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The influence of plant phenolic compounds on the host range of Coniatus tamarisci Fabr. (Coleoptera: Curculionidae): a potential biological control agent of invasive Tamarix L. (Caryophyllales: Tamaricaceae) Taxa in South Africa

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The Influence of Plant Phenolic Compounds on The Host Range of *Coniatus tamarisci* Fabr. (Coleoptera: Curculionidae): A Potential Biological Control Agent of Invasive *Tamarix* L. (Caryophyllales: Tamaricaceae) Taxa in South Africa



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A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science, December 2022

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Declaration

I declare that this thesis* is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy** at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



_____

(Signature of candidate)

_____day of ____December____2022____at__Johannesburg_____

* dissertation or research report as applicable

** or Master of Science as applicable.

Dedication

"To win in spite of struggle implies much greater magnification…" – The Internet

This dissertation is dedicated to my late grandmother, Elsie N. Hatile, who taught me the importance of education through her words, "*Akho mntwana wam ozokuba liqaba*", but will sadly never get to see what uNtemekane has accomplished. To my grandfather, David K. Hatile, *njenge ndoda ephume kwezozandla yonke ethi yenzwa zezi zam yeyakho, nalona ngumsebenzi wakho, enkosi Tata*. To my mother, Noluthando Hatile, all I have to say is uMarko 1 and thank you.

Perseverance wins the crown.

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Abstract

Tamarix L. (Caryophyllales: Tamaricaceae) are halophyte species that are native to Eurasia, North Africa, and southwestern Africa. These phreatophyte trees or shrubs have become prominent and widespread invaders in North America, South America, Australia, and South Africa. In South Africa, the Alien and Invasive Species regulations of the National Environmental Management: Biodiversity Act 2014 (NEM:BA) has classified invasive *Tamarix* as category 1b invader, which require control. Thus, three potential biological control agents have undergone laboratory-based host-specificity trials for the long-term sustainable control of *Tamarix.*

The first two of these biocontrol agents, *Diorhabda carinulata* (Desbrochers.) (Coleoptera: Chrysomelidae) and *Trabutina mannipara* (Hemprich & Ehrenberg) (Hemiptera: Pseudococcidae), both failed the laboratory-based host-specificity trials because they completed their life cycle on the indigenous *T. usneoides* E. Mey ex Bunge. Previously, potential biocontrol agents were selected based on their native distribution and the phylogenetic relatedness of the invasive weed to indigenous nontarget species. However, it has recently been suggested that secondary metabolites also play a major role in insect host selection, and thus should be considered to improve the selection criteria of potential biocontrol agents.

The current study is based on the third biocontrol agent that recently underwent laboratory-based host-specificity trials in South Africa, *Coniatus tamarisci* (Fabr.) (Coleoptera: Curculionidae). This was in conjunction with an analysis of the potential influence *Tamarix* phenolic compounds have on insect host selection. The results show that although *C. tamarisci* could complete its' life cycle on *T. usneoides*, the weevil had a low affiliation/risk

associated with the indigenous *Tamarix* taxon. This advocates for the conduction of open field host-specificity trials, which will allow for a better understanding of *C. tamarisci* behaviour in a natural setting. Regarding phenolic compounds, three phenolic acids have been identified as being significantly prominent in *T. usneoides* compared to the invasive *Tamarix* taxa present in South Africa. These are gallic acid, dehydrodigallic acid, and syringic acid. These compounds are associated with protection from plant herbivory, which could explain the behaviour of *C. tamarisci* when exposed to and reared on *T. usneoides*.

Keywords: Biological control, host specificity, invasive *Tamarix* taxa, phenolic compounds, secondary metabolites.

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Acronyms and Abbreviations

Name	Description
ANOVA	Analysis of variance
CAF	Central Analytical Facilities
CO_2	Carbon Dioxide
ERH	Enemy Release Hypothesis
HPLC	High Performance Liquid Chromatography
IAP	Invasive Alien Plant
LC–MS	Liquid Chromatography – Mass Spectrometry
NEM:BA	National Environmental Management: Biodiversity Act 2014
PCA	Principal Component Analysis
TLC	Thin -layer Chromatography
WfW	Working for Water

Chapter One

General Introduction and Literature Review

1.1. Invasive Alien Plants

1.1.1. The arrival of invasive alien plants

Invasive alien plants (hereafter, IAPs) are characterized by their ability to survive and eventually dominate in regions outside their native geographical ranges (Vermeij, 1996). These plants are introduced to new regions through agriculture, forestry, mariculture, horticulture, recreation, and unintentionally (Vitousek *et al*., 1997; Reichard & White, 2001). For example, *Pontederia crassipes* (Mart.) (Commelinales: Pontederiaceae) (commonly known as water hyacinth) and *Pistia stratiotes* L. (Alismatales: Araceae) (i.e., water lettuce) were introduced from South America into areas of Japan as popular ornamentals and for water purification (Miyawaki & Washitani, 2004). In the 1900s, water hyacinth was also introduced into South Africa as an ornamental plant (Cilliers, 1991). In some instances, migratory frugivorous animals consume IAP seeds and relocate them to a new region (Ansong *et al*., 2019). This is considered a secondary pathway of unintentional dispersal as the initial introduction is facilitated by human activity . Invasive alien plant seeds are also unintentionally dispersed via footwear and vehicles (Barney *et al*., 2006; Veldman & Putz, 2010).

Invasive alien plants are renowned for their ability to escape confinement and integrate themselves into novel ecosystems (Bossard *et al*., 2000; Blackburn *et al*., 2011; Hoffmann & Courchamp, 2016). After escape, IAPs often spread and negatively affect surrounding native biodiversity (Vitousek *et al*., 1997; Miyawaki & Washitani, 2004; Lazzaro *et al*., 2020).

1.1.2. The establishment and spread of invasive alien plants

For alien plants to be considered 'invasive', they need to establish themselves and expand their ranges post introduction (Blackburn *et al*., 2011; Hoffmann & Courchamp, 2016). Blackburn *et al.* (2011) provide a unified framework on the barriers alien species need to overcome in order to become invasive. The framework suggests that post introduction, alien plants must: survive, successfully reproduce, and disperse their propagules outside the initial point of introduction to be considered invasive (Blackburn *et al*., 2011).

Invasive alien plants often possess physiological, genetic, and biochemical characteristics that ensure their survival in the new environment (Callaway & Aschehoug, 2000; Ruwanza, 2021). This is often to the detriment of native biota. For example, invasive *Solanum mauritianum* (Scopoli.) (Solanales: Solanaceae) has been reported to form monostands that result in the displacement of surrounding biota (Olckers, 2011; Atkinson *et al*., 2013; Ruwanza, 2021). In the eastern parts of South Africa, *S. mauritianum* invades forestry plantations where it displaces surrounding *Pinus Patula* (Schiede ex Schltdl. & Cham.) (Pinales: Pinaceae) amongst other effects (Atkinson *et al*., 2013).

The absence of natural enemies in colonized areas also aids in the proliferation of IAPs (Keane & Crawley, 2002; Vilà *et al*., 2005; Heger & Jeschke, 2014). This is referred to as the Enemy Release Hypothesis (ERH) and was proposed by the Swiss botanist Albert Thellung in 1915 (Heger & Jeschke, 2014). Vilà *et al.* (2005) tested this hypothesis by showing that invasive *Hypericum perforatum* L. (Malpighiales: Hypericaceae) populations occurred at greater densities with less damage by insect herbivores in their newly found environment than in their native region.

In addition to the ERH, IAPs possess advantageous characteristics, such as allelochemicals, that have been suggested to affect their establishment and spread (Callaway & Aschehoug, 2000; Ruwanza, 2021).

Unfortunately, successful IAPs are a financial burden globally (Luque *et al*., 2014; van Wilgen *et al*., 2020). The economic cost of IAPs is estimated at US$ 300 billion per year worldwide (Luque *et al*., 2014; van Wilgen *et al*., 2020). Consequently, countries like South Africa invest approximately ZAR 2 billion (US$ 118 million) per year in the control of IAPs (van Wilgen *et al*., 2020).

1.2. Biological control of invasive alien plants

Biological control (biocontrol) is a pest management strategy that relies on the human mediated release of host specific natural enemies (mainly insects, mites, and fungal pathogens) to control the spread of invasive alien organisms (McFadyen, 1998; Zachariades *et al*., 2017). This strategy is considered to be an environmentally friendly and cost-effective alternative to both mechanical and chemical control (Blossey *et al*., 1994; Zachariades *et al*., 2017). Globally, 1555 biocontrol agents have been released against 175 target environmental weeds (Schwarzländer *et al*., 2018). South Africa has released 136 classical biocontrol agents against 90 IAP species, with 92 agents established in the field on the target IAPs (Zachariades, 2021).

The biggest criticism directed towards the use of biocontrol is that released agents could exhibit nontarget effects (Marohasy, 1998; van Wilgen *et al*., 2004; Winston *et al*., 2014; Schwarzländer *et al*., 2018; Hinz *et al*., 2020). Nontarget affects are unwanted impacts caused by intentionally released natural enemies on nontarget species (Suckling & Sforza, 2014; Van Driesche & Hoddle, 2016; Schwarzländer *et al*., 2018; Hinz e*t al*., 2020; Peterson *et al*., 2020). However, Briese (2000) provides a framework for biocontrol implementation where

emphasis is placed on conducting host-specificity tests before releasing a potential biocontrol agent to avoid nontarget effects.

Briese's (2000) framework is divided into six steps, namely to: (1) study the ecology of the target weed in its native region, (2) investigate the potential biocontrol agents in its native region, (3) import and quarantine the potential biocontrol agents, (4) evaluate the host range of the potential agent (while in quarantine), (5) release and evaluate the biocontrol agent's effectiveness in the field and impact on target weed (once adequate evidence shows the potential agent to be host specific), and (6) distribute tested biocontrol agents throughout the invaded regions.

If followed correctly, the steps maximize the impact biocontrol agents have on target weeds, while minimizing the occurrence of nontarget effects (Briese, 2000).

1.3. Nontarget effects

Nontarget effects are classed into indirect and direct impacts (Hinz *et al*., 2020). Indirect impacts are difficult to predict and assess, hence they are rarely studied (Hinz *et al*., 2020). However, examples of indirect impacts have been reported, for example,the successful *Diorhabda* species, released against invasive *Tamarix* taxa in the United States. The successful control of *Tamarix* was overshadowed by the indirect impacts on the endangered southwestern willow flycatcher, which prefers *Tamarix* stands as a habitat for reproduction (Bean & Dudley, 2018). Direct impacts are more obvious and can be categorized into five types i.e., direct attack on other biocontrol agents, direct attack on native plant species, outcompeting native herbivores, benefiting nontarget plant species, and hybridization with native plant species (Van Driesche & Hoddle, 2016). Some examples of direct impacts include the attack by *Rhinocyllus conicus* (Frölich.) (Coleoptera: Curculionidae) and *Larinus carlinae* (Olivier.) (Coleoptera: Curculionidae) on native *Cirsium* thistle species, as well as

attacks by *Cactoblastis cactorum* (Berg.) (Lepidoptera: Pyralidae) on some native *Opuntia* species in the United States (Schwarzländer *et al*., 2018). Fortunately, less than one percent of direct nontarget impacts, which are a result of biocontrol programmes, lead to population threatening effects (Hinz *et al*., 2020).

1.4. Host range selection

The fundamental host range of herbivorous insects constitutes a list of plants these insects could feed and complete their life cycle on (Schaffner, 2001). This host range can be divided into two groups, namely, the physiological and realized host ranges (Schaffner, 2001). Physiological host range represents the list of plants that the herbivorous insect can complete its immature development on, while the realized host range refers to a list of plants that have usually co-evolved with the herbivorous insects and are used by the insects under natural conditions (Schaffner, 2001).

1.5. Host-specificity trials

In the implementation of biocontrol, host-specificity trials are used to estimate the fundamental host range of potential biocontrol candidates (Marohasy, 1998). Commonly, monophagous insects and pathogens are selected as potential biocontrol agents to in order to avoid nontarget effects (Schaffner, 2001). During host specificity trials, the feeding intensity and oviposition behaviour of monophagous insects on taxonomically closely related plant species is monitored to predict potential host range expansions (Marohasy, 1998). This is because insects are most likely to select taxonomically closely related plants as hosts, because such plants are both morphologically (i.e., leaf morphology) and chemically similar (i.e., secondary metabolites) (Rasmann & Agrawal, 2011; Nipperess *et al*., 2012). The hybrid combinations of closely related species are also included as test plants during host specificity tests, as herbivorous insects have been reported to utilize hybrids to increase their host range (Pilson, 1999). Host-specificity trials are commonly composed of two tests; namely, the no-

choice test and the choice test (Schaffner, 2001). The no-choice test is used to determine whether a potential biocontrol agent can complete its life cycle on a selected test plant;while the choice test is used to determine the host preference of the potential biocontrol agent by supplying a mobile insect with two or more test plants and asking it to choose between them (Schaffner, 2001). Marohasy (1998) has suggested the inclusion of preliminary and/or sequential tests as additional tests, where potential agents spend a relatively short time on test species, to further the understanding of the potential agent's ecology. van Lenteren *et al.* (2006) proposed a framework for host-specificity testing that begins with preliminary tests, and only if nontarget arthropod species are attacked by the biocontrol agent do they then recommend that larger arena tests be conducted. Wood (2006) and Boughton *et al.* (2011) are examples of researchers that followed this advice on invasive alien weeds and conducted preliminary host-specificity tests before whole plant tests on *Endophyllum osteospermi* (Doidge.) (Pucciniales: Pucciniaceae) and *Austromusotima camptozonale* (Hampson.) (Lepidoptera: Crambidae) respectively.

Post host-specificity trials, risk assessments are conducted by pooling data from both the choice and no-choice tests,thereby assessing the overall potential risk the biocontrol candidate poses on target and nontarget species (Wan & Harris, 1997; Olckers, 2004; Barratt *et al*., 2010).

1.6. Phylogenetics and biological control

Wapshere (1974) proposed the centrifugal phylogenetic method for testing potential biocontrol agents. A method that includes plant taxa closely related to the target IAP during host-specificity testing (Wapshere, 1974; Briese & Walker, 2002). Insects are most likely to select closely related plants as hosts (Nipperess *et al*., 2012; Rasmann & Agrawal, 2011). This suggests that plants that are closely related to target IAPs are at the most risk of nontarget effects (Futuyma, 2000). However, some insects have host ranges which include

more distantly related species (Hinz & Diaconu, 2015). Additionally, Briese (2003) pointed out that the centrifugal phylogenetic method was only relevant for the state of knowledge of that time, when fewer studies were done on the behaviour and ecology of host specific insects and host plants. Recently, it has been reported that insect herbivore host ranges are not always phylogenetically constrained but are also determined by plant characteristics such as secondary metabolites (Rapo *et al*., 2019; Barrett *et al*., 2021).

1.7. Secondary metabolites and biological control

Secondary metabolites are compounds that are by-products of nitrogen metabolism, which function in the protection of plants against herbivory while also promoting plant growth and development (Nishida, 2014; Zaynab *et al*., 2018). These compounds are stored in plant tissues and organs, and are released through glandular trichomes (Bourgaud *et al*., 2001; Ramakrishna & Ravishankar, 2011; Pang *et al*., 2021; Wang *et al*., 2021). Plant secondary metabolites are classified according to their chemical structure and can be divided into three major groups, i.e., alkaloids, phenolic compounds and terpenoids (Zwenger & Basu, 2008; Zaynab *et al*., 2018). Phenolic compounds are the most prominent and widely distributed secondary metabolites in higher plants (Zwenger & Basu, 2008; Cheynier *et al*., 2012; Lin *et al*., 2016; Minatel *et al*., 2017). Some insects have co-evolved with host plants containing secondary metabolites by detoxifying the compounds and exploiting the plant for feeding and oviposition (Ryan & Byrne, 1988; Matsuki *et al*., 2011). For example, *Curculio chinensis* (Chevrolat.) (Coleoptera: Curculionidae) weevils detoxify the chemical defences of *Camellia* species in order to feed on the plant's seeds (Zhang *et al*., 2020). Consequently, secondary metabolites have been suggested to be primary determinants of insect host specificity over phylogenetic relatedness of the host plants (Rapo *et al*., 2019; Barrett *et al*., 2021).

1.8. Invasive *Tamarix* taxa

Tamarix L. (Caryophyllales: Tamaricaceae) are halophytic (salt tolerant) plant species that are native to Eurasia, North Africa, and southwestern Africa (Baum, 1978; Mahfoudhi *et al*., 2014; Marlin *et al*., 2017). Several species from this genus have successfully invaded parts of North and South America, Australia, and South Africa (Mayonde *et al*., 2016; Marlin *et al*., 2017). It has been reported that this invasion was because of several *Tamarix* taxa being imported as ornamentals, for erosion control, and phytoremediation of mine dumps (Newete *et al*., 2019). In the United States, the western riparian zones are highly susceptible to *Tamarix* invasion as these areas provide a suitable environment for these phreatophyte trees to thrive (Hultine *et al*., 2010). In South Africa, invasive *Tamarix* populations are widely spread across five of the nine provinces, with the dominant invasive taxon being the hybrids between *T. chinensis* (Lour.) and *T. ramosissima* (Ledeb.) (Mayonde *et al*., 2016; Newete *et al*., 2019). South Africa's *Tamarix* population also includes the indigenous *T. usneoides* E. Mey ex Bunge., which is also being promoted for the phytoremediation of mine dumps (Marlin *et al*., 2017; Mayonde *et al*., 2021). Previous genetic studies showed evidence of hybridization between the indigenous and the two exotic species (Mayonde *et al*., 2016, 2019). Previously, leaf and flower morphology (based on visual appearance) has been used as an indicator in the classification of *Tamarix* taxa, and more recently *T. usneoides* has been reported as a phylogenetically distant relative to *T. chinensis* and *T. ramosissima* (Baum, 1978; Gaskin & Schaal, 2003; Mayonde *et al*., 2015; Villar *et al*., 2019).

1.9. Biological control of invasive *Tamarix*

The biocontrol efforts against invasive *Tamarix* taxa in South Africa follows a "piggyback" strategy of what has been done in the United States (Byrne *et al*., 2021). In the United States, *Diorhabda carinulata* (Desbrochers.) (Coleoptera: Chrysomelidae) underwent host-specificity tests before being released to control the expansion of invasive *Tamarix*

populations (Dudley & Kazma, 2005; Nagler *et al*., 2014; Bean & Dudley, 2018). During these tests, there was a reduction in the beetle's size and delayed oviposition when reared on the nontarget *T. aphylla* L. (a sister taxon to *T. usneoides*) (Mayonde *et al*., 2016; Dudley & Kazma, 2005). These results, coupled with the native distribution of *D. carinulata*, which overlaps with that of *T. ramosissima* prompted the initiation of a biocontrol programme for invasive *Tamarix* taxa in South Africa (Tracy & Robbins, 2009; Mayonde *et al*., 2016; Marlin *et al*., 2019). Unfortunately, during host-specificity tests in South Africa, *D. carinulata* included the indigenous *T. usneoides* in its host range (Marlin *et al*., 2019). Continuing with the rationale of host specificity being dependent on phylogenetic relatedness and native distribution, *Trabutina mannipara* (Hemprich & Ehrenberg.) (Hemiptera: Pseudococcidae) was the next candidate tested (Hatile *et al*., 2022). The scale insect also proved to not be a suitable biocontrol agent of invasive *Tamarix* taxa in South Africa as it could also complete its life cycle on the indigenous *T. usneoides* (Hatile *et al*., 2022). Two possible explanations were given for these results i.e., some insects have phylogenetically disconnected host ranges which include more distantly related species, and the possible influence of secondary metabolites on these insects' host choices (Hinz *et al*., 2008; Hinz & Diaconu, 2015). Studies have been done to confirm the presence of glandular trichomes in both the indigenous and invasive *Tamarix* taxa present in South Africa (Sookbirsingh *et al*., 2010; Wilson *et al*., 2017). However, secondary metabolite analysis has only been done on *T. ramosissima* (Bahramsoltani *et al*., 2020).

1.10. Study rationale

Invasive alien plants are not only a threat to global biodiversity, but also to global economies (Vitousek *et al*., 1997; Miyawaki & Washitani, 2004; Lazzaro *et al*., 2020). Hence, it is important to increase the use of cost-effective invasive alien plant management strategies like biocontrol (Blossey *et al*., 1994; Follet & Duan, 2012; Zachariades *et al*., 2017). The current

study follows work by Marlin *et al.*, (2019) and Hatile *et al.* (2022) to find an effective biocontrol agent against invasive *Tamarix* taxa in South Africa. Previously, nontarget plants that were used in host-specificity testing were selected based on their phylogenetic relatedness to the target plants. However, in addition to plant phylogeny secondary metabolites have been reported to affect host selection. The current study explores the idea that secondary metabolites may be influential in host selection by a new biocontrol agent against invasive *Tamarix* taxa in South Africa, while also conducting host-specificity trials on the potential biocontrol agent.

1.11. Study aims and objectives

The current study aims to investigate the host range of *Coniatus tamarisci* (Germar.) (Coleoptera: Curculionidae), a potential biocontrol agent of invasive *Tamarix* taxa, through host-specificity trials conducted using the indigenous *T. usneoides* and invasive *Tamarix* taxa present in South Africa. It also aims to investigate the influence of the most commonly found secondary metabolites in Kingdom Plantae i.e., phenolic compounds, on the host selection of *C. tamarisci*.

Objective 1: to conduct choice and no-choice host-specificity trials, involving the indigenous *T. usneoides* and invasive *Tamarix* taxa in South Africa, in order to assess the host range of *C. tamarisci* as a potential biocontrol agent of invasive *Tamarix* taxa in South Africa.

Objective 2: to conduct a qualitative and quantitative analysis of the main group of secondary metabolites, specifically phenolic compounds, present in the leaves of *Tamarix* taxa found in South Africa.

1.12. Dissertation outline

The first chapter of this dissertation is a general introduction, which includes a brief literature review on invasive alien plants, biological control, and current biological control strategies against invasive *Tamarix* taxa in South Africa. In chapter two, a series of host-specificity trials were conducted to determine the host range of potential biocontrol agent of invasive *Tamarix* taxa in South Africa, *C. tamarisci* (i.e., objective 1). Chapter three focuses on the qualitative and quantitative analysis of the main groups of secondary metabolites found in the *Tamarix* taxa found in South Africa (i.e., objective 2). Lastly, chapter four includes a general discussion with conclusions and recommendations for future studies on the biocontrol of invasive *Tamarix* taxa in South Africa.

This dissertation has been prepared and written in the format of scientific papers. The repetition of information given this format is unfortunately unavoidable but has been minimised as far as possible.

Chapter Two

Determining The Host Range of *Coniatus tamarisci* Fabr. (Coleoptera: Curculionidae): A Potential Biological Control Agent of Invasive *Tamarix* L. (Caryophyllales: Tamaricaceae) Taxa in South Africa

Abstract

Several species of *Tamarix* (Tamaricaceae), a phreatophyte genus from the Old World, are tenacious competitors which have invaded parts of South Africa. Biological control efforts on invasive *Tamarix* taxa in South Africa have been hampered by the indigenous *T. usneoides*, because the two potential biological control agents tested can complete their life cycles on the species. In the current study, *Coniatus tamarisci* underwent laboratory-based host-specificity trials. Preliminary no-choice tests showed that there was no significant difference in the feeding intensity of *C. tamarisci* across all test *Tamarix* taxa including the indigenous species. However, during preliminary choice tests, *C. tamarisci* showed a significant preference for invasive *Tamarix* taxa (namely, *T. chinensis*, *T. ramosissima*, and their hybrid combinations). Whole plant no-choice testing revealed that the weevil could complete its life cycle on both the invasive *Tamarix* taxa and the indigenous *T. usneoides*. There was no significant difference in *C. tamarisci* pupae and adult weights across the tested *Tamarix* taxa. However, there was a significant difference in the number of F1 generation adults produced between the invasive *Tamarix* taxa and the indigenous *T. usneoides*, with *T. usneoides* producing the least adults. Risk assessments analysis revealed that the weevil poses low risk to the indigenous *T. usneoides*. Despite the nontarget effects to *T. usneoides*, the preliminary choice test; number of F1 generation adults produced per taxon; and the risk assessments results prompt to suggest that *C. tamarisci* should undergo open field host-specificity tests in

Eurasia. This would reveal the true realized host range of the insect in a natural setting before the release as a biological control agent against invasive *Tamarix* taxa in South Africa.

Keywords: Invasive *Tamarix*, biological control, host specificity, nontarget effects, risk assessments.

2.1. Introduction

2.1.1. Biological invasions

Biological invasions describe the arrival, establishment, and dominance of alien species in environments outside their native geographic ranges (Vermeij, 1996; Pyšek *et al*., 2020). According to the IPBES (2019) global assessment, these invasions form part of the top five direct and indirect drivers that exacerbate negative change in nature globally. Globally, the number of invasive alien species per country has risen by approximately 70% since 1970, and the yearly cost of biological invasions has been estimated at US$ 300 billion (Luque *et al*., 2014; IPBES, 2019).

In South Africa, 1400 alien species have been listed, with about 56% of these being invasive alien plants (IAPs) (van Wilgen *et al*., 2020). These alien species cost the country approximately ZAR 15 billion per annum (van Wilgen *et al*., 2020) in lost revenue. Invasive alien plants are introduced from foreign regions through various means, namely, agriculture, forestry, mariculture, horticulture, and recreation (Vitousek *et al*., 1997; Reichard & White, 2001; Faulkner *et al*., 2020). Upon introduction, some of these IAPs escape confinement and thrive as competitors to native biota through the production of allelochemicals and absence of natural enemies (Heger & Jeschke, 2014; Ruwanza, 2021). For example, invasive *Opuntia stricta* (Haw.) (Caryophyllales: Cactaceae) was initially introduced as an ornamental and hedging plant but escaped confinement and now has a negative impact on the growth and regeneration of native biota (Shackleton *et al*., 2017; Witt *et al*., 2020). In South Africa,

invasive *Solanum mauritianum* (Scopoli.) (Solanales: Solanaceae) produces numerous seeds which are bird dispersed, so it rapidly disperses. It also has allelopathic effects on other plants, allowing it to occupy approximately 80 500 ha of the country (Cowie *et al*., 2018; Ruwanza, 2021). Another example of an IAP present in South Africa is the genus *Tamarix* L.

2.1.2. Invasive *Tamarix* taxa in South Africa

The genus *Tamarix* (Caryophyllales: Tamaricaceae) (commonly known as saltcedar) is a halophytic, multibranched tree or shrub native to Eurasia, the Mediterranean, and north and southwestern Africa (Baum, 1978; Mahfoudhi *et al*., 2014; Marlin *et al*., 2017). This genus consists of approximately 60 phreatophyte species that are characterized by their needlelike leaves, which overlap each other along the stem, and white to pinkish flowers (Baum, 1978; Samejo *et al*., 2013; Bahramsoltani *et al*., 2020). *Tamarix* plants grow up to 12 meters high and have a lifespan of 75 to 100 years (DiTomaso, 1998). Moreover, a tree can produce approximately 500 000 seeds per season (Brotherson & Field, 1987). These seeds are light, buoyant, and covered in tiny hairs, which make them well adapted to water and wind dispersal (Tomanek & Ziegler, 1960). Additionally, due to their self-compatibility, *Tamarix* plants can produce seeds even without cross pollination (Brotherson & Field, 1987).

Alien *Tamarix* taxa were introduced into South Africa primarily for use as ornamentals, for erosion control, and phytoremediation of mine dumps (I. Weiersbye pers. comm. in Newete *et al*., 2019). Their mass production of seeds has allowed them to become naturalized and subsequently begin competing with native biota for resources in several parts of the country. Currently, *T. chinensis* (Lour.), *T. ramosissima* (Ledeb.) and their hybrid combinations have established invasive populations in the Northern Cape, Eastern Cape, Western Cape, Free State, and Northwest provinces (Mayonde *et al*., 2016; Newete *et al*., 2019).

Tamarix trees absorb salt through their roots and excrete it through specialized glands on their leaves (Bosabalidis & Thomson, 1985). Occasionally, *Tamarix* plants drop their leaves, depositing salt into the soil which inhibits the germination and growth of surrounding biota (Newete *et al*., 2020). *Tamarix* leaf litter is also highly flammable, thus contributing to seasonal fires that are detrimental to fire intolerant species (Busch & Smith, 1992). These facultative phreatophytes have roots that penetrate as deep as 30 meters into the ground, and this decreases the water availability for co-occurring plants with shallower roots, e.g., in the USA *Salix alba* L. (Malpighiales: Salicaceae) and *Baccharis salicifolia* (Ruiz & Pav.) (Asterales: Asteraceae) (Baum, 1978; DeLoach *et al*., 2000). The daily water usage of *Tamarix* species has been reported to be between 6.1 to 122 litres/day, further affecting the water availability of surrounding biota (Sala *et al*., 1996; Owens & More, 2007). Therefore, *Tamarix* infestations affect soil salinity, fire intensity, and water availability. These attributes coupled with *Tamarix*'s high seed production and tolerance to extreme environmental conditions (i.e., floods and droughts) make these species tenacious invaders. At four sites, in the Eastern Cape and Western Cape, invasive *Tamarix* taxa canopy cover was reported at over 67% (Newete *et al*., 2019).

The Alien and Invasive Species regulations of the National Environmental Management: Biodiversity Act 2014 (NEM:BA) (DEA, 2016) has classified the alien invasive *Tamarix* and its hybrids as category 1b invaders, which require management. Previously, the application of herbicides and mechanical removal have been explored as control options for invasive *Tamarix* worldwide, however these have proven costly and ineffective (Marlin *et al*., 2017). To chemically control an invasive *Tamarix* population costs US$450-550/ha, while mechanical control costs US$1500-1700/ha (McDaniel & Taylor, 2003). Additionally, the vigorous vegetative reproduction of invasive *Tamarix* make the plant difficult to control mechanically (Fornarsari, 2004). Fortunately, there is a less costly and more sustainable

alternative in classical biological control should a suitable biocontrol agent be found that is effective.

2.1.3. Classical biological control

Classical biological control (biocontrol) is the introduction of highly selective natural enemies to control the spread of an invasive organism (McFadyen, 1998; Zachariades *et al*., 2017). In biocontrol, the natural enemies (i.e., insects, mites, parasites, parasitoids, and/or pathogens) are collected from their respective countries of origin and released in the invaded region after having undergone host-specificity trials (McFadyen, 1998; Briese, 2000; Zachariades *et al*., 2017). Weed biocontrol has been in practice for more than a hundred years, with its stand-out benefits being that: (a) it is an environmentally friendlier alternative, when compared to mechanical and chemical control; (b) it is cost effective; and (c) permanent and self – sustaining(Blossey *et al*. 1994; Zachariades *et al*., 2017).

2.1.4. Insect host selection

Leaf morphology, i.e., visual appearance, toughness, waxiness, and the presence or absence of trichomes, has been reported to play a pivotal role in insect host selection (Rivero-Lynch *et al*., 1996; Kang *et al*., 2010). Trichomes are epidermal structures that physically and chemically protect plants from insect herbivory (Kang *et al*., 2010). These structures are classed into two categories; namely, non-glandular and glandular trichomes (Kang *et al*., 2010). Non-glandular trichomes mechanically obstruct the movement of insect herbivores across the plant's leaf surface, while glandular trichomes protect plants from herbivory through the excretion of secondary compounds (Kang *et al*., 2010).

In response to selection pressures, plants synthesize a range of secondary compounds/metabolites which function to protect them against herbivory, microbes, and abiotic factors, whilst encouraging plant growth and development (Nishida, 2014; Wink, 2018; Zaynab *et al.*, 2018). These phytochemicals are derived from nitrogen metabolism and are stored in plant cells, tissues, and organs (Bourgaud *et al*., 2001; Ramakrishna & Ravishankar, 2011).Many herbivorous insects have coevolved with plants that contain secondary metabolites by detoxifying these chemicals and some even utilize them as attractants (Matsuki *et al*., 2011). Specialist herbivorous insects can cope with higher concentrations of qualitative (toxic) secondary chemicals from select host plants; whereas generalist insects have a lower tolerance of these qualitative compounds and a better ability to cope with quantitative secondary compounds from a variety of plants (Mansura *et al*., 2021).For example, *Papilio* butterflies (Linnaeus.) (Lepidoptera: Papilionidae) commonly known as swallowtail butterflies, use secondary metabolites together with other compounds to a select host plant/s for oviposition (Nishida, 2014). Similar, the host selection of *Anoplognathus* beetles (Leach.) (Coleoptera: Scarabaeidae) is dependent on the presence of cyanogenic glycosides (a secondary metabolite) (Gleadow *et al*., 2008; Matsuki *et al*., 2011). If the compound is present in the leaves of the potential host, then the beetle avoids it (Gleadow *et al*., 2008). Rapo *et al.* (2019) suggests that secondary metabolites are the primary determinates of insect host specificity over phylogenetic relatedness.

2.1.5. Biological control of invasive *Tamarix* taxa in South Africa

The biocontrol of invasive *Tamarix* taxa in South Africa is handicapped by the presence of the indigenous *T. usneoides* (E. Mey ex Bunge), which is being promoted for the phytoremediation of mine dumps (Weiersbye *et al*., 2006; Marlin *et al*., 2017: 2019; Mayonde *et al*., 2021). Morphologically, *T. usneoides* have vaginate leaves, which are different to the sessile leaves of the invasive *T. chinensis* and *T. ramosissima* taxa. It also

shows sufficient polymorphic genetic characters to place the indigenous *T. usneoides* in a distinct phylogenetic clade from its invasive counterparts (Baum, 1978; Gaskin & Schaal, 2003; Mayonde *et al*., 2016; Villar *et al*., 2019). Hybrids between the indigenous *T. usneoides* and the invasive *T. chinensis* and *T. ramosissima* (approximately 13%) pose a threat of nontarget effects to the indigenous species because they might create a 'bridge' for the biocontrol agent (Floate & Whitham, 1993; Mayonde *et al*., 2016). Despite the indigenous taxon being phylogenetically distant from the invasive species, host-specificity trials conducted in South Africa on *Diorhabda carinulata* (Desbrochers.) (Coleoptera: Chrysomelidae) and *Trabutina mannipara* (Hemprich & Ehrenberg.) (Hemiptera: Pseudococcidae) have shown substantial nontarget effects, with both insect species completing their life cycles on *T. usneoides* and their hybrids (Marlin *et al*., 2019; Hatile *et al*., 2022). With two potential agents rejected, the search for a biocontrol agent of invasive *Tamarix* species in South Africa continues.

2.1.6. The current potential biocontrol agent: *Coniatus tamarisci* Fabr., 1817

The genus *Coniatus* (Schönherr.) (Coleoptera: Curculionidae) consists of 12 species that are native to the Canary Islands, the Mediterranean region, the Caucasus, and Iraq (Fornasari, 1998: 2004; Eckberg & Foster, 2011). These weevils (average male length is 4.1 mm and average female length is 4.9 mm) have an association with *Tamarix* and a closely related species, *Myricaria germanica* (Desv.) (Caryophyllales: Tamaricaceae) (Fornasari, 2004; Mouna *et al*., 2009). *Coniatus tamarisci* (Fabr.) (Coleoptera: Curculionidae) is native to the Canary Islands and the Mediterranean region, i.e., France, Italy, Algeria, Morocco, and Spain (Fornasari, 1998). This region is also home to *T. gallica* L. and *T. parviflora* DC. (species

closely related to *T. chinensis* and *T. ramosissima*) (Fornasari, 1998; Maldonado *et al*., 2016). The weevil has a green glossy coat, with two alternating trapezium shaped brown/goldish patches on each elytron, and v-shaped black patterns forming across both elytra when they are closed (Fig. 2.1). This colour combination camouflages the weevil with branches and leaves from *Tamarix* trees. *Coniatus tamarisci* females lay their eggs singly at the leaf axils of mature stems and on the tips of newly forming twigs (Fornasari, 1998). These eggs take approximately 41 days to hatch and mature into adult weevils at a temperature and relative humidity of between 20-30°C and 60-100% respectively (Fornasari, 1998). In its native region, *C. tamarisci* is associated with *T. gallica*, feeding on the plant's growing buds, leaves, and stems (Fornasari, 1998). *Tamarix gallica* is phylogenetically a cousin taxon to *T. ramosissima* and *T. chinensis*, the invasive *Tamarix* taxa in South Africa (Villar *et al*., 2019). Additionally, *C. tamarisci* has been reported as a natural enemy of *T. ramosissima* and *T. chinensis* (CAB International, 2022). During host-specificity trials of *C. tamarisci* in the United States, no adults emerged on the nontarget test plant *T. aphylla* (Karst.), meaning that the weevil could not complete its life cycle on the plant (Fornasari, 1998). *Tamarix aphylla* and *T. usneoides* are members of a sister taxon (Villar *et al*., 2019). Thus, the results from Fornasari (1998) suggest that *C. tamarisci* is a promising candidate for the biocontrol of invasive *Tamarix* taxa in South Africa, provided it does not complete its life cycle on *T. usneoides*.

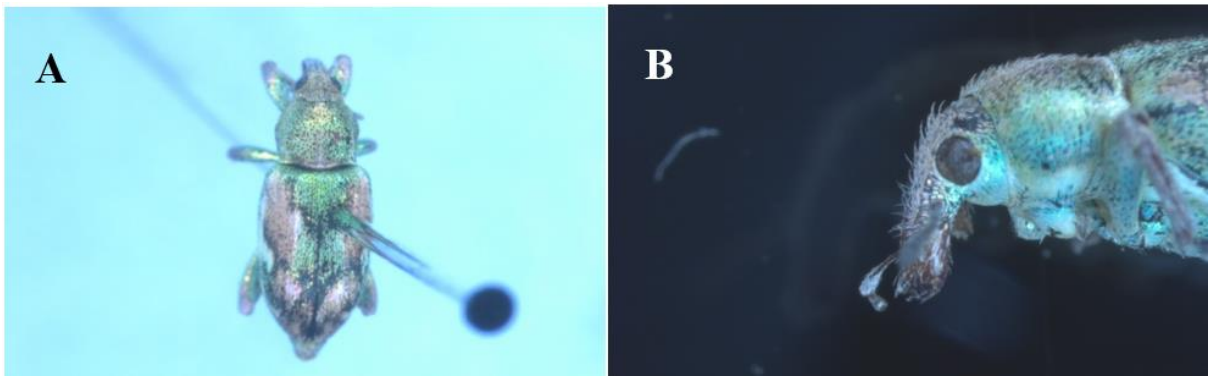

Figure 2.1. Top view of adult *Coniatus tamarisci* (a.), and side profile of adult *Coniatus tamarisci* (b.).

2.1.7. Aims and objectives

This chapter aimed to assess the host specificity of *C. tamarisci* with respect to the *Tamarix* taxa present in South Africa. This was done through a series of choice and no-choice host-specificity trials, involving the indigenous *T. usneoides* and the invasive *Tamarix* taxa (together with their hybrid combinations) present in South Africa. Ultimately, the objective of this study was to assess the potential of *C. tamarisci* as a biocontrol agent of invasive *Tamarix* taxa in South Africa.

2.2. Materials and methods

Tamarix plant and *C. tamarisci* collection

Hardwood cuttings from known South African *Tamarix* populations (namely *T. usneoides, T. chinensis, T. ramosissima, T. chinensis* × *ramosissima,* and *T. chinensis* × *usneoides*) previously identified by Mayonde *et al.* (2016) were cultivated for 18 months at the University of the Witwatersrand nursery, Johannesburg (26.1917°S, 28.0328°E) before use in this study.

Coniatus tamarisci individuals were collected from Sicily, Italy (37.6000˚N, 14.0154˚E) in Sept–Nov 2021 and reared on potted *T. ramosissima* plants at the University of the

Witwatersrand quarantine facilities at an average temperature and relative humidity of 24 °C and 55% respectively, with a photoperiod of 16:8 (L:D).

2.2.1. No–choice host-specificity tests

2.2.1.1. Preliminary no–choice tests

Thirty *Coniatus tamarisci* adults were placed into 30 individual petri dishes containing Whatman 90mm filter paper and one gram of plant material from the experimental *Tamarix* taxa for a week. Thereafter, pictures of the dishes were taken, with an Apple iPhone XS 12-megapixel camera, to count the number of frass particles produced by the weevils as an indicator of feeding intensity. ImageJ was used to count the frass particles.

2.2.1.2. Whole plant no–choice tests

Six potted plants from each of the experimental *Tamarix* taxa were placed in separate 558×432×1012mm tulle cages and inoculated with six *C. tamarisci* adults per plant. The experiment design constituted a total of 30 cages for the five *Tamarix* taxa, containing a total number of 180 *Coniatus* weevils. The life cycle of *C. tamarisci* was monitored for seven weeks across all experimental *Tamarix* taxa. Survival on the experimental *Tamarix* taxa was measured by counting the number of dead *C. tamarisci* weevils (every second day) from the 36 individuals (six weevils per cage) placed onto each taxon (considered as the parental generation). Maturing of *C. tamarisci* on the experimental *Tamarix* taxa was measured by counting the number of hatched F1 ectophagous larvae and counting the number of F1 larvae to successfully go through pupation. Lastly, the weights of adult *Coniatus* weevils from the parental and F1 generations were taken for comparison and to predict future fecundity.

2.2.2. Paired choice host-specificity testing

2.2.2.1. Preliminary choice test

Sixty *C. tamarisci* adults were placed in 60 individual petri dishes with a pair of leaf material bouquets from two of the experimental *Tamarix* taxa. Focal sampling was conducted, by observing the amount of time an individual spent on a particular *Tamarix* taxon bouquet. The choice of the *C. tamarisci* adults was recorded on an hourly basis for 10 hours, the amount of time (hours) *Coniatus* individuals were seen spending on selected taxa was compared.

2.2.3. Data Analysis:

2.2.3.1. No - choice host-specificity tests

2.2.3.1.1. Preliminary no – choice test

ImageJ was used to calibrate frass images (i.e., calibrating a single image against a known scale value, then applying that calibration to the uncalibrated image) from pixels to cm, and a quadrat (2.66 × 2.56cm), which accommodated all the frass produced in one area, was set where the number of frass particles produced by *C. tamarisci* individuals was counted.

A one-way independent variable analysis of variance (ANOVA) was conducted to compare the number of frass particles and area of frass produced by *C. tamarisci* weevils between the test genotypes (i.e., *T. usneoides*, *T. chinensis*, *T. ramosissima*, *T. chinensis* × *ramosissima*, *T. chinensis* × *usneoides*, and *T. ramosissima* × *usneoides*). If there were any significant differences, then a Tukey's HSD post-hoc test was conducted.

2.2.3.1.2. Whole plant no-choice tests

A one-way analysis of variance (ANOVA) was used to compare the means of; (a) the mortality rate of the parental generation; (b) the number of hatched F1 generation larvae; (c) number of pupating F1 generation individuals; (d) F1 generation pupal weights; (e) the

number of F1 generation *C. tamarisci* adults; and (f) F1 generation adult weights. These were followed by Tukey's HSD post-hoc tests to determine where the differences between the means lay. A student t-test was used to compare the mean sizes between adult *C. tamarisci* individuals from the parental and the F1 generation reared on the same *Tamarix* taxon.

2.2.3.2. Paired choice host-specificity testing

2.2.3.2.1. Preliminary choice test

The total amount of time (hours) each *C. tamarisci* individual spent on either *Tamarix* taxa was tallied. The three possible choices (i.e., Taxon A. or Taxon B., or no plant) were treated as three treatments and a one-way repeated measures analysis of variance (ANOVA) was conducted to compare the time a weevil spent on or off the taxa. If there were any significant differences between the *Tamarix* taxa, then a pairwise student t-test was used to determine where the difference was. This post hoc test was considered appropriate, because the data were dependent (i.e., any choice a *Coniatus* individual made affected its response to the other two treatments) and because of the number of outliers in the datasets.

For all statistical tests, R and RStudio (R Core Team 2019, version 3.5.3) was used and the significance level was set at $p\text{-value}<0.05$.

2.2.3.3. Risk assessment

Risk assessments were also conducted to assess the feeding and reproductive risk posed by *C. tamarisci* to the indigenous *T. usneoides* and invasive *Tamarix* taxa in South Africa, following Olckers (2000; 2004). The risk to the nontarget *Tamarix* species was quantified by measuring *C. tamarisci's* performance on each experimental plant, at different stages in the host selection process, as a proportion of that on *T. ramosissima* and *T. chinensis*. The risk of *C. tamarisci* utilizing nontarget *Tamarix* was assessed in two ways. Firstly, the feeding risk was calculated as the product of amount of frass produced by adult (R^1), time spent by adult

on each plant taxon (R^2), and adult mortality (R^3). Secondly, the reproductive risk was calculated as a measure of the number of eggs that reached the larval stage of development (R^4), larvae survival (R^5), and adult weight (R^6).

2.3. Results

2.3.1. Preliminary no-choice test

2.3.1.1. Number of frass particles

Overall, the number of frass particles produced by one adult *C. tamarisci* per plant bouquet (n = 6 per *Tamarix* taxon) over a period of a week was significantly different between the *Tamarix* taxa (Fig. 2.2; F = 54.364, df = 4.000, p < 0.0001). *Coniatus tamarisci* individuals on sprigs of *T. chinensis* × *usneoides* produced the lowest number of frass particles (approx. 135 particles on one sprig). Although the number of frass particles produced by weevils kept on *T. usneoides*, *T. chinensis*, *T. ramosissima* and *T. chinensis* × *ramosissima* were not significantly different, the highest number of frass particles was recorded on *T. chinensis* × *ramosissima* (approx. 811 particles on one sprig). No weevil mortality occurred in any treatment.

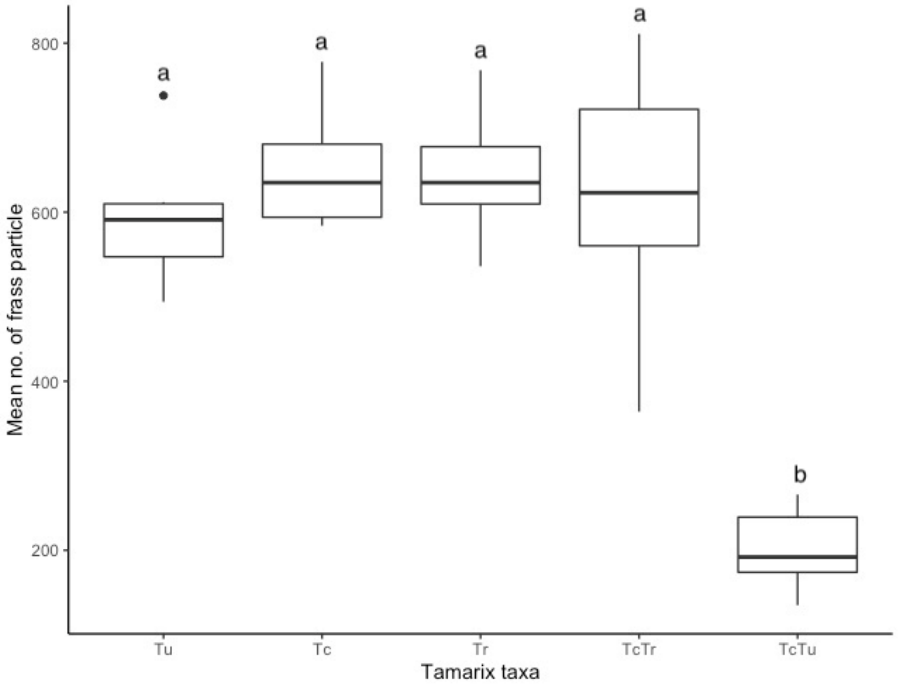

Figure 2.2. Mean number of frass particles produced by individuals *C. tamarisci* weevils after feeding separately on 1g of *Tamarix* leaves from *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu), in one week. n = 6 plants per taxon and 1 insect per plant . Different lowercase letters indicate significant differences between means; ANOVA (p-value <0.05).

2.3.2. Whole plant no-choice test

2.3.2.1. Parental generation adult mortality

There was a significant difference (Fig. 2.3; F = 4.3829, df = 4.000, p < 0.05) in the mortality (i.e., number of dead weevils) of the six adult *C. tamarisci* weevils inoculated onto whole plants of the five *Tamarix* taxa (n = 6 plants per taxon). *Tamarix chinensis* had the highest adult mortality recorded on one plant (approx. 3 individuals from 6), followed by *T. usneoides*, *T. ramosissima*, and *T. chinensis* × *ramosissima* and *T. chinensis* × *usneoides.* However, there was no significant difference between *T. chinensis* × *usneoides* and *T. usneoides*, *T. ramosissima*, and *T. chinensis* × *ramosissima*.

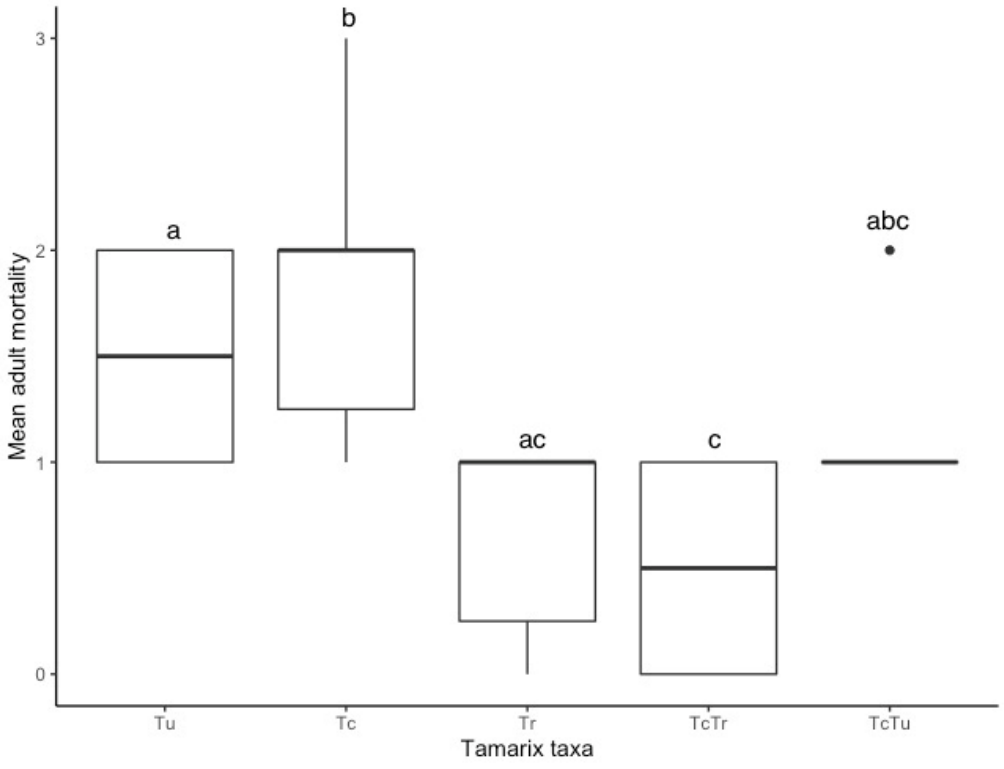

Figure 2.3. The mean mortality of *Coniatus tamarisci* inoculated onto *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) during no – choice testing. n = 6 plants per taxon with 6 insects per plant . Different lowercase letters indicate significant difference between means; ANOVA (p – value <0.05)

2.3.2.2.No choice test: F1 developmental rate

The time taken for the first *C. tamarisci* F1 individual to appear in each sequential developmental stage, on the five experimental *Tamarix* taxa (n = 6 per taxon), showed it took longer for the first egg to hatch and first adult to appear on *T. usneoides* compared to the invasive *Tamarix* taxa (Table 2.1). It took the least amount of time for the first egg to hatch and first adult to appear on *T. chinensis* compared to the other test taxa (Table 2.1).

Table 2.1. The number of days taken for the appearance of the first F1 generation larva, pupa, and adult *C. tamarisci* individuals on five *Tamarix* taxa during whole plant no-choice testing. n = 6 plants per taxon.

Tamarix taxa:	Days to appear		
	Larvae	Pupae	Adult
T. usneoides	18	30	44
T. chinensis	11	24	33
T. ramosissima	14	29	41
T.chinensis ×ramosissima	14	29	41
T. chinensis × usneoides	18	30	41

2.3.2.3. Mean number of F1 larvae

Overall, the number of *C. tamarisci* F1 generation larvae counted across the different *Tamarix* taxa (n = 6 plants per taxon) was significantly different (Fig. 2.4; F = 183.46, df = 4.000, p < 0.0001). *Tamarix chinensis* had the highest mean larval count (20.83), while *T. usneoides* had the lowest mean *C. tamarisci* larval count (3.50) per whole plant.

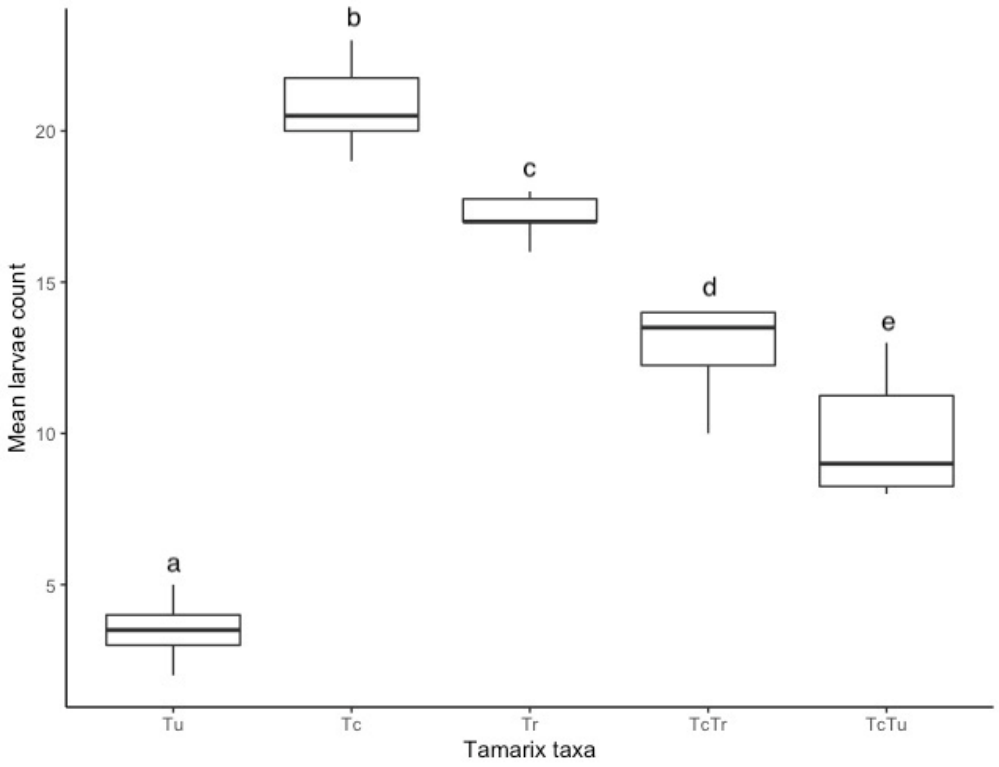

Figure 2.4. Mean number of F1 larvae found after *Coniatus tamarisci* adults were inoculated onto whole plants of *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) during no-choice testing. n = 6 plants per taxon . Different lowercase letters indicate significant differences between means; ANOVA (p – value <0.05).

2.3.2.4. Mean number of F1 pupae

Overall, the number of *C. tamarisci* F1 generation pupae counted across the different *Tamarix* taxa (n = 6 plants per taxon) was significantly different between most taxa (Fig. 2.5; F = 28.617, df = 4.000, p < 0.0001). *Tamarix chinensis* had the highest mean pupal count (8.33), while *T. usneoides* had the lowest (1.67) per whole plant.

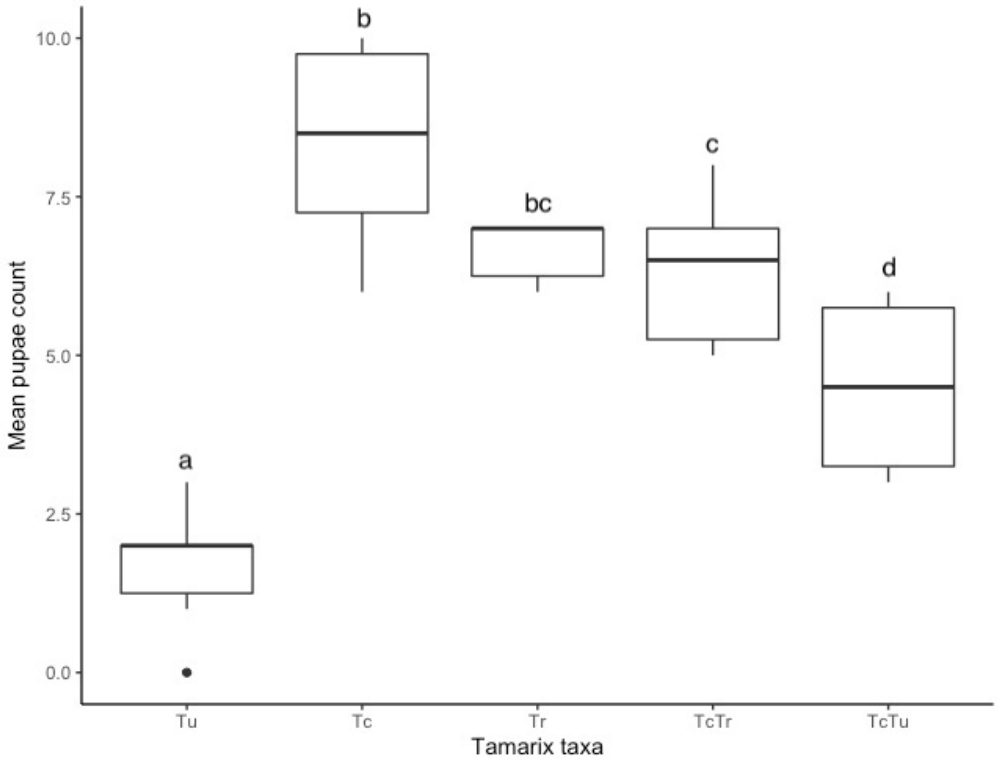

Figure 2.5. Mean number of F1 pupae found after *Coniatus tamarisci* adults were inoculated onto whole plants of *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) during no-choice testing. n = 6 plants per taxon . Different lowercase letters indicate significant differences between means; ANOVA (p – value <0.05).

2.3.2.5. Mean number of F1 adults

Overall, the number of *C. tamarisci* F1 adults counted across the different *Tamarix* taxa (n = 6 plants per taxon) was significantly different (Fig. 2.6; F = 25.393, df = 4.000, p < 0.0001) (Fig. 9). *Tamarix chinensis* had the highest adult count on one plant (4.00), which was only matched by *T. chinensis* × *usneoides*, while *T. usneoides* had the lowest mean number of *C. tamarisci* adults per plant (0.33).

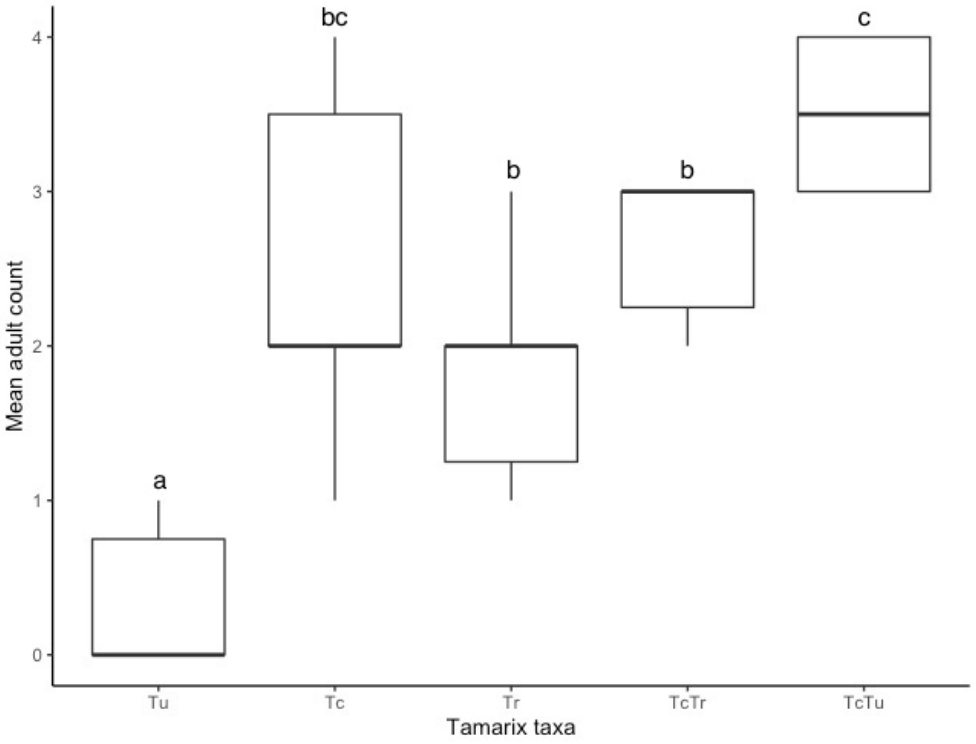

Figure 2.6. Mean F1 generation adult count after *Coniatus tamarisci* P generation individuals were inoculated on different whole plants of *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) during no-choice testing. n = 6 plants per taxon . Different lowercase letters indicate significant differences between means; ANOVA (p – value <0.05).

2.3.2.6. F1 pupal weight

There was no significant difference in the pupal weight of the *C. tamarisci* F1 pupae collected from the different *Tamarix* taxa (n = 6 plants per taxon) (Fig. 2.7; p – value > 0.05).

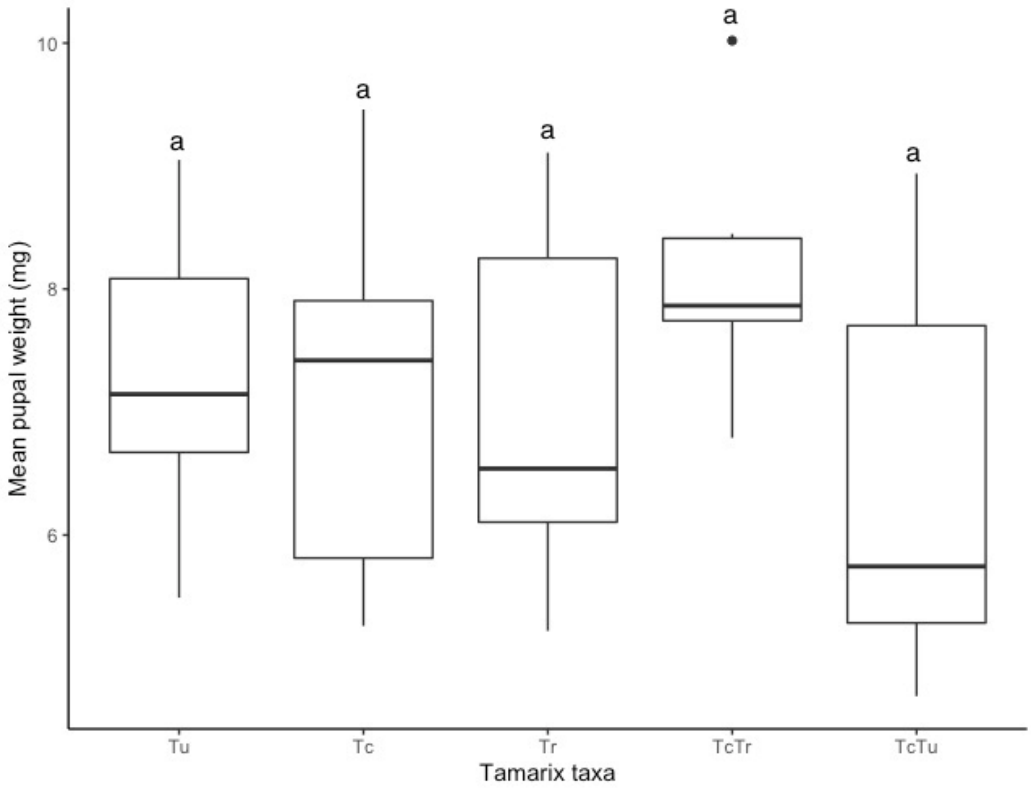

Figure 2.7. Mean pupal weights (mg) of *C. tamarisci* F1 individuals collected from *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) plants. n = 8 per *Tamarix* taxon. Different lowercase letters indicate significant differences between mean; ANOVA (p – value <0.05).

2.3.2.7. F1 adult weight

There was a significant difference in the weights of the eight *C. tamarisci* adults collected from the five experimental *Tamarix* taxa (Fig. 2.8; F = 2.2117, df = 4.000, p < 0.05). The weight of *C. tamarisci* adults collected from *T. chinensis* × *ramosissima* and *T. chinensis* was the highest, followed by weevils from the remaining *Tamarix* taxa. Only two individuals survived to adulthood on *T. usneoides*.

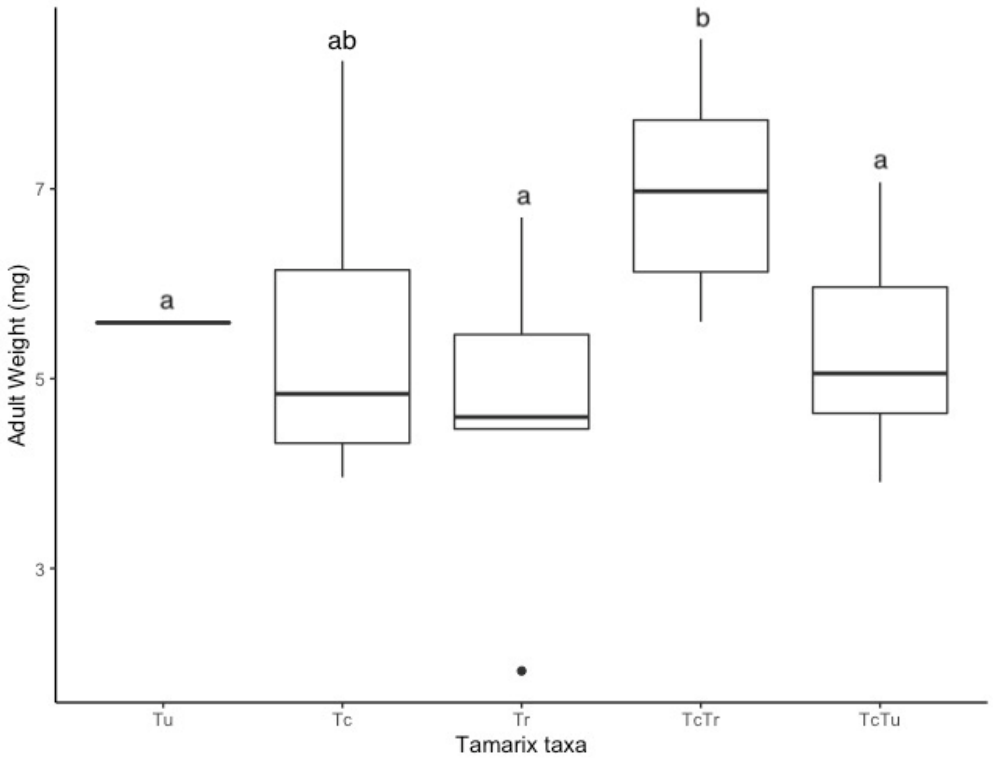

Figure 2.8. Mean weight (mg) of *C. tamarisci* F1 adults collected from *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) plants. n = 8 per *Tamarix* taxon . Different lowercase letters indicate significant differences between mean; ANOVA (p – value <0.05).

2.3.2.8. Parental vs F1 generation adult weight

There were no significant differences between the weight of the *C. tamarisci* adults from the P and F1 generations collected from the different *Tamarix* taxa (n = 8 weevils collected per taxon) (Fig 2.9; $p > 0.05$). Note that *T. usneoides* only yielded two F1 generation adults; therefore, insufficient to statistically compare with the P generation.

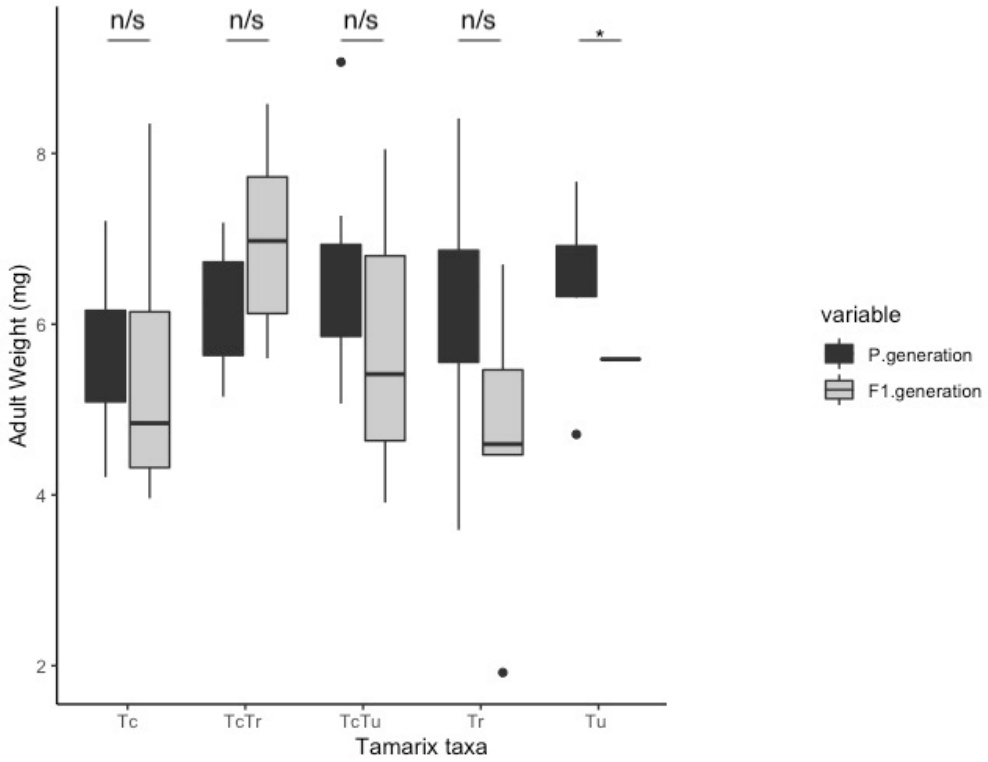

Figure 2.9. Mean adult weight (mg) of *C. tamarisci* from the Parental vs F1 generation collected from *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) plants. n = 8 weevils per *Tamarix* taxon). Different lowercase letters indicate significant differences between mean; t – test (p – value <0.05).

2.3.3. Preliminary choice test

2.3.3.1. *Tamarix usneoides* vs *T. chinensis* and *T. ramosissima*

Coniatus tamarisci individuals spent more time on *T. chinensis* and *T. ramosissima* compared to the indigenous *T. usneoides* ($p < 0.0001$ and $= 0.016$ respectively). When *T. chinensis* and *T. ramosissima* were paired, the weevils spent more time of *T. ramosissima* than *T. chinensis* (Fig. 2.10) ($p = 0.029$).

2.3.3.2. *Tamarix usneoides* vs *Tamarix* hybrids

Coniatus tamarisci individuals spent significantly more time (hours) on the *Tamarix* hybrids (namely, *T. chinensis* × *usneoides* and *T. chinensis* × *ramosissima*) than *T. usneoides* (Fig. 2.11) ($p = 0.0017$ and $p = 0.00143$). Additionally, the weevil spent significantly more time on *T. chinensis* × *usneoides* than *T. chinensis* × *ramosissima* (Fig. 2.11) ($p = 0.0012$).

2.3.3.3. *Tamarix chinensis* vs *Tamarix* hybrids

Coniatus tamarisci individuals spent significantly more time (hours) on the *T. chinensis* × *ramosissima* than *T. chinensis* (Fig. 2.12) ($p = 0.00087$). However, there was no significant difference in the amount of time the weevils spent on *T. chinensis* × *usneoides* and *T. chinensis* (Fig. 2.12) ($p > 0.05$).

2.3.3.4. *Tamarix ramosissima* vs *Tamarix* hybrids

Overall, there was no significant difference in the amount of time *C. tamarisci* weevils spent on *T. ramosissima* and the tested *Tamarix* hybrid (namely, *T. chinensis* × *usneoides* and *T. chinensis* × *ramosissima*) (Fig. 2.13) ($p > 0.05$).

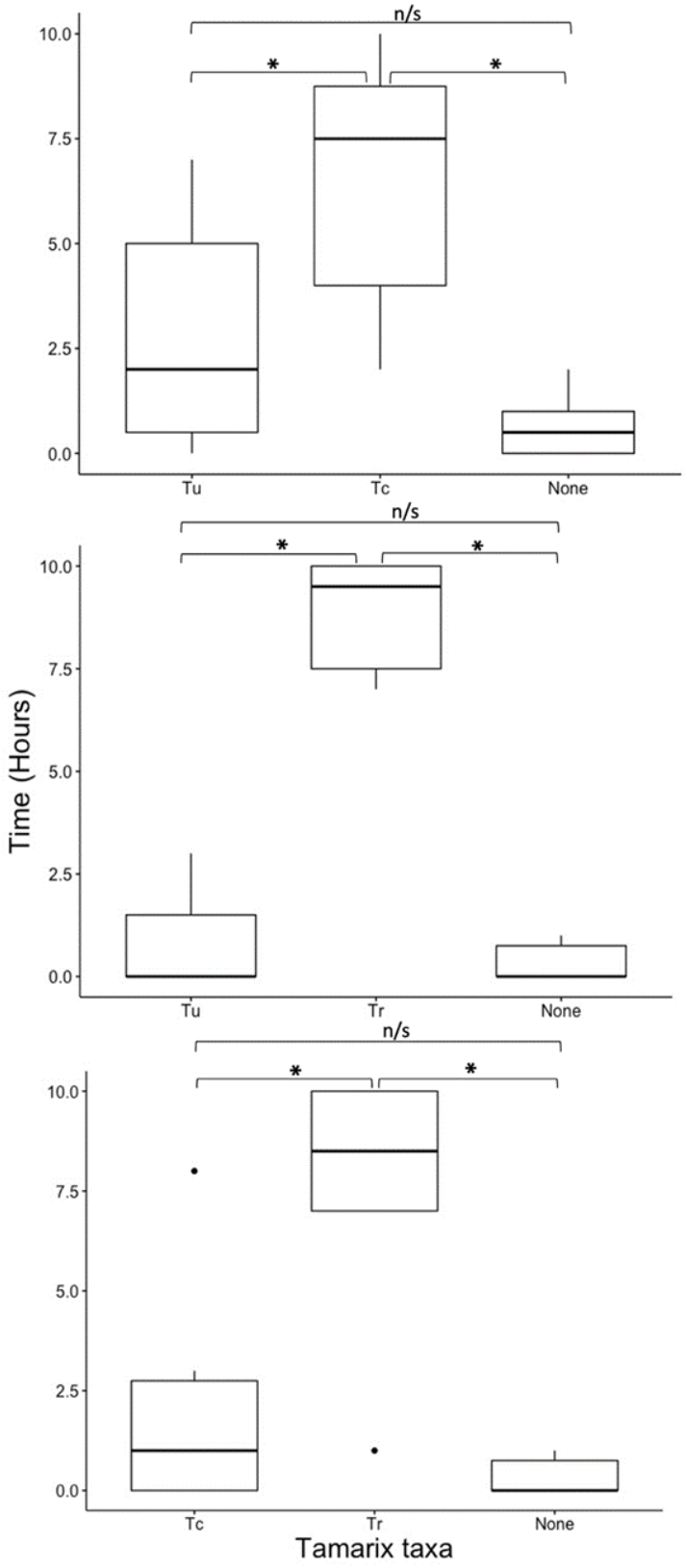

Figure 2.10. Mean amount of time (hours) spent by a *C. tamarisci* individual on paired *Tamarix usneoides* (Tu), *T. chinensis* (Tc), and *T. ramosissima* (Tr) sprigs during preliminary choice testing. n = 6 plants and 1 insect per plant. Asterisks indicate significant differences between paired means, ANOVA followed by pairwise t-test ($p < 0.05$).

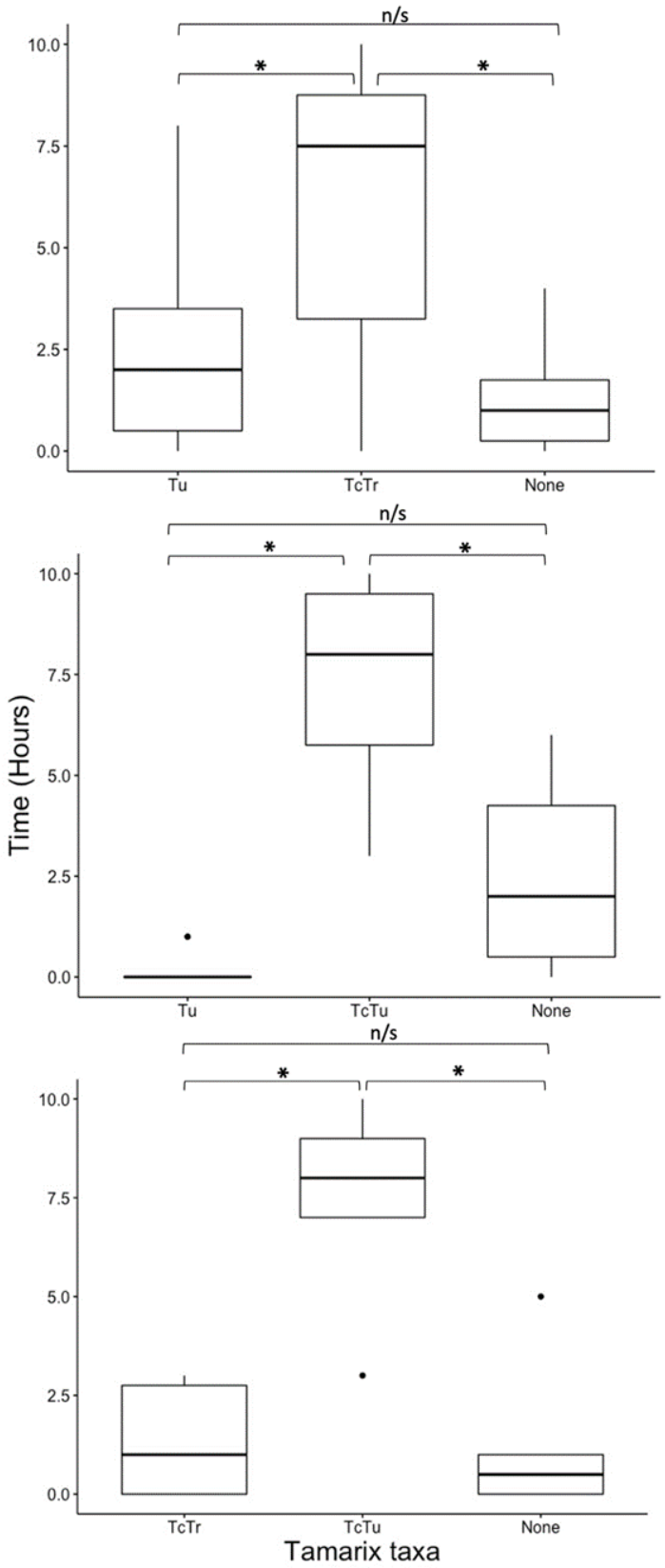

Figure 2.11. Mean amount of time (hours) spent by a *C. tamarisci* individual on paired *Tamarix usneoides* (Tu), *T. chinensis* × *usneoides* (TcTu), and *T. chinensis* × *ramosissima* (TcTr) sprigs during preliminary choice testing. n = 6 plants and 1 insect per plant. Asterisks indicate significant differences between paired means, ANOVA followed by a pairwise t-test ($p < 0.05$).

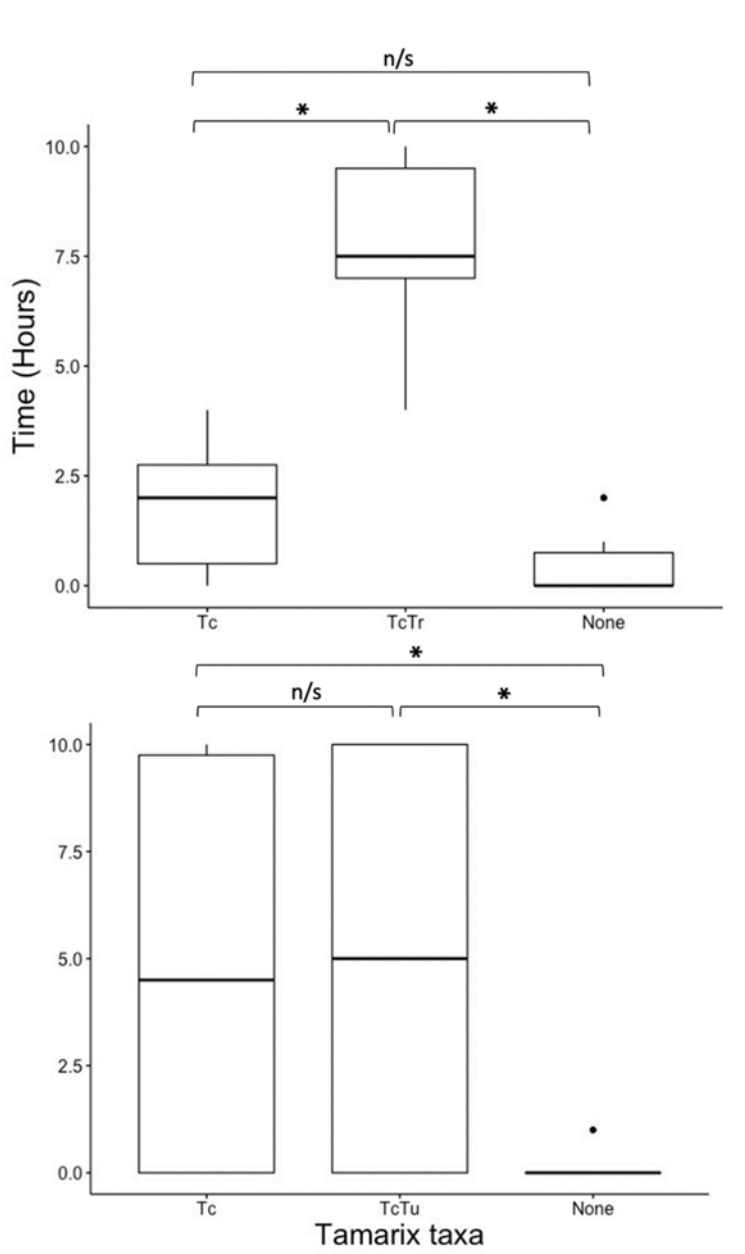

Figure 2.12. Mean amount of time (hours) spent by a *C. tamarisci* individual on paired *Tamarix chinensis* (Tc), *T. chinensis* × *ramosissima* (TcTr), or *T. chinensis* × *usneoides* (TcTu) sprigs during preliminary choice testing. n = 6 plants and 1 insect per plant. Asterisks indicate significant differences between paired means, ANOVA followed by pairwise t-test ($p < 0.05$).

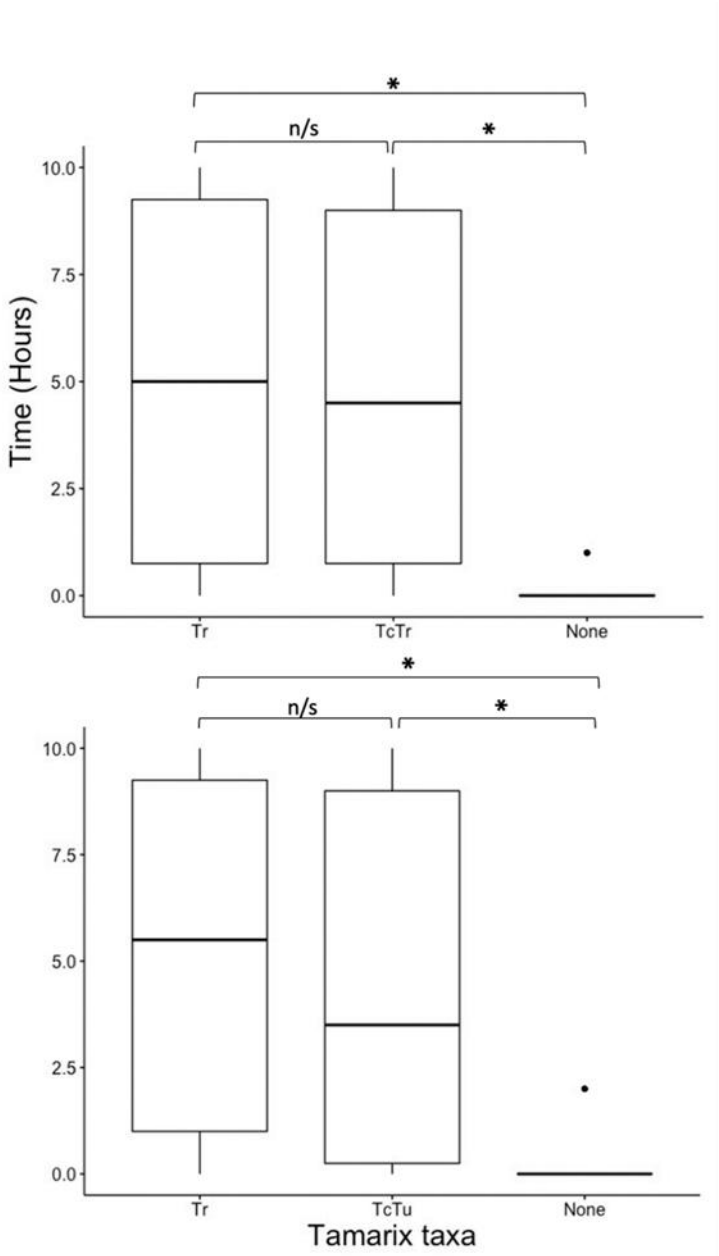

Figure 2.13. Mean amount of time (hours) spent by a *C. tamarisci* individual on paired *Tamarix ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), or *T. chinensis* × *usneoides* (TcTu) sprigs during preliminary choice testing. n = 6 plants and 1 insect per plant. Asterisks indicate significant differences between paired means, ANOVA followed by pairwise t-test ($p < 0.05$).

2.3.4. Risk assessments

2.3.4.1. *Tamarix chinensis* vs *T. usneoides*

The feeding risk analyses indicated that *T. usneoides* has a 42% risk of supporting *C. tamarisci* feeding (Table 2.2), while *T. chinensis* × *ramosissima* and *T. chinensis* × *usneoides* showed a higher risk of supporting *C. tamarisci* feeding (>100% and 54% probability respectively; Table 2.2). The reproductive risk analyses showed that *T. usneoides* has a low probability (13%) of supporting viable populations of *C. tamarisci*, while both *T. chinensis* × *ramosissima* and *T. chinensis* × *usneoides* had a probability >100% of supporting supporting viable populations of *C. tamarisci* (Table 2.2)

Table 2.2. Risk analysis on the performance of *C. tamarisci* on nontarget *Tamarix* plants relative to that on *T. chinensis*.

Test plant species	no. of frass particles (R^1)	Total hours spent (R^2)	Adult mortality (R^3)	Feeding risk ($R^1 \times R^2 \times R^3$)	no. of larvae (R^4)	Larvae survival (R^5)	Adult weight (R^6)	Reproductive risk ($R^4 \times R^5 \times R^6$)
T. chinensis	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
T. usneoides	0.91	0.43	1.08	0.42	0.17	0.79	0.94	0.13
T. chinensis × *ramosissima*	0.95	1.05	1.32	1.31	0.62	1.73	1.15	1.22
T. chinensis × *usneoides*	0.31	1.51	1.16	0.54	0.49	2.87	0.98	1.37

2.3.4.2. *Tamarix ramosissima* vs *T. usneoides*

The feeding risk analyses indicated that *T. usneoides* has a 15% risk of supporting *C. tamarisci* feeding, while *T. chinensis* × *ramosissima* and *T. chinensis* × *usneoides* had a 63% and 29% probability respectively (Table 2.3). The reproductive risk analyses showed that *T. usneoides* has a low risk (21%) of supporting viable populations of *C. tamarisci*, while both

T. chinensis × *ramosissima* and *T. chinensis* × *usneoides* had a probability >100% of supporting supporting viable populations of *C. tamarisci* (Table 2.3)

Table 2.3. Risk analysis on the performance of *C. tamarisci* on nontarget *Tamarix* plants relative to that on *T. ramosissima*.

Test plant species	no. of frass particles (R^1)	Total hours spent (R^2)	Adult mortality (R^3)	Feeding risk ($R^1 \times R^2 \times R^3$)	no. of larvae (R^4)	Larvae survival (R^5)	Adult weight (R^6)	Reproductive risk ($R^4 \times R^5 \times R^6$)
T. ramosissima	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
T. usneoides	0.92	0.19	0.84	0.15	0.20	0.89	1.16	0.21
T. chinensis × *ramosissima*	0.96	0.64	1.03	0.63	0.75	1.95	1.45	2.12
T. chinensis × *usneoides*	0.31	1.02	0.91	0.29	0.59	3.23	1.11	2.12

2.4. Discussion

The unsettling observation throughout the current host-specificity trials was that *C. tamarisci* exhibited nontarget effects on the indigenous *T. usneoides* and hybrids. Similar behaviour was observed when *T. mannipara* and *D. carinulata* underwent *Tamarix* host-specificity trials in South Africa (Marlin *et al*., 2019; Hatile *et al*., 2022). Both potential biocontrol agents were rejected, which suggests the same route of action should be taken with *C. tamarisci*. However, *C. tamariscis'* low risk, when associated with *T. usneoides*, suggests that a population would not sustain itself on the indigenous *Tamarix* taxa in a natural setup.

During the preliminary no-choice test, there was no significant difference in the number of frass particles produced by *C. tamarisci* on the different host taxa, indicating no difference in the feeding intensity of the *Coniatus* weevils across all the experimental *Tamarix* taxa. This would also explain the no significant difference in the mortality rate of the parental generation fed on *T. usneoides* and invasive *Tamarix* taxa (specifically *T.* ra*mosissima* and *T.*

chinensis × *usneoides.*), as starvation has been cited as one of the many causes of insect mortality (Adamo, 2021). These results were unexpected, as Withers and Barton-Browne (2004) and Withers and Mansfield (2005) stress that prior experience with a chosen experimental plant can bias host specificity results. In the current study, *C. tamarisci* was fed on *T. ramosissima* plants prior to use in the host-specificity trials; thus, it was expected to show a higher feeding intensity on *T. ramosissima*. Garcia-Robledo and Horvits (2012) and Carrasco *et al.* (2015), have suggested that insect feeding, and oviposition is affected by the absence of the natural host plant. This is supported by *C. tamarisci* showing no difference in the feeding intensities shown by the weevils across all the experimental *Tamarix* taxa.

Schaffner (2001) states that female monophagous insects are usually selective when choosing a host for oviposition, even when having to choose alternatives in the absence of their natural host plants. Moreover, insects are most likely to select closely related plants if the host is not available, because such plants are both physically and chemically similar (Nipperess *et al*., 2012; Rasmann & Agrawal, 2011). There were significantly higher numbers of emerging; larvae, pupae, and adults on the invasive *Tamarix* species (i.e., *T. chinensis* and *T. ramosissima*) and their hybrid combinations compared to the indigenous *T. usneoides*. Additionally, it took longer for F1 generation weevils to develop on *T. usneoides* compared to the other experimental plants. As distant relatives to the invasive *Tamarix* taxa, *T. usneoides* plants have vaginate leaves which are morphologically different from the sessile leaves present on *T. chinensis* and *T. ramosissima*, and leaf morphology has been reported to affect insect host choice (Baum, 1978; Rivero-Lynch *et al*., 1996; Gaskin & Schaal, 2003; Mayonde *et al*., 2016). Furthermore, the phylogenetic distance between *T. usneoides* and the invasive *Tamarix* taxa present in South Africa means that it is possible that they produce a different array of secondary metabolites, compounds that have also been reported as influential regarding insect host choice (Rapo *et al*., 2019; Barrett *et al*., 2021).

It has been well documented that body size affects arthropod fitness (i.e., survival and fecundity) (Davidowits *et al*., 2003; Akman & Whitman, 2008; Berger *et al*., 2008; Liu *et al*., 2010). For example, larger *Helicoverpa armigera* (Hübner.) (Lepidoptera: Noctuidae) pupae are most likely to survive winter through diapause (Liu *et al*., 2010). Another example is that of *Romalea microptera* (Beauvois.) grasshoppers, where larger adult females produce more eggs compared to smaller adult females (Akman & Whitman, 2008). According to the "mother knows best" hypothesis, the fitness of an insect is dependent on the quality of host plant selected by the mother for oviposition (Garcia-Robledo & Horvits, 2012). In the current study, *C. tamarisci* individuals reared on the indigenous *T. usneoides* were not significantly different in pupal and adult weight when compared to the individuals reared on the invasive *Tamarix* taxa. This suggests that all the experimental plants were of the same quality and produced *Coniatus* weevils of similar fitness, and that the maternal selection of oviposition site is the main mechanism of host selection in this species.

During the preliminary choice test, *C. tamarisci* weevils showed a preference for *T. ramosissima* and *Tamarix* hybrid combinations (i.e., *T. chinensis* × *T. ramosissima* and *T. chinensis* × *T. usneoides*) as they spent more time on the alien plants than the indigenous *T. usneoides*. According to the hybrid bridge hypothesis, exploiting plant hybrid combinations is evidence of a possible host range shift or increase (Floate & Whitham, 1993). For example, a study on a group of leaf-mining moths (Lepidoptera: Tischeridae, Citheraniidae) in seven hybrid zones in central Mexico showed that the insects used the *Quercus* × *dysophylla* (Benth.) (Fagales: Fagaceae) interspecific hybrids, expanding their host range from *Quercus crassifolia* (Bonpl.) (Fagales: Fagaceae) to what it is today (i.e., *Q. crassifolia*, hybrids, and *Q. crassipes*) (Tovar-Sánchez & Oyama, 2006). However, it is important to note that for insects to increase their host range there should be a decrease in food availability (i.e., natural host) and continuous exposure to the newly selected host over generations (Futuyma, 1991).

Notwithstanding the exploitation of hybrid combinations by *C. tamarisci*, the current study's sample period was not long enough to conclude a host range shift. Furthermore, the feeding risk assessment showed that there was a low to moderate feeding risk on *T. chinensis* × *usneoides*, further supporting the inconclusive nature of the current study regarding the occurrence or non-occurrence of a host shift or increase to include *T. usneoides*.

When the measures of insect performance (i.e., results from both the no-choice and choice tests) were used to conduct a risk assessment, it revealed that there is a low feeding and reproductive risk associated with *C. tamarisci* when it is inoculated onto *T. usneoides*. During host-specificity trials in the United States, *C. tamarisci* also showed a low risk when associated with *T. aphylla*, a sister taxon to *T. usneoides*, as the weevil could not complete its life cycle on the *Tamarix* plant (Fornasari, 1998; Villar *et al*., 2019). However, there is not enough evidence to suggest that *C. tamarisci* should be released as a biocontrol agent of invasive *Tamarix* in South Africa as it completed its life cycle on *T. usneoides*. In Olckers (2003), an unidentified species of *Acallepitrix* (Coleoptera: Chrysomelidae), that was a potential biocontrol agent of invasive *S. mauritianum*, exhibited nontarget effects during host-specificity trials, but when a risk assessment was conducted it showed a relatively low risk associated with nontarget *Solanum* plants, which led to the suggestion of open field host-specificity trials. This is because open field studies reveal the insect's realized host range (Balciunas *et al*., 1996).

During the laboratory-based host-specificity trials of *Bagous hydrillae* (O'brian.) (Coleoptera: Curculionidae), a biocontrol candidate of *Hydrilla verticillata* (Royle.) (Alismatales: Hydrocharitaceae), the semi-aquatic weevil utilized 16 phylogenetically related host plants (Balciunas *et al*., 1996). However, during field studies *B. hydrillae* was only found on seven plant species and commonly on one, i.e., the target *H. verticillata* (Balciunas *et al*., 1996). It is because of this narrow host plant range observed in the field that permission

to release *B. hydrillae* in Florida was obtained (Balciunas *et al*., 1996). A similar case is that of *Rhinoncomimus latipes* (Korotyaev.) (Coleoptera: Curculionidae), where during laboratory-based host-specificity trials the weevil fed on 13 plant species, while during open field studies it did not feed on any nontarget species (Frye *et al*., 2010). Generally, laboratory-based host specificity studies report on the physiological host range of a potential biocontrol agent, which may not represent the realized host range that includes plants that the agent will develop on in the field (Balciunas *et al*., 1996). Therefore, open field studies in Eurasia are a necessary follow up to the current study as they will assist in determining the realized host range of *C. tamarisci*, which will hopefully exclude *T. usneoides* and its hybrid combinations.

2.5. Conclusion

The current study presents results obtained from a range of no-choice and preliminary choice tests to determine the host specificity of *Coniatus tamarisci* against *Tamarix* taxa in South Africa. These results show that *C. tamarisci* fed and completed its life cycle on the indigenous *T. usneoides* and its hybrid combinations; however, the risk probabilities of *C. tamarisci* feeding and ovipositing on *T. usneoides* were low in comparison to the invasive *Tamarix* taxa present in South Africa. Open field studies in the *Coniatus* weevil's natural environment are necessary before release, as *C. tamarisci* has a strong association with the *T. chinensis* × *usneoides* hybrid combination. Should *C. tamarisci* fail open field host-specificity trials, then the remaining potential biocontrol agents of invasive *Tamarix* taxa, i.e., the stem-galling midge *Psectrosema noxium* (Marikovskij.) (Diptera: Cecidomyiidae) and the root-galling weevil *Liocleonus clathratus* (Olivier.) (Coleoptera: Curculionidae), should be investigated further (Marlin *et al*., 2019).

Chapter Three

Untargeted UHPLC/Q-TOF-MS Comparative Assessment of Phenolic Compounds of Indigenous and Invasive *Tamarix* L. (Caryophyllales: Tamaricaceae)

Abstract

Tamarix L. (Caryophyllales: Tamaricaceae) is a genus of halophyte tree or shrub that is native to Eurasia, the Mediterranean, north and south-western Africa with several taxa having successfully invaded parts of North America, South America, Australia, and South Africa. In South Africa, there is also an indigenous *Tamarix* taxon, *T. usneoides*. Historically, species from the genus have been reported to be a good source of phenolic compounds, some of which are of commercial importance. Plants also use the toxicity of phenolic compounds to deter generalist herbivores. Numerous qualitative and quantitative studies have been done to confirm the presence of phenolic compounds in *Tamarix* globally. However, there are insufficient qualitative and quantitative studies on the *Tamarix* taxa present in South Africa. The current study aimed to fill this knowledge gap, as these biochemical compounds are determinants of insect host selection; thus, they could help in finding a suitable biological control agent of invasive *Tamarix* in the country. Using Liquid Chromatography–Mass Spectrometry, the qualitative analysis revealed that the same 28 phenolic compound constituents exist in the indigenous *T. usneoides* and the invasive *Tamarix* taxa,while the quantitative analysis showed that three phenolic acids; namely gallic acid, dehydrodigallic acid, and syringic acid, exist in significantly greater amounts in the indigenous *T. usneoides* than the invasive *Tamarix* taxa. *Tamarix usneoides* is an evergreen, and high levels of phenolic compounds could be evidence of a physiological trade-off or evolutionary adaptation to cope with water stress. Regarding biological control, the quantitative analysis

results are significant as insects are adapted to different levels of phenolic compounds. Therefore, there is hope that an insect, pathogen or a mite that is host specific to the invasive *Tamarix* taxa could be found.

Keywords: Evergreen, deciduous, phenolic acids, *Tamarix*, qualitative and quantitative analysis.

3.1. Introduction

3.1.1. Evolution and function of secondary metabolites

Since the Devonian period, plants have had to cope with herbivory, bacterial andfungal infections, and viruses (Wink, 2018). As a result, plants evolved direct defense responses involving physical structures – for example, trichomes (Wink, 2018; Zaynab *et al*., 2018). Trichomes are fine unicellular or multicellular epidermal appendages found on plant leaves and stems (Glas *et al*., 2012; Wang *et al*., 2021). Additionally, they can also occur on plant fruits, flowers, and roots (Glas *et al*., 2012). These structures are classified into two groups i.e., non-glandular and glandular trichomes (Wang *et al*., 2021). Non-glandular trichomes are found on the surfaces of plant leaves and stems where they physically deter insect herbivory and the selection of oviposition sites (Tozin *et al*., 2016). Glandular trichomes are also found on the surfaces of plant leaves and stems, but chemically deter insect herbivory through the secretion of biochemical compounds (Kang *et al*., 2010; Glas *et al*., 2012).

Initially considered waste products, biochemical compounds (hereafter secondary metabolites) are derived from nitrogen metabolism and are stored in plant cells, tissues, and organs (Bourgaud *et al*., 2001; Akula & Ravishankar, 2011). The quantities of these secondary metabolites stored can be dependent on the severity of the stress applied to the plant (Lavola & Julkunen-Tiitto, 1994). Lattanzio (2013) states that the levels of secondary metabolites in plants is environmentally induced. Environmental stresses include, but are not

limited to, elevated carbon dioxide (CO_2) levels, low temperatures, pathogen infection, nutrient deficiency, and herbivores (Lattanzio, 2013). *Betula pendula* (Roth.) (Fagales: Betulaceae) (European white birch) seedlings grown under high CO_2 concentrations (i.e., 1050 and 1400ppm) have been observed to produce greater quantities of secondary metabolites compared to those grown under low CO_2 (i.e., 350 and 700ppm) (Lavola & Julkunen-Tiitto, 1994). Apart from their defensive function, secondary metabolites have also been reported to be insect attractants (Ryan & Byrne, 1988; Matsuki *et al*., 2011; Wink, 2018). For example, glucosinolates are used as cues to lead *Papilio polyxenes* (Fabricius.) (Lepidoptera: Papilionidae) larvae to carrot leaves for feeding (Nahstedt, 1989).

3.1.2. Groups of secondary metabolites

Secondary metabolites are classified into three major groups namely, alkaloids, phenolic compounds, and terpenoids, based on their chemical structure and function (Zwenger & Basu, 2008; Zaynab *et al*., 2018). Alkaloids are simple organic compounds that contain at least one nitrogen atom (Zwenger & Basu, 2008; Jing *et al*., 2014; Ain *et al*., 2016). These compounds are secreted by approximately 20% of higher plants as a defensive measure against herbivory, since many of them are toxic (Jing *et al*., 2014; Ain *et al*., 2016). Terpenoids are modified terpenes (i.e., terpenes containing an additional functional group with oxygen) considered to be by-products of primary metabolism produced in plant vegetative tissues, flowers, and, sometimes, roots (Perveen, 2018). Like alkaloids, terpenoids play an important role in enemy repulsion (Tholl, 2015; Hanus & Hod, 2020). However, unlike alkaloids, terpenoids also play an important role in the attraction of pollinating agents and are influential in the growth and development of plants (Tholl, 2015; Hanus & Hod, 2020). Phenolic compounds are the most prominent phytochemicals in higher plants (± 8 000 types) with diverse functionality (Zwenger & Basu, 2008; Cheynier *et al*., 2012; Lin *et al*., 2016; Minatel *et al*., 2017).

3.1.3. Phenolic compounds

Phenolic compounds are synthesized through the shikimic acid and phenylpropanoid pathways and are distributed throughout the various plant parts (i.e., leaves, stems, roots, and fruits) (Lin *et al*., 2016; Barba de la Rosa *et al*., 2019). These compounds are characterized as simple phenols, phenolic acids and polyphenols that contain 12-16 phenolic hydroxyl groups in a five - to seven – aromatic – ring structure (Zwenger & Basu, 2008; Cheynier *et al*., 2012; Lin *et al*., 2016; Minatel *et al*., 2017). Higher plants synthesize several thousand known different phenolic compounds, and the number known is continually increasing (Boudet, 2007; Lattanzio, 2013). It is estimated that two percent of all carbon photosynthesized by higher plants is converted into phenolic compounds (Robards & Antolovich, 1997). Phenolic compounds are divided into several classes, where some are extremely widespread throughout higher plants while others are specific to certain plant families/genera/species (Cheynier *et al*., 2012). An example of a widely distributed class of phenolic compounds are flavonoids, while mangostanaxanthone VII (a phenolic compound type) is only found on the pericarp of *Garcinia mangostana* L. (Malpighiales: Clusiaceae) and represents an example of a phenolic compound that is exclusive to a species (Garcia–Salas *et al*., 2010; Ibrahim *et al*., 2018).

Some classes of phenolic compounds also differ in their function. For instance, phenolic acids have a key role as defense compounds when environmental stresses, such as high light, low temperature, pathogen infection, herbivores, and nutrient deficiency are prevalent (Barbehenn & Constabel, 2011; Cheynier *et al*., 2012; Lin *et al*., 2016), while polyphenols, such as flavonoids and anthocyanins, are influential in attracting pollinating agents and as colouring for camouflage to protect from natural enemies (Simmonds, 2001; Lin *et al*., 2016).

3.1.4. Other functions of secondary metabolites

Some animals use plant secondary metabolites to protect themselves from predators (Babber, 2015). For example, pyrrolizidine alkaloids found in *Utetheisa ornatrix* (Linnaeus.) (Lepidoptera: Arctiinae) larvae and adults renders these moths unpalatable to insectivores (Babber, 2015). Additionally, Jason (2005) reported that some animals use secondary compounds, received from ingestion of higher plants, to protect dietary proteins from degradation by the symbiotic microflora of foregut fermenters. Babber (2015) has also suggested that some secondary metabolites are important neurotransmitters in animals.

Commercially, secondary metabolites are used as dyes, flavouring agents, glues, oils, perfumes, and waxes (Crozier *et al*., 2006). Furthermore, secondary metabolites possess numerous pharmaceutical properties that aid in the treatment of endocrine disorders and cancers, to name a few (Huang *et al*., 2009; Ansari & Akhtar, 2019; Alnuqaydan & Rah, 2020; Mohammed *et al*., 2021). Alkaloids in *Capsicum annuum* L. (Solanales: Solanaceae), *Curcuma longa* L. (Zingiberales: Zingiberaceae), *Berberis vulgaris* L. (Ranunculales: Berberidaceae) and *Lepidium sativum* L. (Brassicales: Brassicaceae) have been reported to possess antidiabetic properties (Mohammed *et al*., 2021).

3.1.5. The genus *Tamarix* in South Africa

Tamarix L. (Caryophyllales: Tamaricaceae) consists of approximately 60 phreatophyte species, several of which have successfully invaded parts of North America, South America, Australia and South Africa (Mayonde *et al*., 2016). In South Africa, there is the evergreen indigenous *T. usneoides* (E. Mey ex Bunge), with the deciduous *T. chinensis* (Lour), *T. ramosissima* (Ledeb) and their hybrid combinations having established invasive populations in the Northern Cape, Eastern Cape, Western Cape, Free State, and Northwest provinces (Mayonde *et al*., 2016; Newete *et al*., 2019:2020). *Tamarix* plants excrete salts through

specialized glandular trichomes called vesiculated trichomes (Sookbirsingh *et al*., 2010). This gives *Tamarix* taxa a competitive advantage as it makes the soil too saline for co-occurring plant species resulting in a decrease in the population densities(Newete *et al*., 2019: 2020). Like other glandular trichome groups, vesiculated trichomes have also been reported to secrete secondary metabolites (Fernandes *et al*., 2016). Studies have confirmed the presence of vesiculated trichomes in two *Tamarix* taxa present in South Africa, namely, *T. usneoides* and *T. ramosissima* (Sookbirsingh *et al*., 2010; Wilson *et al*., 2017). A review of *Tamarix* secondary metabolites, which included *T. ramosissima*, revealed the presence of phenolic acids, flavonoids, and tannins (Bahramsoltani *et al*., 2020). Hatile *et al.* (2022) reported that the bark of *T. usneoides*, *T. chinensis*, *T. ramosissima*, and *T. chinensis* × *ramosissima*, contains phenolic compounds (specifically tannins). *Tamarix* taxa have also been reported to possess healing properties against heart disease, ulcers, hypertension, skin diseases, hair loss, and gastrointestinal disorders, indirectly confirming the presence of secondary metabolites in the genus (Alnuqaydan & Rah, 2020). However, more studies on the present secondary metabolites in the *Tamarix* species present in South Africa need to be done, not only for commercial benefit, but also to aid in the search of a biological control agent of invasive *Tamarix* taxa in South Africa (Rapo *et al*., 2019; Hatile *et al*., 2022).

3.1.6. Secondary metabolite analysis

The extraction method for these volatile compounds is dependent on their composition and their boiling point (Roopashree & Naik, 2019). Thus, numerous extraction procedures have been reported, for example, distillation, maceration, expression (squeezing plant material under great pressures to press out oils or other liquids), and solvent extraction – to accommodate differences in volatility (Roopashree & Naik, 2019). Commonly, solvent extraction is used because it has low energy consumption, large production capacity, fast action, easy continuous operation, and ease of automation, and it is done in three stages

(Roopashree & Naik, 2019). Firstly, solvent penetration into plant cells/tissue needs to occur to dissolve secondary metabolites into the solvent of extraction (Roopashree & Naik, 2019). The second stage is fractionation, while the third stage is achieved by High Performance Liquid Chromatography (HPLC) or Thin–layer Chromatography (TLC) which involves the separation and identification of the various components (Roopashree & Naik, 2019).

3.1.7. Aims and objectives

This chapter aimed to assess (i.e., qualitative and quantitative analyses) one of the main groups of secondary metabolites present in *Tamarix* taxa available in South Africa. This was done using Liquid Chromatography–Mass Spectrometry (LC–MS) (a technique under HPLC) to analyze the presence of phenolic compounds present in *Tamarix* taxa in South Africa.

3.2. Materials and methods

3.2.1. Plant material

3.2.2. Fresh leaf material from five *Tamarix* taxa (namely, *T. usneoides*, *T. chinensis*, *T. ramosissima*, *T. chinensis* × *ramosissima*, and *T. chinensis* × *usneoides*) previously identified by Mayonde *et al.* (2016) was collected in triplicates of 10g from: Salt Lake/Northern Cape (29.2800°S, 24.0037°E), Bosjesmans Vlei/Northern Cape (29.7333°S, 23.0500°E), Waterkloef En Kruisrivier/Western Cape (33.61667°S, 21.28333°E), and De Rust/Western Cape (33.4907°S, 22.5305°E) in February 2022. After collection, the fresh leaf material was stored in foil and cooler bags to preserve the volatiles. Later, the collected triplicates were divided into three pseudo replicates of five grams fresh leaf material for each *Tamarix* taxon, and oven dried for 48 hours before being crushed into powder with a mortar and pestle at the University of the Witwatersrand nursery, Johannesburg (26.1917°S, 28.0328°E).**LC–MS analysis**

From the pseudo replicates, three 3g samples of the powdered material from *T. usneoides*, *T. chinensis*, *T. ramosissima*, *T. chinensis* × *ramosissima*, and *T. chinensis* × *usneoides* were sent to the Central Analytical Facilities (CAF) of Stellenbosch University, Western Cape (33.9328°S, 18.8644°E) for LC–MS analysis. The samples were analysed using an untargeted

UHPLC/Q-TOF-MS approach and ran in negative ion mode ESI to scan for all compounds between 120 and 1 200 Da(m/z). Fragmented and unfragmented spectra were also submitted to a host of databases for tentative identification of the major compounds present.

3.2.3. Qualitative and Quantitative analysis

The phenolic compound data were received in a Microsoft Excel spreadsheet. Thereafter, the data were categorized, and present compounds were listed and compared. The compound weights (μg/kg) of individual phenolic compounds that were present in the test samples were also compared between the test plants.

3.2.4. Data Analysis

The Principal Component Analysis (PCA) statistical procedure was conducted using SIMCA to uncover the quantitative relationship (i.e., if there are any similarities or differences in the quantities of the phenolic compounds in the tested plants) between the indigenous *T. usneoides* and the invasive *Tamarix* taxa. Thereafter, a one-way independent variable analysis of variance (ANOVA) was conducted to compare the molecular weights of prominent phenolic compounds (if any) across the tested *Tamarix* taxa. If there were any significant differences between the compounds from tested *Tamarix* plants, then a Tukey's HSD post-hoc test was conducted.

For statistical tests, R and RStudio (R Core Team 2019, version 3.5.3) was used, and the significance level was set at $p\text{-value}<0.05$.

3.3. Results

3.3.1. Qualitative analysis

The results of the phenolic compound screening of leaf material from five *Tamarix* taxa (namely, *T. usneoides*, *T. chinensis*, *T. ramosissima*, *T. chinensis* × *ramosissima*, and *T.*

chinensis × *usneoides*.) revealed that the same classes of 27 phenolic compounds are present throughout the tested plants (Table 3.1).

Table 3.1. Phenolic compound constituents detected by LC–MS from the leaf extracts of *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu).

Phenolic compounds	***Tamarix* taxa**				
	Tu	**Tc**	**Tr**	**TcTr**	**TcTu**
2-(3,4-dihydroxyphenyl)-5-hydroxy-10-(4-oxo-4H-chromen-3-yl)-4H,8H,9H,10H-pyrano[2,3-h] chromene-4,8-dione	+	+	+	+	+
2-O-dehydrodigallic acid monocarboxyloyl-3-O-galloyl-(alpha/beta)-glucose	+	+	+	+	+
3,4-Dihydroxymandelic acid	+	+	+	+	+
3,4,8,9,10-pentahydroxy dibenzo [b, d] pyran-6-one	+	+	+	+	+
3,5,7-trihydroxy-8-methoxy-2-(4-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	+	+	+	+	+
(2S,3S,4S,5R,6R)-6-[(E)-4-keto-4-methoxy-but-2-enoyl]oxy-3,4,5-tripropioloyloxy-tetrahydropyran-2-carboxylic acid	+	+	+	+	+
Auriculoside	+	+	+	+	+
Caffeoyl glucose sulfate I	+	+	+	+	+
Caffeoyl glucose sulfate II	+	+	+	+	+
Coumaroyl glucose sulfate	+	+	+	+	+
Dehydrodigallic acid	+	+	+	+	+
Demethoxyegonol	+	+	+	+	+
Ellagic acid	+	+	+	+	+
Gallic acid	+	+	+	+	+
Methylisocitric acid	+	+	+	+	+
MINEs-324828	+	+	+	+	+
Phenylpyruvic acid	+	+	+	+	+
Quercetin 3-O-glucuronide	+	+	+	+	+
1-Salicylate glucuronide	+	+	+	+	+
Shoyuflavone C	+	+	+	+	+
Syringic acid	+	+	+	+	+
Syringic acid isomer	+	+	+	+	+
Syringic acid sulfate	+	+	+	+	+

Tellimagrandin I	+	+	+	+	+
Tricrozarin A	+	+	+	+	+
UNPD94347	+	+	+	+	+
Uralenneoside	+	+	+	+	+

Key: + = Present, - = absent

3.3.2. Quantitative analysis

3.3.2.1. Principal Component Analysis

The Principal Component Analysis using the LC–MS phenolic screened data resulted in clusters separating the indigenous *T. usneoides* from the remaining invasive *Tamarix* taxa that are grouped together. The following phenolic classes: gallic acid, dehydrodigallic acid, and syringic acid were obtained in different quantities separating *T. usneoides* from the rest (Fig. 3.1).

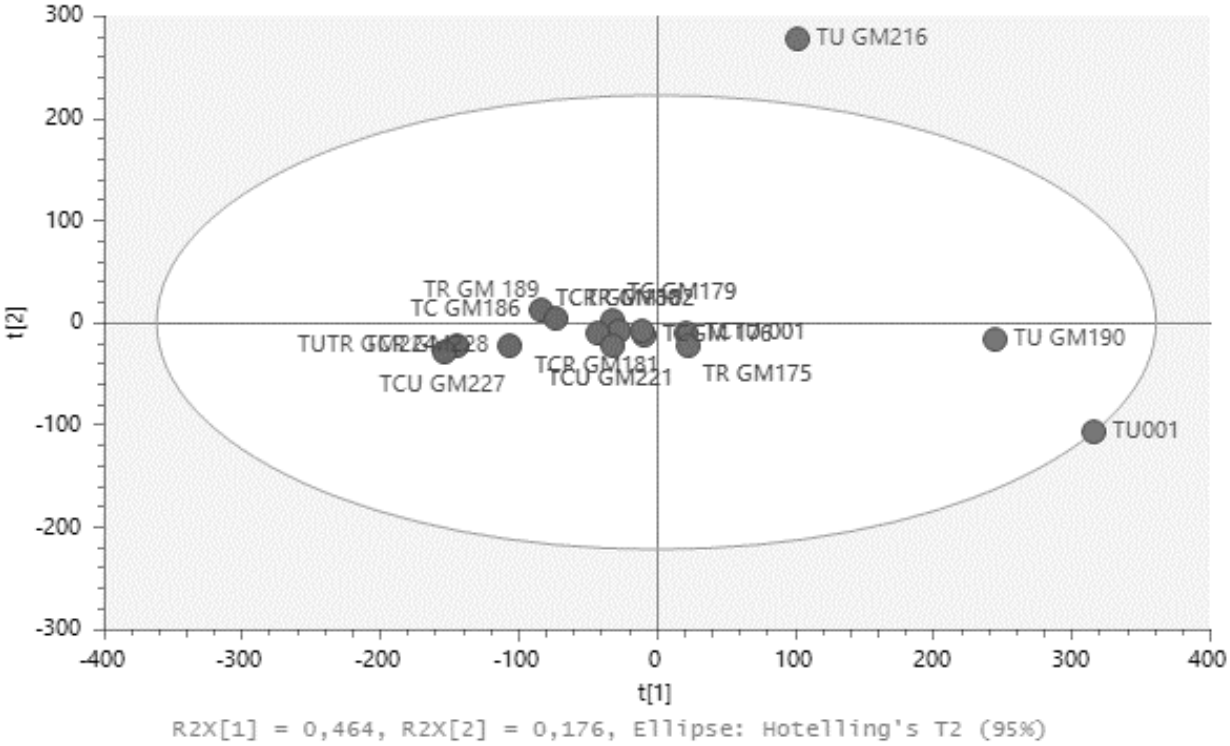

Figure 3.1. A PCA score plot of MS data of leaf samples from five *Tamarix* taxa obtained under the same extraction techniques and conditions.

3.3.2.2. Phenolic acid: Gallic acid

Overall, the molecular weight of gallic acid present in the experimental samples (3g each) across the different *Tamarix* taxa (n = 3 per taxon) was significantly different ($F = 11.24$, $df = 4.000$, $p < 0.05$). *Tamarix usneoides* samples had the greatest amount of gallic acid, while there was no significant difference between the invasive *Tamarix* taxa and their hybrids (Fig. 3.2).

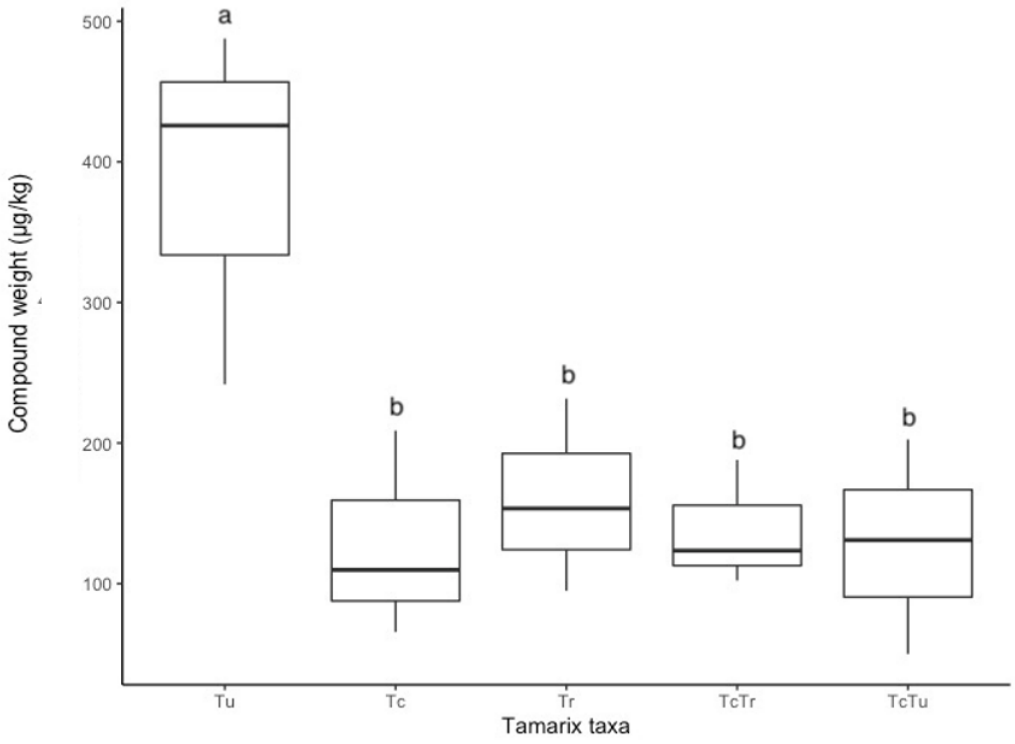

Figure 3.2. Mean gallic acid weights (µg/kg) of 3g *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) found in South Africa. n = 3 samples per *Tamarix* taxon. Different lowercase letters indicate significant differences between mean; ANOVA (p – value <0.05).

3.3.2.3. Phenolic acid: Dehydrodigallic acid

There was a significant difference in the molecular weight of dehydrodigallic acid detected in the samples (3g each) of the five experimental *Tamarix* taxa ($F = 5.394$, $df = 4.000$, $p < 0.05$). *Tamarix usneoides* had the greatest amount of dehydrodigallic acid, while there was no significant difference between the invasive *Tamarix* taxa and their hybrids (Fig. 3.3).

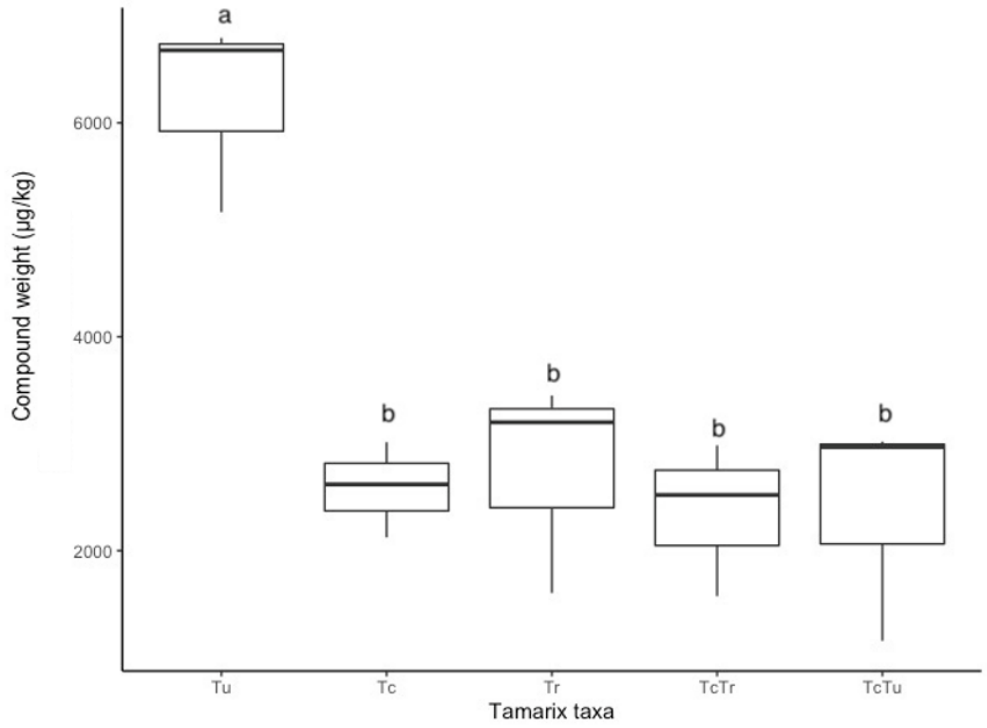

Figure 3.3. Mean dehydrodigallic acid weights (µg/kg) of 3g *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) found in South Africa. n = 3 samples per *Tamarix* taxon. Different lowercase letters indicate significant differences between mean; ANOVA (p – value <0.05).

3.3.2.4. Phenolic acid: Syringic acid

There was a significant difference in the molecular weight of syringic acid detected in the samples (3g each) of the five experimental *Tamarix* taxa ($F = 5.394$, $df = 4.000$, $p < 0.05$). *Tamarix usneoides* had the greatest amount of syringic acid, while there was no significant difference between the invasive *Tamarix* taxa and their hybrids (Fig. 3.5).

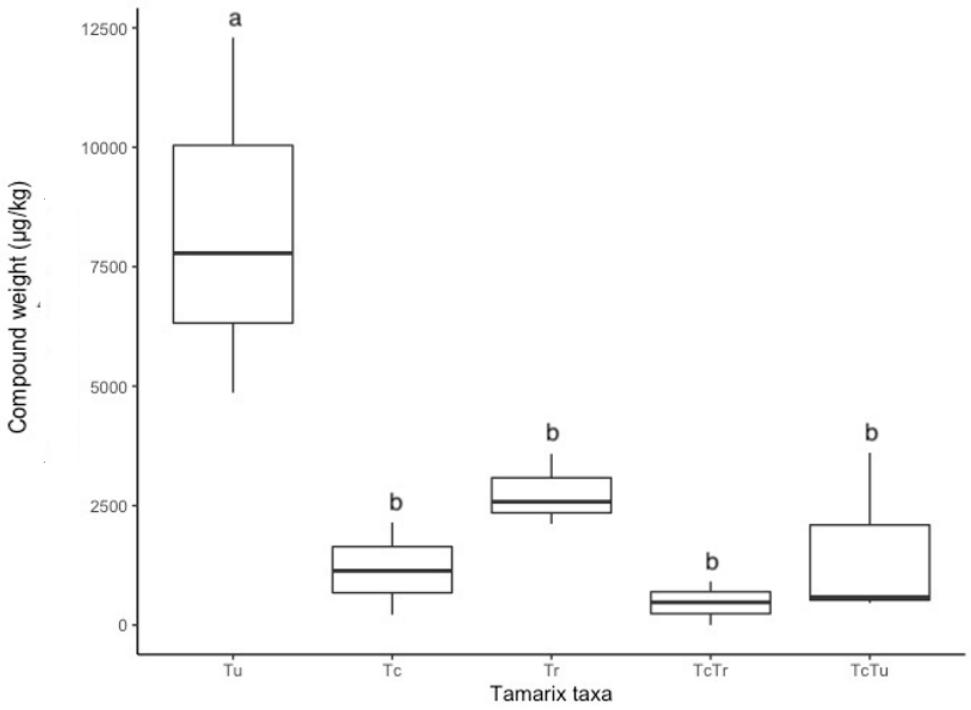

Figure 3.4. Mean syringic acid weights (µg/kg) of 3g *T. usneoides* (Tu), *T. chinensis* (Tc), *T. ramosissima* (Tr), *T. chinensis* × *ramosissima* (TcTr), and *T. chinensis* × *usneoides* (TcTu) found in South Africa. n = 3 samples per *Tamarix* taxon. Different lowercase letters indicate significant differences between mean; ANOVA (p – value <0.05).

3.4. Discussion

The qualitative analysis results indicated the same phenolic compound constituents across the *Tamarix* taxa present in South Africa. These results are not surprising as the principles of chemotaxonomy dictate that the presence of certain secondary metabolites is dictated by various enzymes present in a plant family or genus (Sarker & Nahar, 2006). On the contrary, the quantitative analysis results revealed that there are three phenolic compound classes whose quantities are higher in the indigenous *T. usneoides* than in invasive *Tamarix* taxa. These hydroxybenzoic acids, namely gallic acid, dehydrodigallic acid, and syringic acid, function as a part of a plant's defence against herbivory by deterrence through toxicity (Balasundram *et al*.,2006; Barbehenn & Constabel, 2011; Cheynier *et al*., 2012; Lin *et al*., 2016). In addition to being present in *Tamarix* taxa in South Africa, qualitative analyses have revealed the presence of these acids in *Tamarix amplexicaulis* (Ehrenb.) and *T. aphylla* (Karst.) (Pal *et al*., 2018; Alhazmi *et al*., 2019).

The quantity of phenolic compounds, like phenolic acids, has a positive correlation with levels of toxicity (Nahstedt, 1989). For instance, the feeding intensity of *Papilio polyxenes* (Fabricius.) (Lepidoptera: Papilionidae) larvae on carrot leaves is reduced depending on the amount of glucosinolates present; at higher concentrations intoxication and death of larvae occurs (Nahstedt, 1989). Recent studies state that secondary metabolites are primary determinants of insect host selection (Rapo *et al*., 2019; Barrett *et al*. 2021), with exploitation of plant hosts being dependent on an insect's ability to ingest the host's concentration of phenolic compounds (Nahstedt, 1989). Some insects can take in high concentrations of phenolic compounds making them fit to exploit certain plants, while others can only tolerate low concentrations of these compounds. For example, *Spodoptera eridania* (Stoll.) (Lepidoptera: Noctuidae) individuals feed on the leaves of the lima bean that have higher concentrations of glucosinolates than *P. polyxenes* can cope with (Nahstedt, 1989).

According to Silva *et al.* (2015), plants of different growth forms (namely, evergreen and deciduous plants) possess different leaf characteristics, which reflect physiological trade-offs and/or evolutionary adaptations that affect feeding intensity. Through the comparison of three evergreen species (namely, *Aspidosperma polyneuron* Müll.Arg. (Gentianales: Apocynaceae), *Goniorrhachis marginata* Taub. (Fabales: Fabaceae), and *Ziziphus joazeiro* Mart. (Rosales: Rhamnaceae)) and three deciduous species (namely, *Sapium glandulosum* L., *Handroanthus reticulatus* Mattos., and *Combretum duarteanum* Cambess.), it was discovered that evergreen plant leaves were thicker and had greater concentrations of phenolic compounds compared to deciduous plant leaves (Silva *et al*., 2015). Lattanzio (2013) stated that phenolic compounds are elevated by environmental stress (e.g., water stress), and Silva *et al.* (2015) concluded that evergreen plants had the aforementioned characteristics to combat water stress and predation throughout the year. Bautista *et al.* (2015) also compared the levels of total phenolic compounds and antioxidant flavonoids in many plant species from different families growing under varied environmental conditions (including altitude and water stress) and discovered a significant difference in phenolic compound levels between plants from different environments, with those in regions of low rainfall having more phenolic compounds.

In South Africa, *T. usneoides* is abundant in regions of low rainfall and is an evergreen, unlike the exotic *Tamarix* taxa (Mayonde *et al*., 2016; Marlin *et al*., 2017; Newete *et al*., 2020). This might explain the significantly high amounts of phenolic acids (i.e., the three hydrobenzoic acids) found in the indigenous taxon. It might also explain why Drude (2019) discovered a significant difference in the insect communities that visit *T. usneoides* compared to those that visit the invasive *Tamarix* taxa in South Africa, as insects are adapted to different levels of phenolic compounds.

3.5. Conclusion

Using LC–MS, the current study confirms the presence of the same phenolic compounds in five *Tamarix* taxa present in South Africa, with a distinction in the quantities present. The qualitative analysis results align with the principles of chemotaxonomy that state that the same secondary metabolites are present in plants of the same family or genus (Sarker & Nahar, 2006). Therefore, the high levels of gallic acid, dehydrodigallic acid, and syringic acid found in *T. usneoides* can be probably attributed to the indigenous taxa being an evergreen, as it is in the same genus as the invasive *Tamarix* taxa which are deciduous plants. Evergreen plants have thicker leaves and greater levels of phenolic compounds than deciduous plants (Silva *et al*., 2015). The quantitative analysis provides a possible explanation for the different insect communities found visiting *T. usneoides* and the invasive *Tamarix* taxa in South Africa (Drude, 2019).This is of importance to biological control, because secondary metabolites are determinants of insect host selection (Rapo *et al*., 2019; Barrett *et al*., 2021), and the different insect communities already noted suggest that an insect that is host specific to the invasive *Tamarix* taxa could be found.

More studies on secondary metabolites present in *Tamarix* taxa in South Africa need to be done, with a focus on alkaloids and terpenoids which serve similar functions to phenolic compounds (Tholl, 2015; Hanus & Hod, 2020). Furthermore, factors that influence the quantities of secondary metabolites, such as leaf traits and morphology, also need to be investigated. Like the current study, such findings will contribute to the implementation of effective and safe biological control.

Chapter Four

General Conclusion

The ongoing sixth mass extinction may be the most serious environmental threat to ecosystem functionality, because it is irreversible (Ceballos *et al*., 2020). According to the Living Planet Index, which synthesizes trends in vertebrate populations, species have declined rapidly since 1970, with an approximated reduction of 40% for terrestrial species, 84% for freshwater species and 35% for marine species (IPBES, 2019). Additionally, angiosperms have been disappearing at a rate of three species a year – worldwide – since 1900 (Ledford, 2019).

South Africa has been reported as one of the world's most biodiverse regions, with an estimated 7% of the world's vascular plants, 5% of mammals, and 7% of birds found in the region (Le Roux, 2002). Moreover, more than half the plants, butterflies, amphibians, and reptiles native to South Africa are endemic (Colville *et al*., 2014). Unfortunately, like the rest of the world, South Africa's biodiversity is in decline. It has been reported that between 1990 and 2018 the country's plant richness decreased by 0.12%, with a faster rate of decline (i.e., 0.24%) being observed between 2014 and 2018 (Skowno *et al*., 2021). According to van Wilgen *et al.* (2008), one of the major threats to biodiversity in South Africa is the presence of invasive alien plants (IAPs). Invasive alien plants have also been reported to affect water availability (Chamier *et al*., 2012). With the frequent droughts in South Africa in the last four decades, the presence of IAPs consequently places more pressure on the available water resources (Meza *et al*., 2021). In 1995, the Working for Water (WfW) programme was established by the South African government to address issues raised by the presence of IAPs (Chamier *et al*., 2012).

The WfW programme is allocated ZAR 500 million per annum to monitor and control IAPs throughout South Africa (Chamier *et al*., 2012) including *T. chinensis* and *T. ramosissima*. The Alien and Invasive Species regulations of the National Environmental Management: Biodiversity Act 2014 (NEM:BA) (DEA, 2016) and Henderson (2020) have classified invasive *Tamarix* as category 1b invaders, which require management. This is because invasive *Tamarix* plants act as drivers of ecosystem change (Gaffke *et al*., 2022). For example, dry *Tamarix* foliage ignites readily and burns with high intensity, thereby increasing the frequency and extent of wildfires, consequently affecting native flora that is not adapted to frequent wildfires (Gaffke *et al*., 2022).

In efforts to manage the spread of invasive *Tamarix* taxa in South Africa, two potential biological control agents (namely, *Diorhabda carinulata* (Desbrochers.) (Coleoptera: Chrysomelidae) and *Trabutina mannipara* (Hemprich & Ehrenberg.) (Hemiptera: Pseudococcidae) underwent laboratory-based host-specificity trials, and failed (Marlin *et al*., 2019; Hatile *et al*., 2022). Potential biocontrol agents are usually selected for host-specificity testing based on their native distribution, and the phylogenetic relatedness of the invasive weed and the invaded areas' indigenous plants (nontarget). This system has proven mostly successful (Wapshere, 1974; Briese & Walker, 2002; Tracy & Robbins, 2009; Mayonde *et al*., 2016; Marlin *et al*., 2019). However, recently secondary metabolites have been reported as potential determinants of insect host selection (Rapo *et al*., 2019; Barrett *et al*. 2021).

Secondary metabolites are biochemical compounds that are synthesized during nitrogen metabolism and contribute to plant growth, development, and defense (Bourgaud *et al*., 2001; Kang *et al*., 2010; Akula & Ravishankar, 2011; Glas *et al*., 2012). One of the most common groups of secondary metabolites are phenolic compounds, which have different classes that play a significant role in insect attractance and/or deterrence (Balasundram *et al*., 2006; Zwenger & Basu, 2008; Cheynier *et al*., 2012; Lin *et al*., 2016; Minatel *et al*., 2017).

The current study followed the centrifugal phylogenetic method and considered the possible influence phenolic compounds could have on host selection during laboratory–based host-specificity trials of *Coniatus tamarisci* F. (Coleoptera: Curculionidae). These trials revealed that *C. tamarisci* can complete its life cycle on nontarget *T. usneoides*, but it has a low preference and a low reproductive risk on the South African indigenous *Tamarix*. During whole plant laboratory–based host-specificity trials in the United States, similar behaviour was observed in *C. tamarisci* as the weevil could not complete its life cycle on *T. aphylla* (Karst.), a sister taxon to *T. usneoides* (Fornasari, 1998).

Silva *et al.* (2015) has reported that evergreen species have thicker leaves and greater levels of phenolic compounds than deciduous species to combat extended periods of environmental stress (e.g., water stress), Silva *et al.* (2015) also stated that because of lower phenolic compounds, deciduous plants experience more herbivory than evergreen plants. Unlike *T. chinensis* and *T. ramosissima*, *T. usneoides* is an evergreen that commonly grows in regions of low rainfall (Mayonde *et al*., 2015; Marlin *et al*., 2017; Newete *et al*., 2020). *Tamarix aphylla* is also an evergreen species (Sharma *et al*., 2018). The quantitative analysis of *Tamarix* taxa present in South Africa revealed that gallic acid, dehydrodigallic acid, and syringic acid were found in significantly greater amounts in the indigenous *T. usneoides* than the invasive *T. chinensis* and *T. ramosissima*. Gallic acid, dehydrodigallic acid, and syringic acid have also been reported as the main groups of phenolic *compounds* present in *T. aphylla* (Alhazmi *et al*., 2019). The plant growth form and high phenolic compounds detected in *T. usneoides* could possibly explain the low preference and low reproductive risk exhibited by *C. tamarisci* during laboratory–based host-specificity trials.

Alkaloids and terpenoids are other groups of secondary metabolites that have been detected in the *Tamarix* genus (Umbetova *et al*., 2006; Hebi & Eddouks, 2017; Alhazmi *et al*., 2019). These compounds play a significant role in insect attraction and/or deterrence (Tholl, 2015;

Hanus & Hod, 2020). More studies need to be done on factors influencing secondary metabolites (leaf traits) and other secondary metabolites (i.e., alkaloids and terpenoids) in *Tamarix* taxa present in South Africa. Regarding the biocontrol of invasive *Tamarix* taxa in the country, open field host-specificity trials in Eurasia are needed to reveal the realized host range of *C. tamarisci* in a natural setting. Ultimately, assessing plant secondary metabolites should also continue being common practice in biocontrol research.

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