Poster presentation

Impact of the sap-feeding *Opsius stactogalus* is not influenced by elevated salt concentrations in five different *Tamarix* taxa

Solomon W. Newete[1,2]*, Stawm Allem[2], Nic Venter[2], Marcus J. Byrne[2,3]

[1]*Agricultural Research Council-Institute for Soil, Climate and Water, Geo-Information Science Program, Pretoria, South Africa; Newetes@arc.agric.za/solomonnewete@gmail.com*
[2]*University of the Witwatersrand, School of Animal, Plant and Environmental Sciences, Johannesburg, South Africa*
[3]*DST-NRF, Centre of Excellence for Invasion Biology, Johannesburg, South Africa*

Abstract

Salt cedar, *Tamarix*, is a genus of many Eurasian shrubby tree species in the family Tamaricaceae that is represented by one native (*Tamarix usneoides* E.Mey.) and two exotic (*Tamarix ramosissima* Ledeb. and *T. chinensis* Lour.) species in South Africa. The exotic *Tamarix* spp. have become invasive in South Africa and are targeted for biological control. *Tamarix* spp. are tolerant to extreme soil salinity, but it is unknown how elevated salt levels in the plant will affect coevolved insect herbivores. This study investigated whether elevated salt concentrations influenced the impact of the leafhopper *Opsius stactogalus* on five different *Tamarix* taxa. The results showed that *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa. The high level of salt in the plants' tissues, however, did not show any significant effect on the feeding performance of *O. stactogalus* and subsequently on plant growth. There are about 25 insects that have evolved to strictly feed and survive on the halophytic *Tamarix* species, and among these are *O. stactogalus* and the *Tamarix* beetle (*Diorhabda* spp). The fact that the feeding performance of *O. stactogalus* across the five *Tamarix* taxa in this study was not significantly affected by salt addition may suggest that the *Tamarix* beetle (*Diorhabda* spp.), which is currently under quarantine investigation in South Africa, will not be influenced by the various salt concertation levels of the two exotic *Tamarix* species or their putative hybrids either.

Keywords: *Tamarix*, phytoremediation, *Opsius stactogalus*, electroconductivity, *Diorhabda*

Introduction

The exotic saltcedar, *Tamarix* spp., native to the Old World (Eurasia) and the northern (e.g., Morocco, Senegal), eastern and southern regions of Africa (Marlin et al., 2017), is listed as one of the 100 most problematic invasive species in the world (Lowe et al., 2000). Its invasion is most pervasive in the USA, Mexico, Argentina, Australia and South Africa, among others (Marlin et al., 2017; Newete et al., 2019). According to the National Environmental Management: Biodiversity Act 2014, the plant is declared as a category "1b" weed in South Africa, necessitating its immediate removal (Henderson 2001; Newete et al., 2019). This phreatophitic shrubby tree is well known for its adaptation to extreme saline soils, which is believed to give *Tamarix* a competitive advantage over many co-existing riparian native species, besides its tolerance to inundation, fire and drought combined with rapid seed germination and dispersion abilities (Brock 1994; Natale et al., 2010). Morphological identification of *Tamarix* species is often confounded by the cryptic hybrids resulting from hybridization between the species in the genus which in the past has led to misclassification of many *Tamarix* species. The advent of advanced molecular analysis techniques has, however, allowed accurate discrimination between *Tamarix* taxa; *Tamarix ramosissima* Ledeb. and *T. chinensis* Lour. have been recognized as the prominent invaders in the USA, South Africa and many other countries in the world and have a marked impact on riparian ecosystems and biodiversity (Zavaleta et al., 2001; Natale et al., 2010; Newete et., 2019). With the indigenous *T. usneoides* E.Mey., a total of three *Tamarix* species are currently known to occur in South Africa (Mayonde et al., 2015; Newete et al., 2019). The proper identification of *Tamarix* species is particularly important for the potential introduction of biocontrol agents.

There is no clear record of when and how the two exotic *Tamarix* species, *T. ramosissima* and *T. chinensis*, were introduced to South Africa, but they were most likely introduced in the early 1900s as ornamental or phytoremediation plants in mining sites (Marlin et al., 2017; Newete et al., 2019). *Tamarix ramosissima* and *T. chinensis* were the two most commonly planted species in mining sites across the country at least until 2005 when strict regulation was enforced to prohibit their further use, promoting the native *T. usneoides* instead. *Tamarix* are capable of removing mining contaminants such as sodium, manganese, potassium, calcium, nitrate, copper, sulphur, aluminium, silica, barium and lithium salts (DiTomaso, 1998). This raises the question as to whether salt might affect the feeding performance of herbivorous insects and thus their effectiveness as potential biocontrol agents. Since no biological control agents have been released yet in South Africa, the cosmopolitan *Tamarix* leafhopper *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae) was used as a surrogate to test this hypothesis.

The taxonomic isolation of *Tamarix* has allowed many insect species to co-evolve 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*, of which the *Tamarix* leafhopper, *Opsius stactogalus*, is found exclusively on *Tamarix* plants (Virla et al., 2010). This cosmopolitan small-sized (0.81–4.5mm) insect is native to Europe and takes about a month to develop from a neonate to an adult through five instars (Harding, 1930). The *Opsius* spp. are known to have 3–4 generations depending on the species and the altitude where they occur (Louden, 2010; Siemion and Stevens, 2015). Although *O. stactogalus* is often found in high numbers, damage caused by the leafhopper is generally considered insignificant (DeLoach et al., 2004; Virla et al., 2010) and, therefore, it is not regarded to be a viable biocontrol agent. High concentrations of salts or metals in plant tissues is known to deter herbivory in some insects (Coleman et al., 2005; Boyd, 2010; Davis et al., 2001; Newete et al., 2014). But such information is not available for the *Tamarix* leafhopper. This study investigated the effect of *O. stactogalus* on *Tamarix* spp. with and without elevated salt concentrations as a surrogate for potential biological control agents.

Methods and materials

Five *Tamarix* taxa consisting of three pure *Tamarix* species and two hybrids, identified by Mayonde et al. (2015), were propagated from 15 cm cuttings in a greenhouse at the University of the Witwatersrand, Johannesburg (Table 1). Cuttings were first dipped in root growth hormone (Dynaroot Hormone powder Efekto) and rooted in river sand and allowed to grow for three months until the roots and leaves grew well. The saplings were then transferred into 1-liter plastic pots with potting soil and allowed to acclimate for two months before the experiment began. Five to ten plants from each taxon were used as controls (no salt or leafhopper), and ten for each of leafhopper only and leafhopper + salt treatments. Five replicates were used for *T. chinensis* and *T. chinensis* x *T. ramosissima* due to cutting (sapling) mortality before the start of the experiment.

Salt treatment and measurement

Salt water was prepared by adding 480 g of table salt to 16 liters of tap water to make a salt concentration of 3% (w/w). A volume of 400 ml of this salt water was added to each pot (i.e. 180 mmol-1) over four days (100 ml per day) to reduce physiological shock to the plants. Control treatments received only tap water

Table 1: The five *Tamarix* taxa, with their respective number of replicates, used in this study. Note: For the "leafhopper + salt" treatment, the plants were first exposed to salt for 21 days before the leafhoppers were added.

Tamarix taxa	Treatments			Total Replicates
	Control	Leafhopper	Leafhopper + Salt	
T. chinensis (Tc)	5	5	5	15
T. ramosissima x *T. chinensis* (TrTc)	5	5	5	15
T. chinensis x *T. usneoides* (TcTu)	10	10	10	30
T. ramosissima (Tr)	10	10	10	30
T. usneoides (Tu)	10	10	10	30

(see Table 1). Salt uptake and excretion was recorded from approximately 0.2 g of leaf sample harvested from the leading branch of each *Tamarix* before the inoculation of the *Tamarix* leafhopper on day 21. The harvested leaf biomass was placed in a test tube containing 10 ml deionized water and swirled for 1 minute before measuring salt content of the water using an electro-conductivity meter.

Insect inoculation and plant measurement

A total of ten *Tamarix* leafhoppers collected from a stand of *T. ramosissima* in Germiston, Johannesburg (26°12'37.80"S and 28°2'12.06"E) in October 2016 were inoculated onto the longest branch of each potted plant (5–10 plant replicates per *Tamarix* taxa as indicated in Table 1), and sealed with a small organza net bag. The change in length of the leading branch of each sapling was measured between day 1 and 42.

Results

Electro-conductivity

Salt excretion of salt-treated plants was significantly higher (> 50%) than salt excretion of controls ($F_{4, 66} = 8.1$, $P = 0.0002$) (Figure 1). *Tamarix chinensis* showed the highest electro-conductivity of all the *Tamarix* taxa tested.

Plant growth

Plants onto which leafhoppers were released showed reduced branch growth compared to control plants for all *Tamarix* taxa (Figure 2). However, this difference was only significant for *T. chinensis*, where leafhopper feeding reduced average percentage increase in branch length by 90.6% compared to that of the control (Figure 2). The control plants showed an average percentage increase in branch length of 3.9% compared to those in the leafhopper treatment (0.2%) (P = 0.006) and leafhopper/salt treatment (0.3%) (P = 0.008). The reduction in plant growth caused by the leafhoppers was not significantly different on *Tamarix* plants with or without elevated salt concentrations.

Discussion

Electro-conductivity

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). In this study *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa. However, since this species is not indigenous to South Africa and is a declared invasive species (Marlin at al., 2017), propagation of this plant for phytoremediation should not be promoted in South Africa. Leaves of all the other taxa, including the indigenous *T. usneoides*, also excreted significant amounts of salts.

Plant growth

None of the *Tamarix* taxa tested showed a significant increase in branch length between the leafhopper and leafhopper + salt treatment plants suggesting no effect of salt on leafhopper feeding.

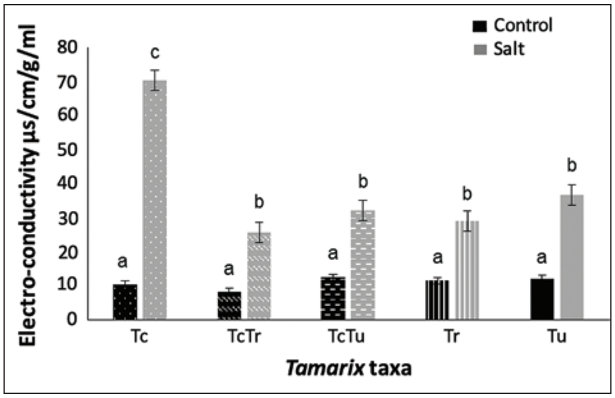

Figure 1. The five *Tamarix* taxa, with their respective number of replicates, used in this study. Note: For the "leafhopper + salt" treatment, the plants were first exposed to salt for 21 days before the leafhoppers were added.

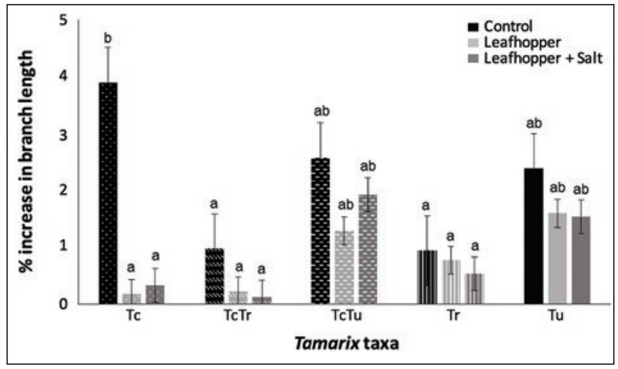

Figure 2. Percentage increase in length of the leading branch of *Tamarix* taxa taken before insect inoculation in day 21 and 21 days after inoculation. Means compared by one-way ANOVA; those followed by the same letter(s) across all bars are not significantly different ($P > 0.05$; Fisher's Least Significant difference 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).

The only significant change in plant vigor was seen in *T. chinensis* where the increase in branch length of the control was significantly greater than those in the leafhopper or the leafhopper + salt treated plants. This indicates that the treatments (leafhopper and leafhopper + 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 all the *Tamarix* taxa tested. 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).

Conclusions

In conclusion, leafhopper impacts were not significantly affected by the addition of salt. There are about 25 insects that have evolved to strictly feed and survive on the halophytic *Tamarix* species, and among these are *O. stactogalus* and the *Tamarix* beetle (*Diorhabda* spp). The fact that the feeding performance of *O. stactogalus* across the five *Tamarix* taxa in this study was not significantly affected by salt addition may suggest that the *Tamarix* beetle, which is currently under quarantine investigation in South Africa, will not be influenced by the various salt concentration levels of the two exotic *Tamarix* species or their putative hybrids. Although high levels of salt and heavy metals in different plants are known to reduce or deter insect herbivory (Davis et al., 2001; Coleman et al., 2005; Boyd, 2010; Newete et al., 2014), the insects that feed on *Tamarix* seem to circumvent such physiological stress, since they have evolved and adapted to feed and thrive on strictly halophytic plants (Kovalev 1995).

Acknowledgments

We would like to extend our special gratitude to Wits School of Governance (WSG) at the University of the Witwatersrand for the financial support to attend and present the findings of this study at the XVth International Symposium on Biological Control of Weeds in Engelberg, Switzerland through the "Life in City" project (Project #: 2858), to the ISBCW team, and Hariet Hinz in particular, for all the support and review of these proceedings.

References

Boyd, R.S., 2010. Heavy metal pollutants and chemical ecology: exploring new frontiers. J. Chem. Ecol. 36, 46–58. https://doi.org/10.1007/s10886-009-9730-5

Brock, J.H., 1994. *Tamarix* spp. (salt cedar), an invasive exotic woody plant in arid and semi-arid riparian habitats of western USA. In: de Waal, L.C., Child, L.E., Wade, P.M., Brock, J.H. (Eds.), Ecology and Management of Invasive Riverside Plants, Chichester: Wiley, pp. 27–44.

Coleman, C.M., Boyd, R.S., Eubanks, M.D., 2005. Extending the elemental defense hypothesis: dietary metal concentration below hyperaccumulator levels could harm herbivores. J. Chem. Ecol. 31 (8), 1669–1681. https://doi.org/10.1007/s10886-005-5919-4

Davis, M.A., Pritchard, S.G., Boyd, R.S., Prior, S.A., 2001. Developmental and induced responses of nickel-based and organic defences of the nickel hyperaccumulating shrub, *Psychotria douarrei*. New Phytol. 150, 49–58.

DeLoach, C.J., Carruthers, R.I., Dudley, T.L., Eberts, D., Kazmer, D J., Knutson, A. E., Bean, D. W., Knight, J., Lewis, P.A., Milbrath, L.R., Tracy, J.L., Tomic-Carruthers, N., Herr, J.C., Abbott, G., Prestwich, S., Harruff, G., Everitt, J.H., Thompson, D.C., Mityaev, I., Jashenko, R., Li, B., Sobhian, R., Kirk, A.. Robbins, T.O., Delfosse, E.S., 2004. First results for control of saltcedar (Tamarix spp.) in the open field in the western United States. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K. (Eds.), Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Entomology, Canberra, Australia, pp. 505–513.

DeLoach, C.J., Carruthers, R.I., Lovich, J.E., Dudley, T.L., Smith, S.D., 2000. Ecological interactions in the biological control of Saltcedar (*Tamarix* spp.) in the United States: toward a new understanding. In: Spencer, N. R. (Ed.), Proceedings of the X International Symposium on Biological Control Weeds. Montana State University, Bozeman, Montana, U.S.A., pp. 819–873.

DiTomaso, J.M., 1998. Impact, biology, and ecology of Saltcedar (*Tamarix* spp.) in the Southwestern United States. Weed Technol. 12(2), 326–336.

Harding, L., 1930. The Biology of *Opsius stactogalus* Fieber (Homoptera, Cicadellidae). J. Kans. Entomol. Soc. 3(1), 7–22.

Henderson, L., 2001. Alien weeds and invasive plants: A complete guide to declared weeds and invaders in South Africa. Agricultural Research Commission, Cape Town, South Africa.

Kovalev, O.V., 1995. Co-evolution of the tamarisks (Tamaricaceae) and pest arthropods (Insecta; Arachnida: Acarina), with special reference to biological control prospects. Pensoft Publishers, Moscow, Russia.

Louden, N.P., 2010. Asymmetric Interspecific Competition Between Specialist Herbivores That Feed On Tamarisk In Western Colorado. Master thesis, Utah State University.

Lowe, S. Browne, M., Boudjelas, S. and De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12 pp. First published as special lift-out in Aliens 12, December 2000. Updated and reprinted version: November 2004.

Manousaki, E., Kadukova, J., Papadantonakis, N., Kalogerakis, N., 2008. Phytoextraction and phytoexcretion of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non-saline and saline soils. Environ. Res. 106(3), 326–332.

Marlin, D., Newete, S.W., Mayonde, S.G., Smit, E.R., Byrne, M.J., 2017. Invasive *Tamarix* (Tamaricaceae) in South Africa: current research and the potential for biological control. Biol. Invasions 1–22.

Mayonde, S.G., Cron, G.V., Gaskin, J.F., Byrne, M.J. 2015. Evidence of *Tamarix* hybrids in South Africa, as inferred by nuclear ITS and plastid trnS-trnG DNA sequences. S. Afr. J. Bot. 96, 122–131.

Natale, E., Zalba, S.M., Oggero, A., Reinoso, H., 2010. Establishment of *Tamarix ramosissima* under different conditions of salinity and water availability: implications for its management as an invasive species. J. Arid Environ. 74, 1399–1407.

Newete, S.W., Erasmus, B.F.N., Weiersbye, I.M., Byrne, M.J., 2014. The effect of water pollution on biological control of water hyacinth. Biol. Control 79 (2014) 101–109.

Newete, S.W., Mayonde, S., Byrne, M.J., 2019. Distribution and abundance of invasive *Tamarix* genotypes in South Africa. Weed Res. https://doi.org/10.1111/wre.12356.

Siemion, G.M., Stevens, L.E., 2015. Interactions among *Tamarix* (Tamaricaceae), *Opsius stactogalus* (Cicadellidae), and Litter Fungi Limit Riparian Plant Establishment. Advances in Entomology, 2015, 3, 65–81.

Virla, E.G., Logarzo, G.A., Paradell, S.L., 2010. Occurrence of the *Tamarix* Leafhopper, *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae), in Argentina. J. Insect Sci. 10(23), 23.

Zavaleta, E.S., Hobbs, R.J., Mooney, H.A., 2001. Viewing invasive species removal in a whole-ecosystem context. Trends Ecol. Evol. 16 (8): 454–459.