3.4: Illustration of **(a)** *Tamarix* leafhoppers (*Opsius stactogalus*) collection using sweep nets in Germiston (Ekhuruleni) for the insect feeding experiment in the lab, and **(b)** Net draw-bags used to ensleeve the longest branch of each *Tamarix* with ten leafhoppers for a period of three weeks.

3.3.2 Measurements and sampling

The same plant physiology and plant vigour measurements taken in Phase 1 were then repeated for Phase 2 immediately after Phase 1. These measurements were taken on the branch inoculated with the leafhoppers after a period of three weeks (at the end of Phase 2, day 42).

3.4 Data Analysis

Data was analysed using STATISTICA© 13.0 software. Factorial ANOVA was applied in instances where multiple categorical independent variables were present and One-way ANOVA was applied for single categorical independent variables. Fischer LSD post-hoc tests were applied where statistical significance was determined at $P \leq 0.05$.

CHAPTER 4

4 RESULTS OF SALT EXCRETION AND LEAFHOPPER HERBIVORY INTERACTIONS IN *TAMARIX* SPECIES

4.1 Phase 1: Salt trial results

This section reflects the results of the various measurements taken in response to salt treatment. The physiological plant parameters measured include stomatal conductance, water potential and chlorophyll fluorescence, as discussed in Chapter 3.

4.1.1 Stomatal conductance (g_s)

Tamarix usneoides showed a significant decrease in stomatal conductance (g_s) in the salt treated plants from week one to week three ($P < 0.0036$) (Figure 4.1e). The remaining *Tamarix* taxa showed no relevant significant difference between the salt treated plants and their controls over the duration of Phase 1. In week one the salt treated *T. usneoides* plants showed an average g_s of 197.8 mmol.$H_2Om^{-2}s^{-1}$ compared to the control plants, which showed an average g_s of 238.9 mmol.$H_2Om^{-2}s^{-1}$, resulting in a significant 17% decrease in stomatal conductance ($P = 0.001$). In week two the average g_s in the salt treated *T. usneoides* plants decreased further to 166.0 mmol.$H_2Om^{-2}s^{-1}$ with the control plants averaging 238.9 mmol.$H_2Om^{-2}s^{-1}$, showing a significant decrease of 31% ($P = 0.045$). In the final week of Phase 1 stomatal conductance in the salt treated plants decreased significantly by 19% ($P = 0.001$), with the average g_s in salt treated *T. usneoides* levelled out and increased slightly to 177.0 mmol.$H_2Om^{-2}s^{-1}$ differing significantly from the control plants which increased to 217.2 mmol.$H_2Om^{-2}s^{-1}$.

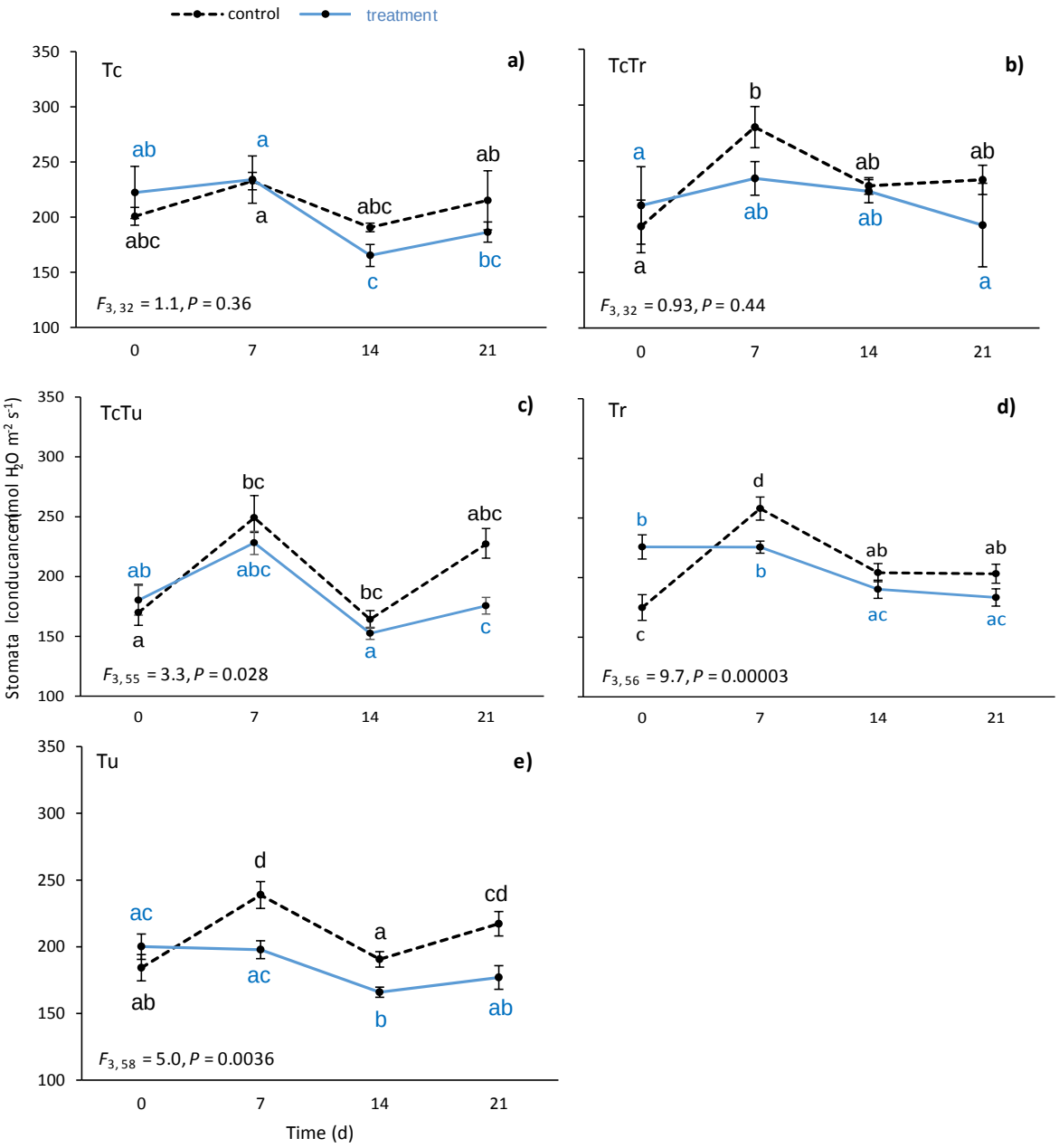


Figure 4.1: Stomatal conductance (mmol.$H_2O m^{-2} s^{-1}$) of *Tamarix* taxa after salt treatment. Different lowercase letters indicate significant differences in stomatal conductance between salt treated taxa and the control plants between sampling dates within the taxa. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA; Fischer LSD test). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.1.2 Water potential (Ψ)

Overall, *T. usneoides* did not show a significant decrease in leaf water potential (Ψ) in the treated plants compared to its control plants (P = 0.28) (Figure 4.2e). *Tamarix chinensis* displayed the lowest Ψ in the treated plants on day 21 with a significant decrease of 79% (*P* = 0.004) with the treated plants recording a Ψ of -0.397 Mpa compared to the control plants which showed a Ψ of -0.082 Mpa (Figure 4.2a). *Tamarix chinensis x Tamarix ramosissima* decreased significantly by 62% (*P* = 0.037) with the treated plants showing a Ψ of -0.321 Mpa compared to -0.123 Mpa in the controls. *Tamarix chinensis x Tamarix usneoides* (Ψ) decreased to -0.131 Mpa compared to the control plants at -0.058 Mpa, showing a decrease (not significant) of 56%. *Tamarix ramosissima* showed a significant 62% decrease (*P* = 0.003) in Ψ to -0.287 Mpa in the treated plants and -0.110 Mpa in the control.

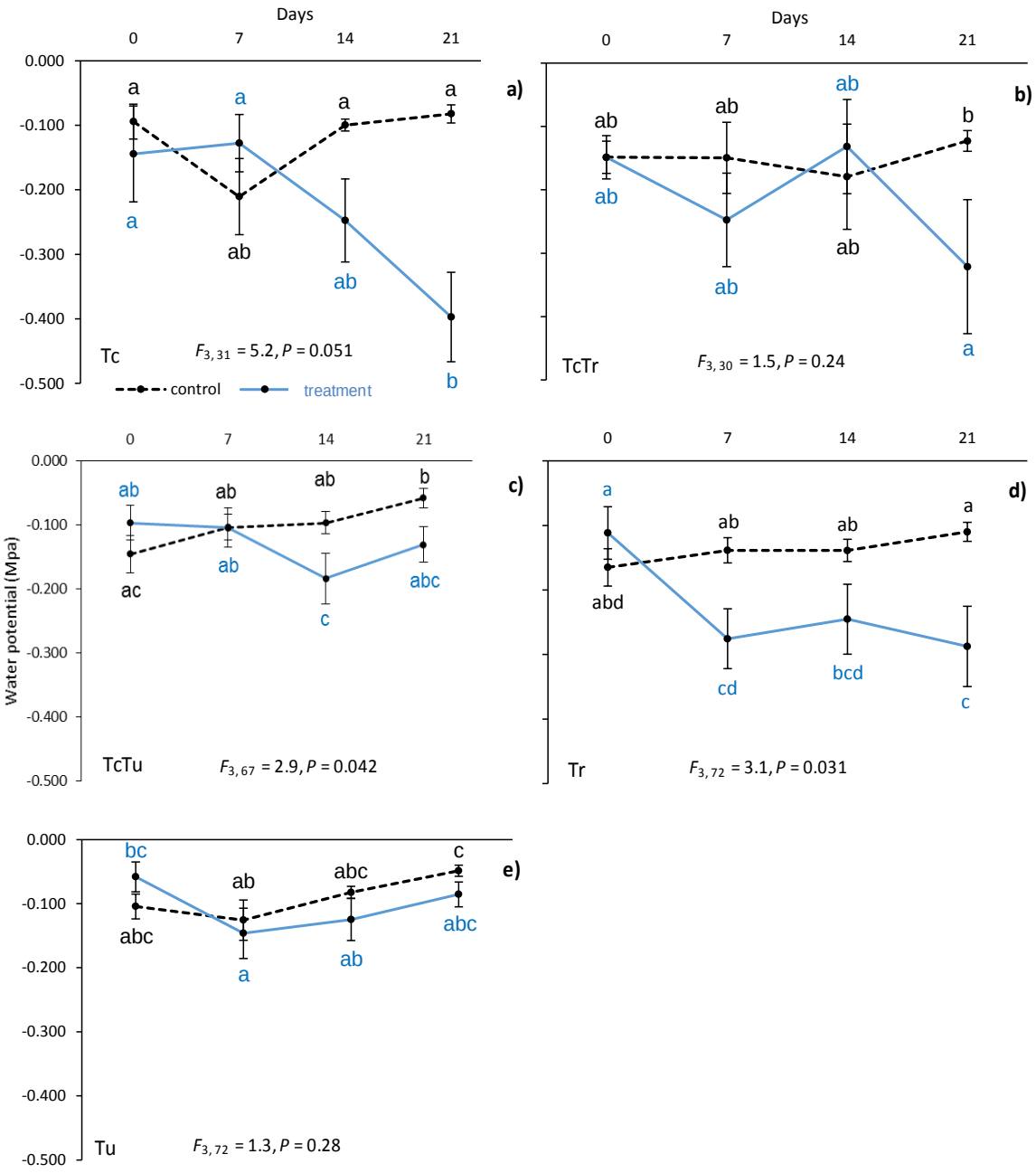


Figure 4.2: Leaf water potential (Mpa) of *Tamarix* taxa after salt treatment. Different lowercase letters indicate significant differences in water potential between salt treated taxa and the control plants between sampling dates within the taxa. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA; Fischer LSD test). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.1.3 Chlorophyll fluorescence

Tamarix usneoides showed a significant 2% increase in chlorophyll fluorescence (P = 0.012) on day 21 in the treated plants as compared to its controls, with an average chlorophyll fluorescence of 0.850 (F_v/F_m) in the treated plants compared to the control of 0.836 (F_v/F_m) (Figure 4.3e). *Tamarix chinensis x Tamarix ramosissima* showed a significant decline of 2% (P = 0.012) in the treated plants on day 14, with the treated plants showing an average chlorophyll fluorescence of 0.808 (F_v/F_m) compared to 0.825 (F_v/F_m) in the controls (Figure 4.3b).

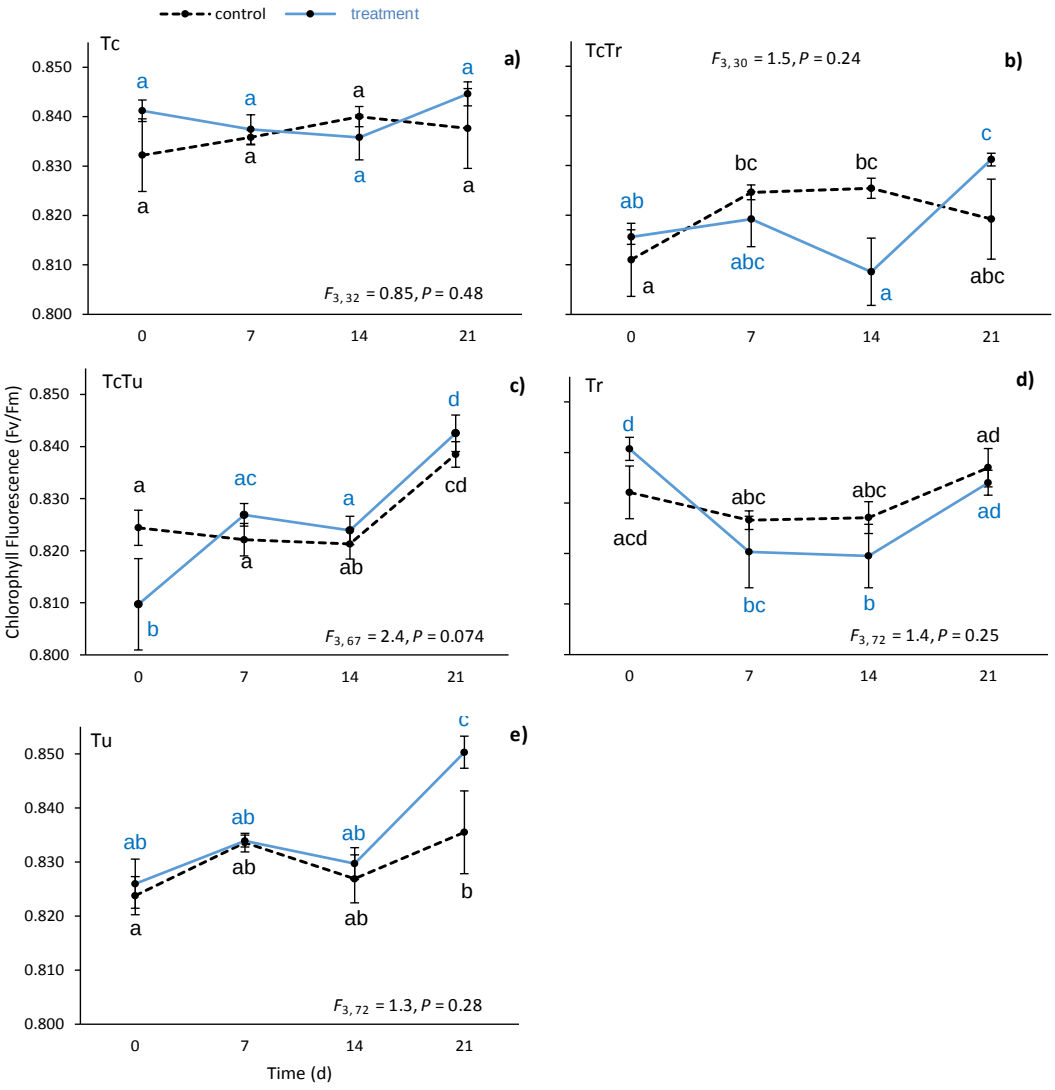


Figure 4.3: Chlorophyll fluorescence (Fv/Fm) of *Tamarix taxa* after salt treatment. Different lowercase letters indicate significant differences in chlorophyll fluorescence between salt treated taxa and the control plants between sampling dates within the taxa. (F_{stat} and P-value indicate overall difference between treatment and control plants using factorial ANOVA; Fischer LSD test). n = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.1.4 Electroconductivity

Electroconductivity (EC) was measured at the end of Phase 1 of the experiment, and is shown in in Figure 4.4. *Tamarix chinensis* showed a significantly higher average EC than the other *Tamarix* taxa. The EC values for the other *Tamarix* taxa treated with salt did not vary significantly and ranged from a minimum of 26 µs/cm/g/ml (*T. chinensis x T. ramosissima*) to a maximum of 37 µs/cm/g/ml (*T. usneoides*).

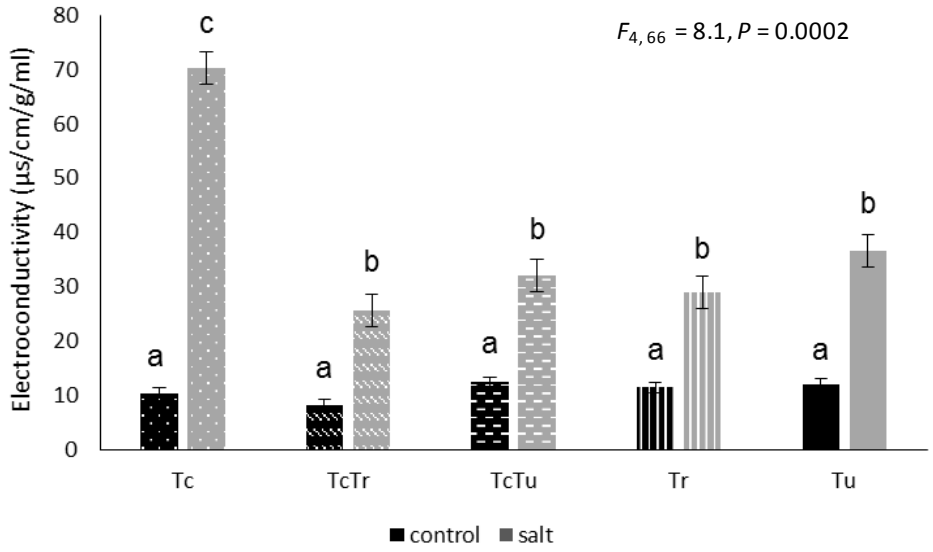


Figure 4.4 :Electroconductivity (µs/cm/g/ml) in the control and salt treated taxa. Readings taken directly after Phase 1 (day 22). Different lowercase letters indicate significant differences in electroconductivity between salt treated taxa and the control plants. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu).

Micrographs of the leaves of the *Tamarix* taxa showing salt gland density appear to mimic the EC results, with *T. chinensis* (Figure 4.5a) having more obvious salt glands at greater density compared to the other *Tamarix* taxa (Figure 4.5)

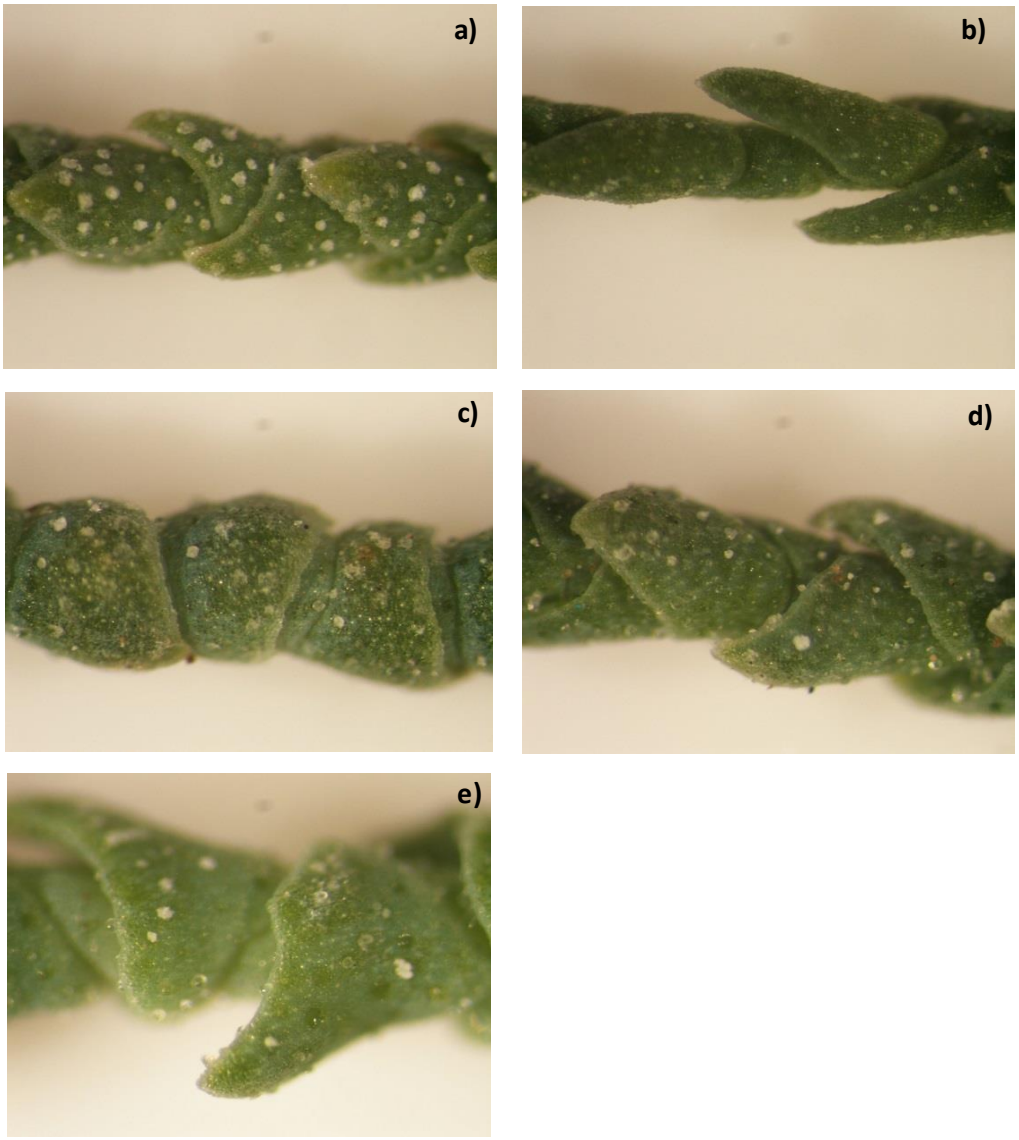


Figure 4.5: Leaves of salt treated *Tamarix* taxa. (40X magnification) after Phase 1 of the experiment. The salt glands are seen as white crystalline structures on the bract-like leaves. **(a)** *T. chinensis* **(b)** *T. chinensis x T. ramosissima* **(c)** *T. chinensis x T. usneoides* **(d)** *T. ramosissima* **(e)** *T. usneoides*

4.1.5 Chlorophyll content

Total chlorophyll content (chlorophyll a + chlorophyll b) was measured at the end of Phase 1. There was no significant difference in average chlorophyll content between the salt treated and control plants across all *Tamarix* taxa and hybrids ($P = 0.38$) (Figure 4.6). The average total chlorophyll content in the salt treated plants ranged from a minimum of 0.98 g/ml^{-1} (*T. chinensis*) to a maximum of 1.50 g/ml^{-1} (*T. usneoides*).

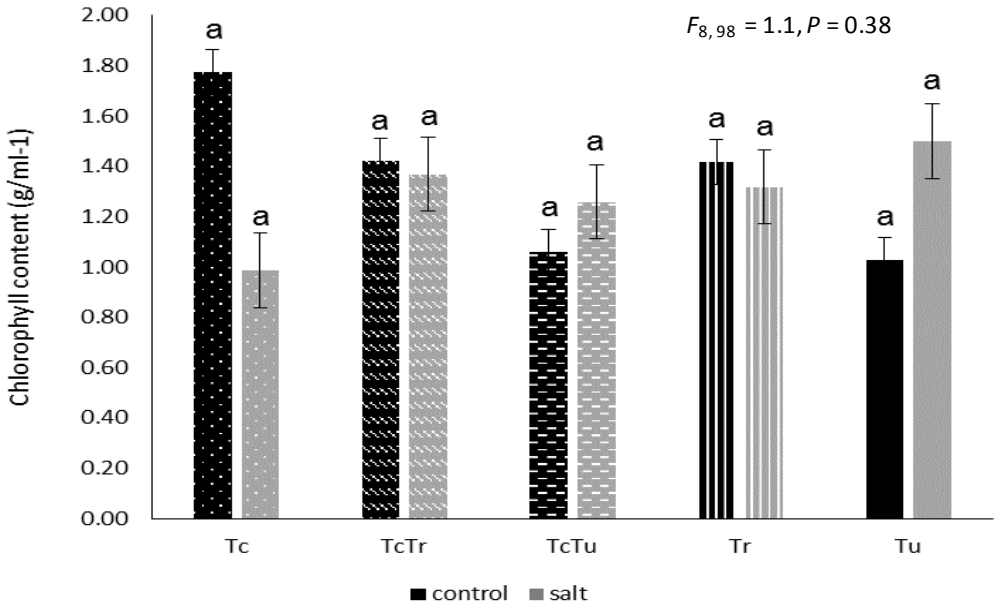


Figure 4.6: Average total chlorophyll content (g/ml^{-1}) in the control and salt treated *Tamarix* taxa. Readings taken directly after Phase 1 (day 22). Different lowercase letters indicate significant differences in average total chlorophyll content between salt treated taxa and the control plants. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu).

4.1.6 Plant growth

Salt treated *T. usneoides* showed a 20% increase in leading branch length over the course of Phase 1 compared to an 18% increase in the control. *Tamarix chinensis x Tamarix ramosissima* showed an 8% average increase in branch length compared to a 6% increase in the control plants. All other taxa (*T. ramosissima, T. chinensis, T. chinensis x T. usneoides)* showed lower levels of growth in the salt treated plants (Figure 4.7), but none of these changes were statistically significant.

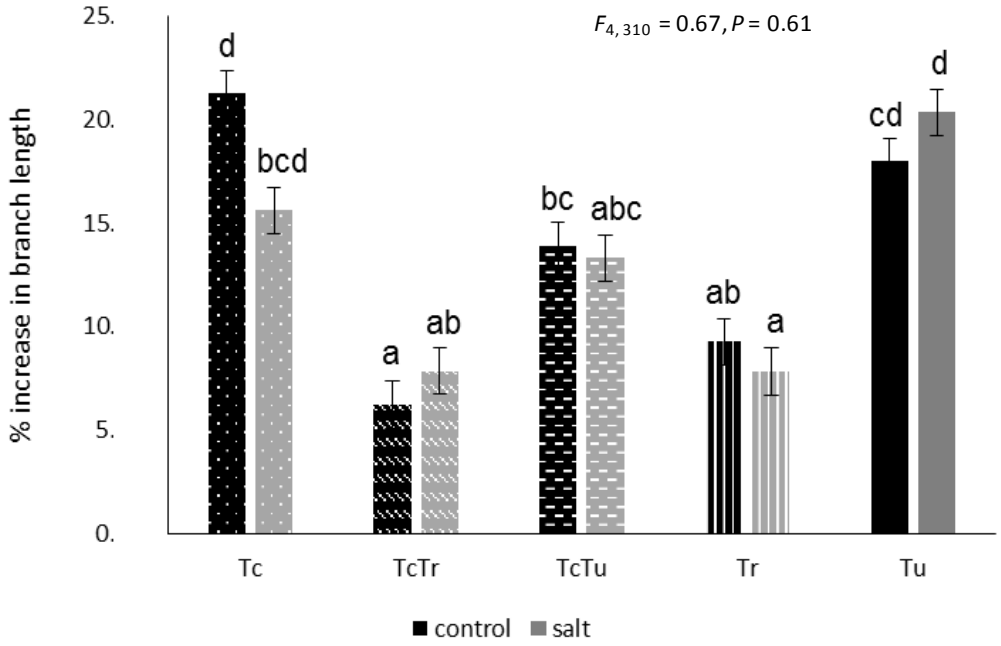


Figure 4.7: Percentage increase in length of the leading branch for the control and salt treated *Tamarix* taxa during the final week of Phase 1. Readings taken during the first three weeks of the experiment. Different lowercase letters indicate significant differences in the average percentage increase in length of the leading branch between salt treated taxa and the control plants. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu).

4.2 Phase 2: Insect trial results

This section reflects the results of the various measurements taken in response to insect only and insect/salt treatment. The physiological plant parameters measured include stomatal conductance, water potential and chlorophyll fluorescence, as discussed in Chapter 3.

4.2.1 Stomatal conductance (g_s)

Overall *T. usneoides* showed the greatest decline in stomatal conductance (g_s) in the control, insect only and insect/salt treatments. However, none of the values were significantly different between the different *Tamarix* taxa when compared with the treatments (Figure 4.8). The average percentage change in g_s in the insect treated plants (Figure 4.8b) varied from a minimum decrease of 22.1% (*T. ramosissima*) to a maximum of decrease of 48.1% (*T. usneoides*). The average percentage change in g_s in the insect/salt treated plants (Figure 4.8c) varied from a

minimum of 34.8% (*T. ramosissima*) to a maximum of 43.6% (*T. usneoides*). It is noted, however, that these changes were not statistically significant.

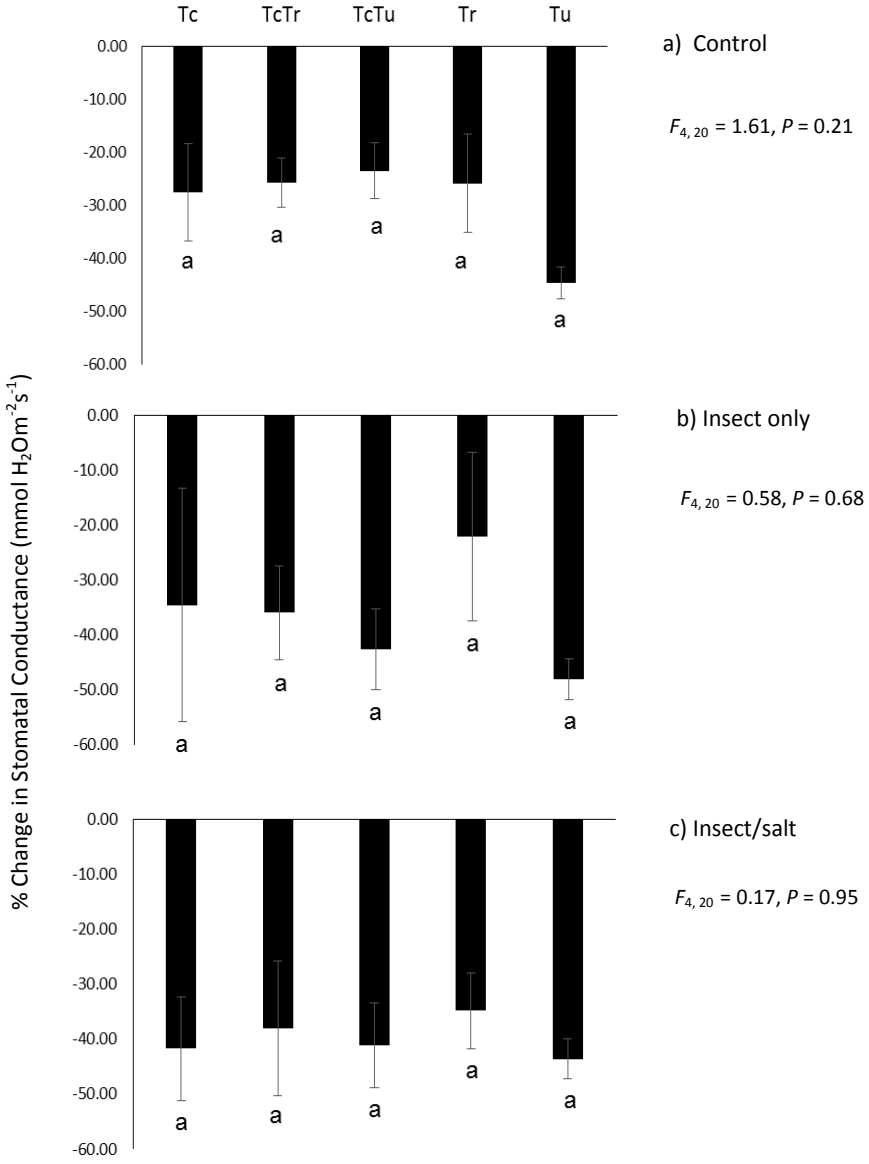


Figure 4.8: Percentage change in stomatal conductance (mmol $H_2Om^{-2}s^{-1}$) of *Tamarix* taxa after salt and insect treatment. **(a)** Control **(b)** Insect **(c)** Insect/salt treatment. Different lowercase letters indicate significant differences in the percentage change in stomatal conductance between treatment and control plants between the first and final day of Phase 2. (F_{stat} and *P*-value indicate overall difference between taxa and hybrids for indicated treatment using one-way ANOVA; Fischer LSD test). *n* = 5 plants for *T. chinensis* (Tc) and T. *chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.2.2 Water potential (Ψ)

The percentage change in Ψ for all treatments did not vary significantly between taxa within the same treatments throughout Phase 2 of the experiment (Figure 4.9). However, within same taxa between different treatments, the insect treatment resulted in positive percentage changes in Ψ for all *Tamarix* taxa, whereas Ψ in the control and insect/salt treatment plants fluctuated around zero.

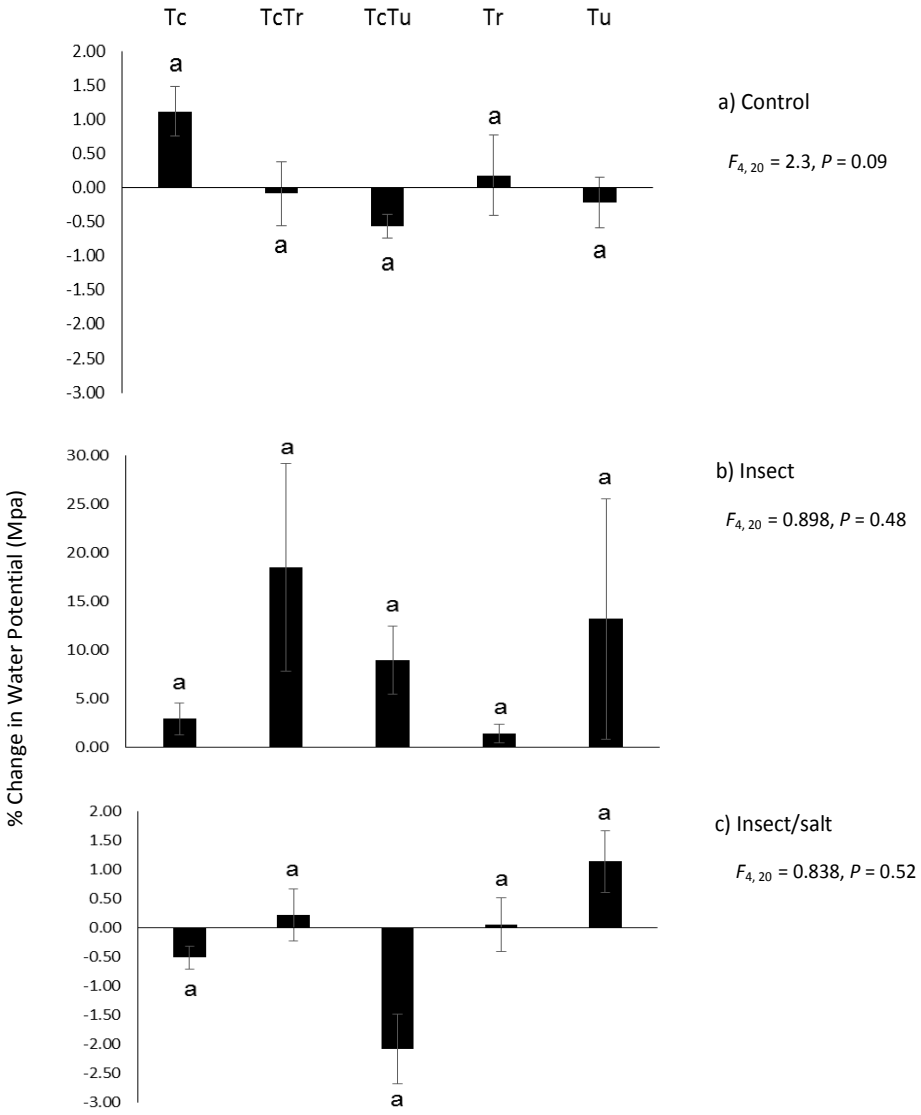


Figure 4.9: Percentage change in leaf water potential (Mpa) of *Tamarix* taxa after salt and insect treatment. **(a)** Control **(b)** Insect **(c)** Insect/salt treatment. Different lowercase letters indicate significant differences in the percentage change in water potential between treatment and control plants between the first and final day of Phase 2. (F_{stat} and P-value indicate overall difference between taxa and hybrids for indicated treatment using one-way ANOVA; Fischer LSD test). n = 5 plants for *T. chinensis* (Tc) and *T. chinensis* x *T. ramosissima* (TcTr); n = 10 plants for *T. chinensis* x *T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.2.3 Chlorophyll fluorescence

There were no significant changes in chlorophyll fluorescence observed in any of the *Tamarix* taxa in response to the insect or insect/salt treatments (Figure 4.10). The average percentage change in chlorophyll fluorescence over the course of Phase 2 in the insect treated plants varied from a minimum decrease of -12.1% (*T. chinensis x T. usneoides*) to a maximum decrease of -17.4% (*T. chinensis x T. ramosissima*). The average percentage change in chlorophyll fluorescence in the insect/salt treated plants varied from a minimum decrease of -9.11% (*T. chinensis x T. usneoides*) to a maximum decrease of -14.7% (*T. usneoides*). It is noted, however, that none of these variations were statistically significant.

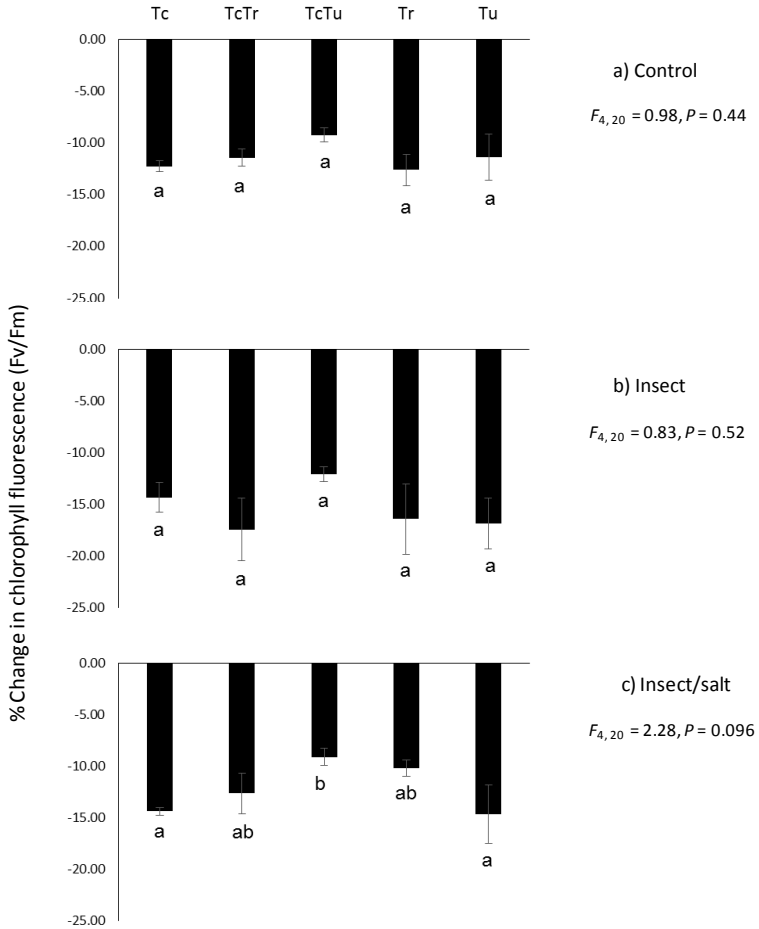


Figure 4.10: Percentage change in chlorophyll fluorescence (Fv/Fm) in *Tamarix* taxa after salt and insect treatment. **(a)** Control **(b)** Insect **(c)** Insect/salt treatment. Different lowercase letters indicate significant differences in the percentage change in chlorophyll fluorescence between treatment and control plants between the first and final day of Phase 2. (F_{stat} and *P*-value indicate overall difference between taxa for indicated treatment using one-way ANOVA; Fischer LSD test). n = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); n = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.2.4 Chlorophyll content

There was no significant difference in average chlorophyll content in the treated and control plants across all *Tamarix* taxa ($P = 0.79$) (Figure 4.11). The average total chlorophyll content in the insect/salt treated plants ranged from a minimum of 1.56 g/ml^{-1} (*T. usneoides*) to a maximum of 1.48 g/ml^{-1} (*T. chinensis x T. ramosissima*). The average total chlorophyll content in the insect treated plants ranged from a minimum of 1.02 g/ml^{-1} (*T. usneoides*) to a maximum of 1.42 g/ml^{-1} (*T. chinensis x T. ramosissima*).

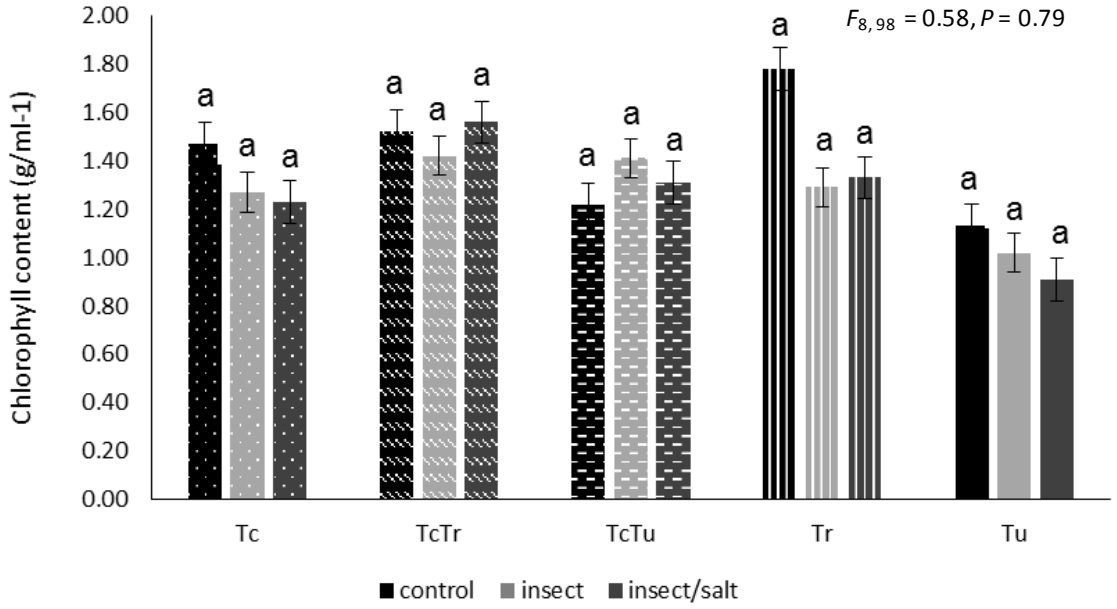


Figure 4.11: Total chlorophyll content (g/ml^{-1}) in the control, salt and insect/salt treated *Tamarix* taxa. Readings taken after Phase 2 on day 43. Different lowercase letters indicate significant differences in total chlorophyll content between treatment and control plants. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA; Fischer LSD test). $n = 5$ plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); $n = 10$ plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu) for each data point.

4.2.5 Plant growth

There were no significant differences in plant vigour noted between the various treatments (insect and insect/salt) across all *Tamarix* taxa (Figure 4.12) except for *T. chinensis* where the control plants showed an average percentage increase in branch length of 3.90% compared to the insect treatment (0.18%) ($P = 0.006$) and insect/salt treatment (0.33%) ($P = 0.008$). The treated plants (insect and insect/salt) showed reduced branch growth compared to the control plants for

all *Tamarix* taxa. Although this variation was not significant, it still indicates that the treatments had some effect on reducing plant growth.

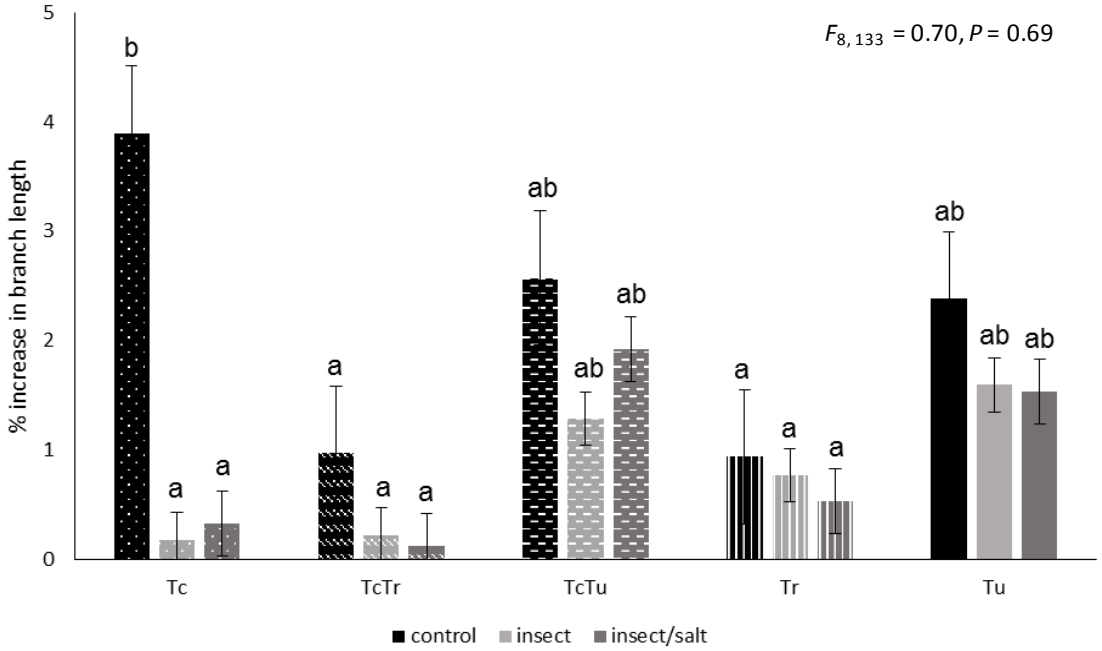


Figure 4.12: Percentage increase in length of the leading branch for the control, salt and insect/salt treated *Tamarix* taxa. Readings taken over a three week period over Phase 2 from day 21 to 42. Different lowercase letters indicate significant differences in the percentage increase in length of the leading branch between treatment and control plants. (F_{stat} and *P*-value indicate overall difference between treatment and control plants using factorial ANOVA; Fischer LSD test). *n* = 5 plants for *T. chinensis* (Tc) and *T. chinensis x T. ramosissima* (TcTr); *n* = 10 plants for *T. chinensis x T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu).

CHAPTER 5

5 DISCUSSION OF SALT EXCRETION AND LEAFHOPPER HERBIVORY INTERACTIONS IN *TAMARIX* SPECIES

5.1 Phase 1: Salt trial

5.1.1 Stomatal conductance *(g_s)*

The only *Tamarix* taxon to show a significant change in average g_s in the salt treated plants as compared to the respective control over the course of Phase 1 was *T. usneoides*, which showed a significant decrease in g_s in response to salt treatment. Stomatal conductance is the relative rate at which water vapour exits the stomata while CO_2 enters the leaf through the stomata (Craine et al., 2016). Stomatal conductance can be used as a proxy to assess photosynthetic rates in plants because it is reasonably correlated to plant photosynthesis and yield (Pask et al., 2012). Soil salinity has been shown to reduce g_s in halophytes, although less so than would be expected in non-halophytic plants (Shabala and Mackay, 2011). Since *Tamarix* are classified as halophytes (Brock, 1994; DiTomaso, 1998) this may indicate that *T. usneoides* was more affected by soil salinity than the other *Tamarix* taxa during Phase 1 of the experiment as a lower g_s implies a lower photosynthetic rate and increased plant stress. However, reductions in g_s may represent adaptive mechanisms to cope with soil salinity rather than an indication of a negative effect of salinity (Koyro, 2006; Flanagan and Jefferies, 1988; Clark et al., 1999). This indicates that the reduction in g_s in response to salinity by *T. usneoides* may be an adaptive mechanism to cope with salt stress.

5.1.2 Water potential (Ψ)

Water potential (Ψ) can be used as a proxy for plant water stress in vascular plants (Waring and Cleary, 1967), the more negative Ψ becomes the more stressed the plant is considered to be. *Tamarix usneoides* was the only *Tamarix* taxa that did not show a significant difference in Ψ throughout Phase 1. This may be due to the significant decrease in g_s shown by *T. usneoides*, since g_s is related to leaf Ψ by a feedback process, such that reductions in g_s prevent further decreases in Ψ by reducing transpiration (Gimenez et al., 2005). This indicates that although *T. usneoides* appears to be more affected by salt stress due to a significant decrease in g_s, *T.*

usneoides is able to maintain Ψ in response to the high saline condition. All the other *Tamarix* taxa showed a significant decrease in Ψ in the salt treated plants compared to the control plants by the end of Phase 1; this is an indication of the effect of salt stress on these plants. For comparison Bolaños and Longstreth (1984) reported average Ψ values of -2.20 Mpa in *Alternanthera philoxeroides* when exposed to salt concentrations of 200mM and found that Ψ decreased with increasing salt concentration. This is comparable with the Ψ shown in all *Tamarix* taxa except for *T. usneoides* and may indicate that *T. usneoides* uses different physiological mechanisms to adapt to salt stress, which is supported by the notion that halophytes show a diversity of growth responses to increasing salinity, from a dramatic stimulation to inhibition (Flowers and Colmer, 2008).

5.1.3 Chlorophyll fluorescence

Changes in chlorophyll fluorescence in response to salinity can indicate whether or not leaf metabolism has been affected by increases in soil salinity and can therefore be used as an indicator of plant stress (Smillie and Norr, 1982). Broetto et al. (2007) found that a salinity concentration of 400mM NaCl in the facultative halophyte *Mesembryanthemum crystallinum* had negligible adverse effects on its performance and that there was not any chronic photoinhibition (reduced potential quantum yield of photosystem II (F_v/F_m). Bacarin et al. (2011) found that there were no significant differences in chlorophyll fluorescence (F_v/F_m) in response to salinity in *Brassica napus* L., exposed to concentrations varying between 0 to 200mM. These results are largely reflected in this study as significant variations in chlorophyll fluorescence (F_v/F_m) in response to salinity at the end of Phase 1 (day 21) which was limited to *T. usneoides*, with all other *Tamarix* taxa showing no significant changes in average chlorophyll fluorescence in response to salinity. A significant decrease in the average chlorophyll fluorescence in the salt treated plants of *T. chinensis x T. ramosissima* was noted on day 14 of Phase 1, this physiological response is most likely due to the effects of salt stress, as is noted by Bacarin et al. (2011), the main effect of salt stress is to decrease photosynthesis which reduces CO_2 fixation rates and plant growth. Maricle et al. (2007) found that the processes responsible for harvesting solar energy in plants are mostly unaffected by increasing salinity in estuarine grasses (*Spartina atens, S. alterniflora, S. densiflora, and Distichlis spicata*) and that there is no notable relationship between fluorescence parameters and salt sensitivity in these species. A significant increase in

average chlorophyll fluorescence (F_v/F_m) in the salt treated *T. usneoides* plants as compared to the control in the final week of Phase 1 could therefore be due to *T. usneoides* retaining high functioning photosystems despite the salt treatment, since it is well adapted to high salt concentrations.

5.1.4 Electroconductivity

Heavy metal accumulation in the roots of *Tamarix* plants increases with increasing salinity (Manousaki et al., 2008). This is because salinity improves the availability of metals in sediments and stimulates transport of metals from the roots to the leaves of the plant (Fitzgerald et al., 2003). *Tamarix* taxa capable of withstanding higher salt concentrations and excreting more salt should be considered more effective phytoremediators as these plants would be capable of decreasing high soil salt concentrations and soils with high heavy metal concentrations. In this study *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa and should therefore be considered as possibly the *Tamarix* taxa best suited for phytoremediation, because it is capable of excreting more salt and will therefore be able to tolerate high soil salinity and potentially excrete more heavy metal contaminants. However, since this species is not indigenous to South Africa and is a declared invasive species (Marlin at al., 2017) propagation of this plant should not be advocated in South Africa.

5.1.5 Chlorophyll content

There was no significant difference in the total chlorophyll content between the salt treated and control plants across all *Tamarix* taxa ($P = 0.38$). It would appear that salinity had no impact on chlorophyll biosynthesis. This is comparable to Manousaki et al. (2008) who found total chlorophyll content in *Tamarix smyrnensis* was not significantly affected by salinity, with salt concentrations ranging from 0.0% to 3.0% NaCl. Brugnoli and Lauteri (1991) found that the primary effect of salt stress on photosynthesis is caused by stomatal closure and chloroplast reactions are not affected until after other plant processes have become significantly impacted. This may indicate that since the other plant processes were not significantly impacted by salt stress the chloroplast, and hence chlorophyll content, have remained unaffected and if the *Tamarix* were exposed to higher salt treatments for a longer time period chlorophyll content would eventually be impacted.

5.1.6 Plant growth

Halophytes show a variation of growth responses to high concentrations of soil salinity, from a dramatic stimulation to inhibition (Flowers and Colmer, 2008). The slight decrease in growth in some of the salt treated plants can be attributed to excess energy expended by the plants to transport salt from the roots to the salt glands, which results in less energy being available for plant growth (Manousaki et al., 2008). Salt treated *T. usneoides* plants showed the highest growth rate of the taxa tested. Although the higher growth rate of the salt treated *T. usneoides* plants compared to the control was not significant. This could still be used as an incentive to use *T. usneoides* for phytoremediation in South Africa as opposed to the exotic species or hybrids because this plant is capable of generating more biomass under saline conditions, and given that propagation and growth of *Tamarix* on contaminated land is a difficult (Wilson, et al., 2017). High plant biomass turnover is an important feature to the success of any phytoremediation technique (Newete and Byrne, 2016; Manousaki et al., 2008) as the biomass is able to return nutrients to the soil on decomposition. In addition, higher biomass yield significantly reduces the time needed to remediate contaminated soils (Jiang et al., 2015), and it is also recognized that the biomass resulting from phytoremediation processes can be used as locally produced renewable fuel for bioenergy and other bio-products (Volk et al., 2006).

5.2 Phase 2: Insect Trial

5.2.1 Stomatal conductance (g_s)

No significant variations in g_s were noted for any of the treatments in any of the taxa in Phase 2. Feeding by the *Tamarix* leafhoppers (*O. stactogalus*) appears to have had a negligible effect on g_s across all *Tamarix* taxa. Insect herbivory is expected to have an effect on g_s. Craine et al. (2016) found that the tamarisk leaf beetle (*Diorhabda carinulata*) increased g_s in *T. ramosissima* by about 14% over a period of three years and decreased chlorophyll content significantly. Their data indicated that beetle herbivory decreased photosynthesis and produced leaves that were unable to regulate water loss due to beetle defoliation. These findings are contiguous with Snyder et al. (2010) and (Pattison et al., 2011) who also found that *D. carinulata* increased stomatal conductance and inhibited water regulation in *Tamarix*. These studies imply that beetle herbivory reduces drought tolerance in *Tamarix*. In addition, Craine et al. (2016) found that *D. carinulata* caused a significant loss of photosynthetic area and suggested that *Tamarix* leaves

might increase chlorophyll production to improve photosynthesis in the remaining foliar tissues. Their results show that *D. carinulata* has a negative impact on photosynthetic potential and they suggest that a tradeoff exists between photosynthetic rate and drought response where individuals experiencing reduced photosynthetic potential must increase g_s to maintain carbon fixation rates. *Tamarix* leaves in the Craine et al. (2016) study responded to herbivory by decreasing chlorophyll content which required the *Tamarix* leaves to open stomata for extended periods of time increasing transpiration and water loss. In this study, *O. stactogalus* herbivory decreased g_s, albeit not significantly, and had no significant effect on chlorophyll content in all *Tamarix* taxa tested. This might indicate that the herbivory of *O. stactogalus* is not as effective as *D. carinulata* as it was unable to significantly reduce chlorophyll content, which would explain why g_s did not increase in the *Tamarix* taxa tested as the *Tamarix* had no need to increase photosynthetic potential.

5.2.2 Water potential (Ψ)

There were no significant variations in average Ψ for all treatments (insect and insect/salt) during Phase 2 of the experiment. This may indicate that insect herbivory has no effect on Ψ and that the effects of salt in the insect/salt treated plants was starting to stabilise as there were no significant changes in average Ψ experienced by the insect/salt treated plants indicating that the insects have no additional effect on average Ψ in the insect/salt treated plants. The insect treatment was the only treatment to show a positive percentage increase in Ψ across all *Tamarix* taxa. The effect of the insects on Ψ appears to have been negated by the presence of salt as the Ψ variations in the insect/salt treatment were far less pronounced than those seen in the insect only treatment.

5.2.3 Chlorophyll fluorescence

The insect treatment (*O. stactogalus*) had no notable effect on chlorophyll fluorescence in any of the *Tamarix* taxa during Phase 2 of the experiment. This would imply that *O. stactogalus* herbivory had no impact on the functionality of the photosystems of the selected *Tamarix* taxa in this study.

5.2.4 Chlorophyll content

There was no significant difference in total chlorophyll content in the treated (insect and insect/salt) and control plants across all *Tamarix* taxa. It would appear that *O. stactogalus* had no significant impact on chlorophyll biosynthesis in this study. This might be due to the limited time period allowed for *O. stactogalus* feeding on the *Tamarix* or might be an indication that *O. stactogalus* is not an effective biocontrol agent because it is unable to affect chlorophyll content as other insects have been shown to reduce chlorophyll content in *Tamarix* leaves. Craine et al. (2016); Snyder et al. (2010) and Pattison et al. (2011) all found that *D. carinulata* herbivory on *Tamarix* decreases chlorophyll content.

5.2.5 Plant growth

The only significant change in plant vigour was seen in *T. chinensis* where the average percentage increase in branch length for the control was significantly higher than the treatment (both the insect and insect/salt treated plants). All other *Tamarix* taxa showed no significant increase in percentage growth in branch length in the control plants as compared to the treatment plants. This indicates that the treatments (insect and insect/salt) had a greater impact in decreasing the growth rate of *T. chinensis* as compared to the other taxa. This might be explained by *T. chinensis* excreting the most salt of the *Tamarix* taxa tested; hence *T. chinensis* expelled more energy excreting salt and had less available energy for growth. Excess energy is expended by *Tamarix* to transport salt from the roots to the salt glands, which results in less energy being available for plant growth (Manousaki et al., 2008).

CHAPTER 6

6 Conclusions

Heavy metal accumulation in the roots of *Tamarix* plants increases with increasing salinity (Manousaki et al., 2008). This is because salinity improves the availability of metals in sediments and stimulates transport of metals from the roots to the leaves of the plant (Fitzgerald et al., 2003). *Tamarix* taxa capable of withstanding higher salt concentrations and excreting more salt should be considered more effective phytoremediators as these plants would be capable of decreasing high soil salt concentrations and soils with high heavy metal concentrations. In this study, *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa and should therefore be considered the *Tamarix* taxa best suited for phytoremediation. Although the indigenous *Tamarix* spp. (*T. usneoides*) excreted the same amount of salt as *T. ramosissima, T. chinensis x T. ramosissima* and *T. chinensis x T. usneoides*, the salt treated *T. usneoides* plants showed the highest growth rate (plant vigour) albeit not significantly so, this could still stand as an incentive to use *T. usneoides* for phytoremediation in South Africa as opposed to the exotic taxa as this plant is capable of generating more biomass under saline conditions especially considering that propagation and growth of *Tamarix* on contaminated land is a challenge (Wilson et al., 2017), and since high plant biomass turnover is considered an important feature to the success of phytoremediation (Newete and Byrne, 2016; Manousaki et al., 2008).

Tamarix usneoides was the only *Tamarix* taxa to show a significant decrease in stomatal conductance (g_s) in response to salt treatment, however in Phase 2 of the experiment *T. usneoides* plants treated with salt and inoculated with *O. stactogalus* did not show a significant decrease in g_s when compared to the other *Tamarix* taxa. This may indicate that *T. usneoides* was affected by salt stress initially but then employed physiological mechanisms to mitigate negative impacts, this is further supported by (Koyro, 2006; Flanagan and Jefferies, 1988; Clark et al., 1999) who suggest that reductions in g_s are indicative of adaptive mechanisms to cope with salt stress. *Tamarix usneoides* was the only *Tamarix* taxa that did not show a significant decrease in Ψ throughout Phase 1. This could be explained by the feedback relationship between g_s and Ψ, where reductions in g_s prevent further decreases in Ψ by reducing transpiration. This may indicate that *T. usneoides* was the least salt stressed plant. *T. usneoides* was also the only

Tamarix spp. to show a significant increase in average chlorophyll fluorescence in the salt treated plants compared to the control plants. This is an indication that the photosystems in *T. usneoides* were unaffected by high salt concentrations and were able to operate more effectively under high salt concentrations than the other *Tamarix* taxa.

Although *T. chinensis* excreted the most salt and is therefore likely to be the most effective phytoremediator, this should not incentivize the use of *T. chinensis* for phytoremediation above the indigenous *T. usneoides* since the latter appears to be the *Tamarix* spp. least affected by high concentrations of salt making it an ideal candidate for phytoremediation in South Africa.

The reason soil salinity has generally not had a significant effect on the *Tamarix* taxa selected for this study, in terms of chlorophyll content, physiological responses and plant vigour, is likely due to the halophytic nature of the *Tamarix* which allows the plant to expel salt through its salt glands. Salt excretion via salt glands is considered to be a key mechanism contributing to salt resistance in halophytes ((Brotherson and Field, 1987; Flowers et al., 1977; Manousaki et al., 2008). Salt secreting halophytes are adapted to soil salinity by three mechanisms: salt avoidance (roots have low permeability to salts); salt tolerance (capability to survive with high intercellular salt levels); salt evasion (excretion of salt) (Manousaki et al., 2008). In comparison, non-halophytes are unable to synchronize compartmentation of ions (salt evasion) within individual leaf cells and any reductions in photosynthesis in response to high salt concentrations is highly likely to be a consequence of reduced growth caused by high salinity (Munns and Tester, 2008).

Although it is likely that soil salinity did not have a significant effect on the *Tamarix* taxa in this study due to the halophytic nature of *Tamarix* (as indicated above), it is noted that using a higher salt concentration for a longer time period is likely to result in more significant physiological responses. However, since Manousaki et al. (2008) found that the *Tamarix* died before the end of the experiment when using a salt concentration of 200 $mmol^{-1}$ a less potent concentration of 180 $mmol^{-1}$ was chosen for this study.

The amount of photosynthetic pigment (total chlorophyll content) was not significantly affected by either the presence of salt or the inoculation of *O. stactogalus*. Plant growth, expressed as a

percentage increase in the length of the leading branch, was also not significantly affected by the addition of salt to the soil nor the inoculation of *O. stactogalus*. Salt did not appear to have a significant effect on the herbivory of *O. stagtogalus* as chlorophyll content in the insect/salt treatment did not differ significantly from the insect treatment. *Opsius stagtogalus* also had no significant effect on plant growth. Therefore, the results indicate that photosynthetic potential was not negatively impacted by *O. stagtogalus* and salt did not affect the herbivory of *O. stagtogalus*.

In conclusion, the effects of salt on *Tamarix* show that *T. usneoides* should be used as a phytoremediator on mine sites instead of the exotic *Tamarix* taxa because *T. usneoides* shows traits indicative of a suitable and promising phytoremediator. *Opsius stactogalus* does not appear to be a suitable biocontrol agent for the control of *Tamarix* taxa and appears to be unaffected by high salinity in the *Tamarix* taxa tested.

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