Biological Control

Comparison of the performance of an eriophyid mite, Aceria salsolae, on nontarget plants in the laboratory and in the field
--Manuscript Draft--

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Abstract:	There is increasing interest in the possibility of using eriophyid mites as biological control agents of invasive alien weeds; however their small size and our lack of knowledge about their general biology present challenges to evaluating their risk to nontarget plants. Aceria salsolae has been proposed as a candidate agent for Russian thistle (Salsola tragus) in the USA. During host specificity testing this mite could sometimes persist on five nontarget species under laboratory no-choice conditions. We conducted a series of no-choice laboratory experiments and a field experiment to try to delineate the physiological and ecological host ranges of this mite and assess its risk to nontarget plants. In the laboratory, A. salsolae increased exponentially on S. tragus , multiplying about 80 fold in 5 weeks. Low levels of reproduction were observed on some plants of Atriplex coronata , Bassia hyssopifolia , B. prostrata , Kochia scoparia and Suaeda calceoliformis in the laboratory during 5 weeks, but mean mite densities remained low (less than 6 fold increase vs. 80 fold on S. tragus). In a field experiment in which plants were inoculated with mites in June and then harvested when they began to produce seed, mites persisted on A. coronata for up to 9 weeks after inoculation , but at extremely low densities, and with no evidence of reproduction. No mites persisted on A. truncata , B. hyssopifolia, or S. calceoliformis. Mite densities were lower on all plants in the field than in the laboratory, probably due to increased mortality and the opportunity to disperse by wind. No signs of damage were observed on any of the nontarget plants in the laboratory or the field experiments. We conclude that this mite is not likely to multiply on any of these plants under field conditions, and that it is not expected to pose a risk to any nontarget plants in the contiguous USA.

Response to Reviewers:	Response to Editor comments regarding the manuscript BCON_2020_391R1, "Comparison of the performance of an eriophyid mite, Aceria salsolae, on nontarget plants in the laboratory and in the field". Our responses are in all capitals text following each of the Editor comments listed below. Editor Comments: As it stands the Discussion is too long, which makes it difficult for the reader to get the message that is being conveyed. It will benefit from being trimmed considerably.

There are two main problems:

(i)repetition of details from the results. Only the broad findings of the results are needed to support points that are being made in the Discussion.
WE DELETED THIS TEXT AND MOVED SOME OF IT TO THE METHODS SECTION.

(ii)Several parts provide details from other studies which are not needed here (e.g. mark-up in lines 453 – 483 of the attached file). While details of species names and measurement values are appropriate for reviews, research articles only need the principle of the findings (e.g. the life span of mites decreases with increasing temperature) and the reference that shows this trend.
WE REDUCED THE TEXT REFERRING TO OTHER STUDIES.

The discussion would also benefit from more paragraph breaks to improve the flow of ideas.
ONE PARAGRAPH WAS SPLIT AT NEW LINE 492.

COMMENTS FROM THE ANNOTATED MANUSCRIPT PDF:
We accepted all proposed changes except the following.

L 71 Keep "important" instead of "necessary" proposed by the Editor.

L293 We agree that a one-sentence section is odd; however the section serves to group the two laboatory experiments, which parallels the structure of the Methods section. The one sentence is to document that there was no contamination of the mite colony. This eliminates the possible hypothesis that another mite species may have infested some plants. de Lillo is mentioned in the Methods section as the taxonomist, so this sentence could be omitted with the unstated assumption that there was no contamination. We do not object to deleting this section but prefer to keep it.

L 298 Keep "fit" instead of "fitted" proposed by the Editor.

United States Department of Agriculture
Research, Education and Economics
Agricultural Research Service

21 Sept. 2020

TO: Editor of Biological Control

Dear Dr. Hoffmann;

Attached please find a revised version of manuscript BCON_2020_391, "Comparison of the performance of an eriophyid mite, *Aceria salsolae*, on nontarget plants in the laboratory and in the field".

Thank you very much for your consideration.

Sincerely,

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Agricultural Research – Investing in Your Future

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Highlights

Aceria salsolae is a prospective agent of Russian thistle (*Salsola tragus*).

Small size and dispersal by wind makes it difficult to asses host specificity.

We conducted laboratory and field experiments to measure host plant specificity.

Mite densities were much lower on target and nontarget plants in the field.

Risk analysis indicates that this mite should be safe to use in the USA.

1 ~~Title –~~ Comparison of the performance of an eriophyid mite, *Aceria salsolae*, on nontarget plants
2   in the laboratory and in the field.

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5   Javid Kashefi[5], Massimo Cristofaro[1, 6], Lincoln Smith[3]

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22

23

Abstract

There is increasing interest in the possibility of using eriophyid mites as biological control agents of invasive alien weeds; however their small size and our lack of knowledge about their general biology present challenges to evaluating their risk to nontarget plants. *Aceria salsolae* has been proposed as a candidate agent for Russian thistle (*Salsola tragus*) in the USA. During host specificity testing this mite could sometimes persist ~~and possibly multiply~~ on ~~a few~~ five nontarget species under laboratory no-choice conditions. We conducted a series of no-choice laboratory experiments and a field experiment to try to delineate the physiological and ecological host ranges of this mite and assess its risk to nontarget plants. In the laboratory, *A. salsolae* increased exponentially on *S. tragus*, multiplying about 80 fold in 5 weeks. Low levels of ~~A little~~ reproduction w~~as~~ere observed on some plants of *Atriplex coronata*, *Bassia hyssopifolia*, *B. prostrata*, *Kochia scoparia* and *Suaeda calceoliformis* in the laboratory during 5 weeks, but mean mite densities remained low (less than 6 fold increase vs. 80 fold on *S. tragus*). In a field experiment in which plants were inoculated with mites in June and then harvested when they began to produce seed, mites persisted on *A. coronata* for up to 9 weeks after inoculation, but at extremely low densities, and with no evidence of reproduction. No mites persisted on *A. truncata*, *B. hyssopifolia, or S. calceoliformis.* Mite densities were lower on all plants in the field than in the laboratory, probably due to increased mortality ~~factors~~ and ~~the ability~~ opportunity to disperse by wind. No signs of damage were observed on any of the nontarget plants in the laboratory or the field experiments. We conclude that this mite is not likely to multiply on any of these plants under field conditions, and that it is not expected to pose a risk to any nontarget plants in the contiguous USA.

Key words: host plant specificity; risk assessment; biological control of weeds; Eriophyidae; *Aceria salsolae*; *Salsola tragus*

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Commented [A2]: Not clear toa reader what "a few" would entail. Why not give the number or use "some".

Commented [A3]: A six fold increase could be considerable without any context. What was the control?

Commented [A4]: An ability is innate. Should this be "and a tendency to disperse"

1. Introduction

Mites in the superfamily Eriophyoidea constitute a relatively underutilized group of potential biological control agents for invasive plants (Smith et al., 2010). About 4,800 species of eriophyoids have been described (de Lillo and Skoracka, 2010; Amrine & de Lillo unpubl. data). A review of 3,331 species concluded that 80% of them have only one known host plant species, and 95% of them have known hosts that are all in one plant genus (Skoracka et al., 2010). Although the high rate of apparent monospecificity may be partly due to the lack of sampling for mites on other plants (i.e., lack of knowledge), it nevertheless suggests that many species may be highly specific. Furthermore, some eriophyoid mites are important crop pests, which indicates that at least some could have enough impact to be effective as classical biological control agents (Lindquist et al., 1996; de Lillo et al., 2018).

Improving methods for identifying mites has helped to foster increasing research to evaluate them as prospective biological control agents (e.g., Denizhan et al., 2008; Vidović et al., 2018). However, their very small size (< 200 μm) and the fact that they disperse primarily by wind (e.g., Bergh, 2001; Valenzano et al., 2019; Kuczyński et al., 2020) make it challenging to design laboratory experiments that simulate realistic conditions that allow mites to move among plants. Thus, while no-choice laboratory experiments may be relatively easy to conduct, they delineate the physiological (or fundamental) host range and may overestimate the risk of attack under field conditions (ecological host range) (Schaffner, 2001). In cases where a nontarget plant species is attacked under no-choice conditions, assessing its risk of attack under more natural conditions may provide convincing evidence of its safety as a biological control agent (Hinz et al., 2014). Furthermore, it is also important to quantify any reduction in fitness (size, survivorship or reproduction) of the nontarget plant by the agent (Sheppard et al., 2005). Field garden host specificity tests may be the best way to assess the ecological host range of prospective arthropod agents of plants (Schaffner et al., 2018).

Russian thistle (*Salsola tragus* L. *sensu lato*, Chenopodiaceae) is an invasive annual forb in the western USA that originates from Central Asia (Young, 1991; Mosyakin, 1996). The taxonomy of this plant has been very confusing (Rilke, 1999), and recently it has been determined that five closely related species occur in California: *S. australis* R. Br., *S. gobicola* Iljin, *S. paulsenii* Litv., *S. ryanii* Hrusa and Gaskin, and *S. tragus* L. *sensu stricto* (Hrusa and Gaskin, 2008). The differences in the biology and ecology of these species have not been well studied, but *S. tragus* appears to be the most widespread in the western USA. Seeds germinate in the spring, plants flower in late summer and autumn, and eventually die due to lack of water or frost. Russian thistle has been targeted for classical biological control in the USA, and two moths were introduced in the 1970s (*Coleophora klimeschiella* Toll and *C. parthenica* Meyrick, Coleophoridae). Although both species have established, their feeding damage causes relatively minor impact to the weed, and mortality caused by natural enemies and/or poor seasonal synchrony have limited their populations to low densities (Goeden and Pemberton, 1995). Consequently, there is still a need for an effective biological control agent. Exploration for prospective agents led to the discovery of the mite *Aceria salsolae* de Lillo and Sobhian (Eriophyidae) in Turkey (de Lillo and Sobhian, 1996). The mite is multivoltine, and

91 behaves as a vagrant mite on immature *Salsola* plants, occurring primarily in the narrow spaces
92 of leaf axils in branch tips (Smith, 2005). It also forms galls, presumably from flower bud tissue,
93 later in the season (*S. tragus* flowers in autumn). In December and February, live mites have
94 been found under the bracts surrounding seeds that are still attached to dead plants (personal
95 obs.). Thus, it is likely that overwintering mites may be dispersed with the seeds. It is not
96 known how early in the season mites attack new plants, but they are present in early June in
97 northern Greece (Smith et al., 2009). Laboratory no-choice experiments indicated a high degree
98 of host plant specificity (Smith, 2005). Development occurred on all five species of *Salsola*
99 listed above, plus *Salsola collina* Pall. (slender Russian thistle), but not on any of 35 nontarget
100 species, including *Salsola soda* L. that were tested. The original host plant test list for *A.*
101 *salsolae* was based on the taxonomic treatment of Kühn (1993) for the family Chenopodiaceae,
102 which indicated that *Bassia* and *Kochia* (Tribe Camphorosmeae) were in a different subfamily
103 (Chenopodioideae) than *Salsola* (Salsoloideae). Thus, based on the centrifugal phylogenetic
104 hypothesis (Wapshere, 1974), higher priority was placed on testing nontarget species in the
105 Salsoloideae (e.g. *Halogeton*, *Sarcobatus*, *Suaeda*) than the Camphorosmeae. However, after
106 this work was conducted, a phylogeny based on molecular genetics by Berner et al. (2009)
107 showed that the Camphorosmeae is a sister tribe of Salsoleae, which includes *Salsola*. Thus,
108 *Kochia* and *Bassia* are now considered to be the most closely related genera present in North
109 America to *Salsola* after *Halogeton*. Additional no-choice tests conducted in the laboratory
110 indicated some persistence of the mites on *Bassia hyssopifolia* (Pallas) Kuntze and *Kochia*
111 *scoparia* (L.) Schrader 5 weeks after inoculation. Additional tests of *Atriplex coronata* S.
112 Watson, *A. truncata* (Torr. ex S. Wats.) Gray and *Suaeda calceoliformis* (Hook.) Moq. also
113 showed a few live mites on some plants 5 weeks after inoculation. Thus, a field experiment was
114 conducted to further test these nontarget plant species to determine if mites would persist and
115 damage them under natural conditions (Smith et al., 2009). The results showed no live mites on
116 *B. hyssopifolia* or *S. calceoliformis*, and an average of less than 1 mite on *K. scoparia* two
117 months after inoculation.
118   A petition was submitted to the U.S. Department of Agriculture, Animal and Plant Health
119 Inspection Service (APHIS) in 2004, and the Technical Advisory Group for Biological Control
120 Agents of Weeds (TAG) recommended approval for release in 2005. An Environmental
121 Assessment was published in the Federal Register in 2009 (vol. 74(45): 10223-10224), calling
122 for public comments to the proposed release. One public comment received noted that only 3 *S.*
123 *calceoliformis* plants survived to the end of the field experiment, and that more plants should be
124 tested. It also recommended testing some *Atriplex* species in the field.
125   The purpose of this study is to further assess the risk of *A. salsolae* to nontarget plants by
126 comparing its performance on potentially suitable nontarget plant species in the laboratory and
127 versus in the field.

128 **2. Materials and methods:**

129 2.1 Laboratory tests
130   These methods pertain to the two experiments described below (sections 2.2.1 and 2.2.2).
131 Mites for the laboratory experiments came from a colony of *A. salsolae* that originated from
132 Kozani, Greece (Smith, 2005) and were maintained on cuttings of *S. tragus* inside glass-topped
133 wooden sleeve boxes at room temperature (23°C, range 16 - 25°), 16 h L: 8 h D photoperiod, in a

134 certified containment laboratory in Albany, CA. Individual plants were inoculated with a given
135 number of adult mites by transferring them one-by-one using an eyelash glued to a wooden
136 applicator stick with the aid of a compound microscope. No-choice experiments were conducted
137 inside Dacron chiffon screen cages (70 mesh; 0.3 mm-wide openings; 0.7 x 0.7 x 1.0 m) with a
138 sheet metal bottom that was placed on pedestals standing in water moats to prevent mite escape.
139 The screen cage prevented air drafts which might permit the mites to disperse aerially. Working
140 surfaces were regularly wiped with 95% ethanol to disinfest them, and uninfested sentinel
141 cuttings of *S. tragus* in water vials placed outside the cages were regularly monitored to detect
142 possible escape of mites. Nontarget plants consisted of 5-wk-old plants grown from seed in 15
143 mL plastic flower pots (containing Supersoil™: sand: perlite in 3:1:1 ratio). *Salsola* test plants
144 were 15-cm tall cuttings from potted plants in the vegetative stage (no flowers or fruits) that were
145 held in water vials. Target and nontarget plants were placed in separate cages on a table.
146 Diurnal photoperiod was 16 h using ceiling fluorescent lamps, which were augmented by
147 halogen lamps for 4 h in the middle of the day. Vaseline smeared around the outside of each
148 flower pot provided a barrier against mite movement. Strips of double-sided tape were placed on
149 the floor of the cage in a grid pattern to isolate each plant and restrict mite movement.
150   At the appropriate sample date, the plants were examined for signs of mite damage and for
151 the presence of eggs, nymphs and adult mites under a microscope at 20x magnification. Each
152 plant was cut up and washed in a soapy solution to extract all mites which were transferred to a
153 5.3-cm diameter Petri dish (Monfreda et al., 2007). This procedure was at least twice as
154 effective for finding mites as visual inspection of intact plants. The extract solution was
155 immediately examined under the microscope and only live mites were counted. For samples that
156 had a large number of mites (viz. *S. tragus*), a grid (5 mm x 5 mm cells) was placed under the
157 Petri dish, and mites in 7 diagonal cells were counted. This number counted was multiplied by
158 the ratio of the area of the dish to that of the 7 cells to estimate the population. For the *S. tragus*
159 cuttings in the host plant suitability experiment (section 2.1.2), to reduce work, the top and
160 bottom branches were cut, weighed, and extracted separately to count live mites. The total
161 number of live mites per cutting was estimated by using the formula (# mites on top + (# mites
162 on bottom × (wt whole cutting - wt top) / wt bottom)). Mite numbers are greatest at the top and
163 fewest at the bottom (the mean difference was 6.79 fold in this experiment). This formula tends
164 to underestimate the true number of mites, whereas a simple weighted average of the two values
165 is an overestimate. Mites were identified based on morphology by E. de Lillo.

166 2.1.1 Elapsed time experiment
167   The purpose of this experiment was to assess whether mite populations continued to increase
168 on nontarget plants. Each plant was inoculated with 10 adult *A. salsolae*, and experiments were
169 run between 13 December 2005 and 16 March 2006. The change in mite population over time
170 was measured on *B. hyssopifolia* and *K. scoparia* compared to *S. tragus* following the general
171 procedures described above. Five nontarget plants were destructively sampled at 3, 5 or 7 weeks
172 post-inoculation, and 3 cuttings of the target plant were sampled at 3 and 5 weeks. Because *S.*

173 *tragus* cuttings began to deteriorate due to dense mite populations by 5 weeks, no measurements
174 were taken at 7 weeks.

175 2.1.2 Host plant suitability experiment.
176   The purpose of this experiment was to measure the persistence and/or population growth of
177 *A. salsolae* on *S. tragus*, the biological control target, and on the nontarget species *A. coronata*,
178 *A. truncata*, *B. hyssopifolia*, *B. prostrata* (L.) A.J. Scott, *K. scoparia* and *S. calceoliformis*. The
179 two *Bassia* species were tested because Berner et al. (2009) showed that they were closely
180 related to *Salsola* (in the sister tribe). *Atriplex coronata* had not been tested for the petition
181 (Smith, 2005), and this species has an endangered variety: *A. coronata* var. *notatior*. *Suaeda*
182 *calceoliformis* had been tested in a field experiment with the result that dead mites were found on
183 the plants which had senesced during the course of the experiment (Smith et al., 2009). In the
184 same field experiment, mites were also found on *K. scoparia*, but the authors did not distinguish
185 between live and dead mites. Thus, there was some uncertainty about suitability of these plants
186 for the mite. Five weeks was chosen for the test duration based on the previous observations that
187 *S. tragus* cuttings began to deteriorate after 5 weeks, and that the maximum mite population
188 observed on *K. scoparia* occurred at that time. The number of replicates was 9 for each
189 nontarget plant species, except 12 for *B. prostrata*, which has been released as a forage species
190 (Waldron et al., 2010; Clements et al., 2020). Two *S. tragus* cuttings were used as a positive
191 control for each nontarget test, resulting in a total of 12 replicates for *S. tragus* for this
192 experiment. Each plant was inoculated with 15 adult *A. salsolae*, and experiments were run
193 between 14 October 2010 and 14 December 2012.

194 2.2 Field test

195 2.2.1 Plants and field plots.
196   The experiment was conducted at BBCA (Biotechnology and Biological Control Agency
197 onlus) near Rome, Italy, in 2018. We tested four nontarget plant species on which *A. salsolae*
198 had persisted and/or increased in the laboratory no-choice experiments: *A. coronata*, *A. truncata*,
199 *B. hyssopifolia* and *S. calceoliformis*. All plants were grown from seed, starting in mid-April
200 2018. At the end of May, potted plants were set in holes at two field garden plots: one for
201 nontarget plants to be inoculated with *A. salsolae* (test) and the other not (negative control). The
202 two plots were located at the same site, to provide the same environmental conditions for the test
203 and negative control plants, but were separated from each other by ∼ 5 m ~~with the intention~~ to
204 minimize unintentional infestation of the negative control plants. Since eriophyid mites generally
205 disperse by wind (Nault and Styer, 1969; Lindquist and Oldfield, 1996; Bergh, 2001), inoculated
206 *S. tragus* plants (positive control) were placed in a field plot located ∼ 2 km away from the
207 nontarget plants in order to reduce the risk of contamination of the nontarget plants by mites
208 dispersing from heavily infested *S. tragus* plants following the strategy used by Gandolfo et al.
209 (2007). All plants were arranged ∼ 1.5 m apart. In particular, *S. tragus* was organized in 2
210 columns × 5 rows (10 replicates), whereas nontarget plants were in 4 columns × 7 or 10 rows
211 (one species per column; 7, 10, 7 and 10 replicates for *A. coronata*, *B. hyssopifolia*, *A. truncata*
212 and *S. calceoliformis*, respectively; Fig. 1). The ground of all field plots was covered with green

213 plastic, to prevent growth of weeds, and the plants were watered as needed. By 21 June, 4 + 4
214 (test and negative control) *A. coronata* plants and 1 + 1 (test and negative control) *A. truncata*
215 plants were added to their respective field plots, and 1 *S. tragus* (positive control) that had died
216 was replaced. By 25 June, 5 test *B. hyssopifolia* plants were replaced, because of damage caused
217 by ants and aphids. All plants introduced in the experiment after 7 June had been kept near the
218 field plots until they were used.

219 2.2.2 Eriophyid mite inoculation.
220   On 5 June, cuttings of *S. tragus* naturally infested with *A. salsolae* were collected from
221 Kozani area, Greece (Smith et al. 2009). The material was kept at a cool temperature (4°C) until
222 it was used. Each cutting was checked under stereo-microscope at 20x magnification and only
223 those with at least 10 living adult mites were selected to infest *S. tragus* and test nontarget plants.
224   On 7 June, an infested cutting, kept fresh by insertion in a water vial, was gently attached to
225 each potted plant to allow the voluntary and active movement of mites from infested cuttings to
226 plants (Smith et al., 2009; Schaffner et al., 2018). After the inoculation, water was no longer
227 added to the vials holding the infested cuttings, to allow their desiccation. Finally, the same
228 inoculation procedure was repeated on 21 and 25 June, when some plants were added or
229 replaced, as described above. Mites used to perform these other two inoculations came from a
230 colony originated from *A. salsolae* remaining from the material collected in Kozani, Greece and
231 maintained on young *S. tragus* plants kept outside at natural conditions. On the inoculation day,
232 nontarget plants, *A. coronata*, *A. truncata*, *B. hyssopifolia* and *S. calceoliformis*, were on average
233 3.6, 6.1, 13.3, 12.2 cm tall, respectively, whereas *S. tragus* plants were 16.7 cm. All plants were
234 in the vegetative stage (no flowers or fruits), except for three *A. truncata* test plants, which
235 already had a few flowers.
236   Eriophyid mite sampling. From 17 to 20 July, about 42-days post-inoculation, three cuttings
237 10 cm long were collected from 9 *S. tragus* positive control plants, to evaluate the success of the
238 inoculation procedure. The tenth target plant, which was added on 21 June, was sampled on 1
239 August (= 41 d post-inoculation). Cuttings were cut above and as close to the inoculation point
240 as possible. All mites were extracted from cutting samples as describe above, but transferred to a
241 4.7 cm diameter cellulose nitrate filter with 20 μm mesh openings (Sartorius), instead of a Petri
242 dish (Monfreda et al., 2007). Mites were counted under stereo-microscope at 20x magnification
243 and then stored in 70% ethanol for subsequent morphological identification, separating live and
244 dead mites, *i.e.* motile and sessile, and subsequently identified based on morphology by B.
245 Vidović.
246   On 27 August and 6 September, *i.e.* 81- and 91-days post-inoculation, two surveys using pan
247 traps were carried out at the nontarget field plot area, to identify the mite species present and
248 moving at the site. In particular, 18 pan traps, consist of small trays (17.5x10.5x3.5 cm) filled
249 with 500 ml of a soapy solution (Zhao and Amrine, 1997), were placed between nontarget plants,
250 as shown in Figure 1. The traps were exposed for 24 hours, during each survey. At the end of
251 each exposure, the soapy solution of each trap was collected and stored in a bottle and processed

252 using the same procedure describe above. Mites extracted were counted and stored in 70%
253 ethanol for subsequent morphological identification.
254   As soon as plants reached the mature growth stage (*i.e.* fruits), three 10-cm-long apical
255 branch cuttings were collected from each plant. All mites were extracted from cutting samples,
256 counted, collected and identified as describe above. If plants started to senesce earlier, the
257 sampling was performed in advance. Before collecting the cuttings, the number of secondary
258 branches, plant height and diameters (largest and smallest) were recorded, and any sign of
259 damage by mites or other organisms was noted. After the samplings, all plants were harvested,
260 and the aerial portion was stored in paper bags in dry place, out of direct light. Once the dry plant
261 weight stabilized, weight of plants and of 3 10-cm-long cuttings was measured, using a precision
262 balance.

263 2.3 Statistical analyses

264 2.3.1 Laboratory tests
265 The numbers of mites in the elapsed time experiment were fit to an exponential growth model
266 ($y = a \times e^{b \times x}$, where a represents the initial population size, b the growth rate, and x the week)
267 using nonlinear regression in JMP v. 14.0.0 (© 2018 SAS Institute Inc). The number of mites on
268 plants in the host plant suitability experiment was tested using ANOVA followed by Tukey HSD
269 comparisons of means. The effect of the number of live mites on change in plant height (final
270 height/initial height) was tested by linear regression.

271 2.3.2 Field test
272 Statistical analyses were carried out using the RStudio software Version 1.2.5042 (© 2009-2020
273 RStudio, Inc.; R Core Team 2020). All parameters were tested for normality and homogeneity of
274 error variances, and the data were analyzed with a parametric or non-parametric test according to
275 the results. The differences between the number of live and dead mites on 10 cm of cutting were
276 tested by the Welch test or Mann-Whitney test. The same approach was applied for testing the
277 differences between the number of secondary branches, plant height, diameters (largest and
278 smallest) and volume for inoculated vs. not inoculated plants for each species. Plant ''volume''
279 was calculated by the formula for an ellipsoid solid ($volume = \frac{4}{3} \times \Pi \times \frac{height}{2} \times$
280 $\frac{largest\ diameter}{2} \times \frac{smallest\ diameter}{2}$). A rough estimate of the number of live mites per plant was
281 calculated by the formula: $\left(\frac{number\ of\ live\ mites\ collected}{dry\ weight\ of\ 3\ 10-cm-long\ cuttings} \times \right.$
282 $dry\ weight\ of\ the\ plant \left. \right)$. Differences between the proportion of live juvenile, female and
283 male *A. salsolae* collected from each plant species were tested using the Chi-squared test (χ^2).
284 The effect of the plant species on the density of live mites was determined by a regression model
285 for count data. In particular, a negative binomial GLM with a *log link* function was used
286 [$log(y) = intercept + b_1(x)$], which considers the expected number of live mites on 10 cm of
287 cutting (y) depending on the species (x). Both plant weight and the interactive term for species
288 by plant weight were excluded because neither were statistically significant. The *log link*
289 function ensures positive fitted values, and the negative binomial distribution is typically used

290   for count data with over-dispersion of the dependent variable. Since the model uses a categorial

291   variable (*i.e.* the plant species), *S. tragus* was selected as the control (*i.e. intercept*), against

292   which the data from ~~*A. coronata*~~ test plants were compared.

3. Results

3.1 Laboratory tests

295   All mites examined by E. de Lillo were determined to be *A. salsolae*.

3.1.1 Elapsed time experiment

297   The mite population increased exponentially on *S. tragus* cuttings in both experiments

298   attaining means of 912.3 ($\pm$ 461.6 SE) and 726.3 ($\pm$ 90.2) live mites by week 5 (Fig. 2). An

299   exponential growth model ($y = a \times e^{b \times x}$) fit mite populations on *S. tragus* for both sets of data

300   (parameters for the *B. hyssopifolia* expt.: $a = 13.14 \pm 0.33$ [SE], $p < 0.0001$, $b = 0.849 \pm 0.005$, p

301   < 0.0001; for the *K. scoparia* expt.: $a = 3.34 \pm 0.66$, $p < 0.0001$, $b = 1.076 \pm 0.040$, $p < 0.0001$).

302   The live mite population gradually decreased on *B. hyssopifolia* as indicated by the significantly

303   negative value for parameter b (growth rate) ($a = 12.95 \pm 1.43$, $p < 0.0001$; $b = -0.240 \pm 0.085$, p

304    $= 0.0045$). A maximum of 27 live mites on an individual *B. hyssopifolia* plant was observed at

305   week 3, and on week 7 only one plant had live mites (9 individuals). The mite population did not

306   significantly change on *K. scoparia* (b was not significantly different from 0) ($a = 9.65 \pm 4.59$, p

307    $= 0.036$; $b = -0.064 \pm 0.163$, $p = 0.69$). Live mites were found on *K. scoparia* only once, with

308   100 mites on a plant at week 5.

3.1.2 Host plant suitability experiment

310   The sizes of test plants are presented in Table 1. The mean numbers of live mites on week 5

311   for the 6 nontarget species and *S. tragus* are presented in Figure 3. The number of live mites on

312   *S. tragus* (961.6 $\pm$ 184.0 SE) was significantly higher than that on any of the nontarget plants

313   (ANOVA, $F_{(6, 61)} = 19.53$, $p < 0.0001$, Tukey HSD, $p < 0.0001$). The number of mites on the

314   various nontarget plants did not differ significantly (*A. coronata* 20.5 $\pm$ 3.1, *A. truncata* 0.1 $\pm$

315   0.1, *B. hyssopifolia* 81.0 $\pm$ 27.5, *B. prostrata* 6.4 $\pm$ 2.6, *K. scoparia* 0.0 $\pm$ 0.0, and

316   *S. calceoliformis* 54.4 $\pm$ 7.3). Mite numbers increased on 75% (6 of 8) of the *A. coronata* plants

317   (maximum of 35 mites on one plant), on 78% (7 of 9) of the *B. hyssopifolia* (max. 247), on 17%

318   (2 of 12) of *B. prostrata* (max. 24), and on 100% (9 of 9) of the *S. calceoliformis* (max. 79).

319   There was no correlation between the number of mites and the change in plant size for any

320   nontarget species (linear regression, $\alpha = 0.05$). Impact on *S. tragus* was not tested because only

321   cut branches were tested, which do not grow.

3.2 Field test

323   The two surveys using pan traps, performed 81 and 91 days post-inoculation at the site

324   where the nontarget plants were located, did not show the presence of any *A. salsolae*. Only

325   some other *Aceria* sp., *Tetra* sp. and some eriophyoid mites belonging to the subfamily

326   Rhyncaphytoptinae were recorded.

327   At the end of the experiment, cuttings from test plants and positive and negative control

328   plants were collected when the plants reached the seed production stage, which occurred at

Commented [A5]: This doesn't seem to warrant a complete sub-section. Could it not have been included in the Introduction?

Commented [A6]: The section includes the 2 laboratory experiments. The alternative is to promote 3.1.1 to 3.1 and 3.1.2 to 3.2, and change all subsequent sections. The sentence is a result, indicating that there had been no contamination or misidentification of the mites used, which was one possible hypothesis for why we saw mites persisting on some plants. de Lillo is mentioned in the Methods section, so this could be omitted with the unstated assumption that there was no contamination.

329 different times for the various species. Duration of the experiment and percentage of plants
330 infested for each species tested are presented in Table 2. No mites were found on *A. truncata,*
331 *B. hyssopifolia* or *S. calceoliformis* at 78 to 117 days post-inoculation. However, an average
332 of 0.9 live mites per 10 cm cutting was found on *A. coronata* plants after about 55 days.
333   A total (live and dead) of 59 and 804 *A. salsolae* were collected from *S. tragus* cuttings at
334 42- and 114-days from inoculation, respectively. Whereas, only 18 (live and dead) *A. salsolae*
335 were collected from nontarget plants, and all of them were found on only 5 (of 7) *A. coronata*
336 test plants, despite having been infested with at least 10 adult mites per plant. The estimated
337 number of live mites per plant ranged from 113,961 to 831,327 (mean 394,078 $\pm$ 90,254 [SE])
338 on *S. tragus* and from 0 to 8 on *A. coronata* (2.0 $\pm$ 1.1). Juveniles were found only on *S. tragus*,
339 but and none were found on *A. coronata* (Table 2), even among the dead specimens. No
340 eriophyid mites were found on any uninoculated negative control plants, and *A. salsolae* was the
341 only eriophyid mite species collected from the infested plants.
342   The proportion of live and dead *A. salsolae* collected from the species tested (test and
343 positive control) and the proportion of juveniles, females and males among the live individuals
344 collected are presented in Table 2. The proportion of live and dead mites on *S. tragus* did not
345 change from 42 to 114 days after the inoculation. However, among live individuals collected, the
346 proportion of adult females increased (from 78 to 91%) and the proportion of juveniles decreased
347 (from 16 to 6%) between the two sample dates (female proportion: χ^2= 9.70, df = 1, p = 0.0018;
348 juvenile proportion: χ^2 = 7.06, df = 1, p = 0.0039), whereas the proportion of males did not
349 change (6 and 2%, respectively). On *A. coronata*, which was harvested at about 55 days after the
350 inoculation, the proportion of live *A. salsolae* was lower (33%) than on *S. tragus*, after both 42
351 days (85%, χ^2 = 15.9, df = 1, $p < 0.0001$) and 114 days (87%, χ^2= 35.86, df = 1, $p < 0.0001$)
352 post-inoculation. The proportion of females among live individuals on *A. coronata* (67%) did not
353 differ from *S. tragus* at 42 days post-inoculation (78%), but it was significantly lower compared
354 with *S. tragus* at the end of the experiment (91%, χ^2 = 4.51, df = 1, p = 0.034). On the other
355 hand, the proportion of males was higher than on *S. tragus*, both 42 and 114 days post-
356 inoculation (*A. coronata vs. S. tragus* at 42 days: 33% *vs*. 6%, χ^2 = 4.92, df = 1, p = 0.026; *A.*
357 *coronata vs. S. tragus* at 114 days: 33% *vs*. 2%, χ^2 = 21.56, df = 1, $p < 0.0001$).
358   Average numbers of live and dead *A. salsolae* per 10 cm of cutting of infested plant species
359 are presented in Figure 4. In particular, the average number of live *A. salsolae* per 10 cm of
360 cutting of *S. tragus* at 42 days from the inoculation was 5.6 times higher than for the dead ones
361 (1.7 $\pm$ 0.5 SE *vs*. 0.3 $\pm$ 0.1 ; W = 82, p = 0.015). The same pattern was observed at the end of the
362 experiment, when this difference increased, *i.e.* the average number of live *A. salsolae* per 10 cm
363 of cutting was 6.4 times higher than for the dead ones (23.2 $\pm$ 4.4 SE *vs*. 3.6 $\pm$ 0.7; t = 4.42, df =
364 9.49, p = 0.0014). Moreover, both parameters were higher than what was recorded 42 days after
365 inoculation (live at 42 *vs*. 114 days: t = -4.90, df = 9.20, p = 0.00079; dead at 42 *vs*. 114 days
366 post-inoculation: W = 2, p = 0.00029). On *A. coronata* the number of live and dead eriophyid
367 mites per 10 cm of cutting did not differ significantly (0.3 $\pm$ 0.1 SE and 0.6 $\pm$ 0.3), but the
368 average number of live *A. salsolae* per 10 cm of cutting was lower than that on *S. tragus*, both at

369 42 and 114 days post-inoculation (at 42 days: $W = 12$, $p = 0.025$; at 114 days: $W = 70$, $p =$
370 0.00071), whereas the average number of dead *A. salsolae* per 10 cm of cutting differed from *S.*
371 *tragus* only at the end of experiment ($t = -3.93$, df = 11.18, $p = 0.0023$), and it was lower. The
372 negative binomial regression analysis ($[log(y) = intercept + b_1(x)]$, where $y =$ no. of live
373 mites on 10-cm-long cuttings, and $x =$ plant species [i.e. *S. tragus* vs. *A. coronata*]; Table 3)
374 revealed that the density of live mites was affected by the plant species. The incidence of live *A.*
375 *salsolae* on *A. coronata* was 1% of that on *S. tragus* ($IRR = 0.01$).
376 Evaluation of the impact of the possible presence of *A. salsolae* on plants was performed by
377 comparison of different parameters (*i.e.* volume, height, largest and smallest diameter, and
378 number of secondary branches) of inoculated and uninoculated ~~test~~ nontarget plants (Table 4).
379 None of the parameters differed for any of the four nontarget species tested, except for the height
380 of *S. calceoliformis*, for which the inoculated ~~test~~ plants were taller than uninoculated controls
381 (58.8 *vs.* 40.9 cm, $t = -3.20$, df = 11.97, $p = 0.0076$). Notably, there were no significant
382 differences in plant size for *A. coronata* despite the presence of mites on 5 of the 7 test plants.
383 Moreover, no damage by mites was noted on any of the nontarget plants. However, by early
384 August (~ 60 days post-inoculation) extensive galling was apparent on the positive control *S.*
385 *tragus* plants (Fig. 5).
386 In order to directly compare the laboratory and field experiment results, the number of live
387 mites per 10 cm of plant branch was estimated (Table 5). Mite densities were 99 to 100% lower
388 in the field on the nontarget plants and 97% lower on *S. tragus*.

389 **4. Discussion**
390 In some field experiments on host specificity of eriophyid mites there was some suspicion
391 that mites found on nontarget plants may have recently dispersed from nearby heavily-infested
392 target plants (Stoeva et al., 2008, 2012; Smith et al., 2009; Weyl et al., 2019~~). In a previous field~~
393 ~~experiment with *A. salsolae*, all the uninoculated negative control *S. tragus* plants became~~
394 ~~infested by mites dispersing from nearby inoculated plants (Smith et al., 2009)~~. The dispersal
395 behavior of eriophyid mites is not well known, but mites presumably have little or no control
396 over ~~regarding~~ where they land, so any behavioral selectivity would involve assessing the plant
397 and then either staying to feed and multiply or dispersing on the next available wind (Kiedrowicz
398 et al., 2017. It is important to not mistake 'transitory' live mites found on a nontarget plant as a
399 sign of attack ~~(see discussion below), so we designed the field experiment to minimize this~~
400 ~~possibility. Positive control plants were placed far from the test plot, so that dispersing mites~~
401 ~~would not contaminate the test plants~~. The number of mites dispersing aerially is usually
402 positively correlated to the density of mites on plants (Berg, 2001). So, pan traps to monitor the
403 presence of aerially dispersing mites were exposed late in the summer (27 Aug. and 6 Sept.),
404 when mite populations ~~are~~ normally peak ~~very high~~ on *S. tragus*. Absence of *A. salsolae* in the
405 pan traps confirmed that few if any mites were dispersing during the experiment, which is not
406 surprising given the ~~extremely~~ low numbers present on the inoculated test plants.
407 In the elapsed time laboratory experiment, ~~the *A. salsolae* population grew exponentially on~~
408 ~~*S. tragus*, increasing roughly 80 fold in 5 weeks. In contrast, mite numbers generally decreased~~

Commented [A7]: FM: it would be 63 days! I think is important to specify how many days post-inoculation to help the reader to understand that the damages on S. tragus were recorded just a week later than A. coronata plants were harvested (55 days - mean), which did not show any symptom

Commented [A8]: This should be in the Methods section.

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409 ~~on *B. hyssopifolia* and *K. scoparia*, with mites on only 1 of 4 remaining *B. hyssopifolia* plants~~
410 ~~and on none of the *K. scoparia* on week 7. However, mite numbers did increase on two plants of~~
411 ~~*B. hyssopifolia* to 14 and 27 mites on week 3, and on one *K. scoparia* plant to 100 mites on week~~
412 ~~5. Thus, this~~ *A. salsolae* populations generally decreased on *B. hyssopifolia* and *K. scoparia*, but

413 the mite ~~is~~ was sometimes able to reproduce on each of these species~~these two plants under~~
414 ~~laboratory conditions~~. Seven weeks may not be long enough to show that this mite goes to
415 extinction on these two nontarget plants under our laboratory conditions. However, 5 weeks was
416 long enough to reveal a possible increase in the mite population (80 fold on *S. tragus*), and it was
417 chosen for the laboratory host plant suitability experiment as a compromise between a duration
418 long enough to show mite extinction and short enough so that test plants would not get too big
419 and touch their neighbors, which could cause possible cross-contamination.
420   In the 5-week-long host plant suitability laboratory experiment there was some reproduction
421 on at least some plants of *A. coronata*, *B. hyssopifolia*, *B. prostrata* and *S. calceoliformis,* but
422 final numbers were only 0.7 to 8.4% of those on *S. tragus*. There was only one live mite on one
423 *A. truncata* plant and none on *K. scoparia*, indicating that these plants could not support this
424 mite. Persistence of live mites on nontarget plants in the two laboratory tests was not completely
425 consistent. Live mites were found on 1 of 10 *K. scoparia* plants (sampled at weeks 5 and 7) in
426 the elapsed time experiment, but on none of the 9 plants in the host plant suitability experiment.
427 However, there were 100 mites on that one plant, which was an increase from the inoculated
428 number of 10 mites. Live mites were found on 5 of 9 *B. hyssopifolia* plants sampled at 5 or 7
429 weeks in the elapsed time experiment, but they were found on all 9 plants at 5 weeks in the host
430 plant suitability experiment. It is not clear why the results of the two experiments differed, but
431 the variability suggests the importance of doing enough replication.
432   In the field experiment, the only nontarget plant that had mites was ~~no mites were found on~~
433 ~~*A. truncata*, *B. hyssopifolia* or *S. calceoliformis*~~ at 78 to 117 days post-inoculation. ~~However, an~~
434 ~~average of 0.3 live mites per 10 cm cutting was found on~~ *A. coronata* ~~plants after about 55 days~~,

435 but no juveniles were present. This suggests that ~~this mite~~*A. salsolae* can persist for a long time
436 on this plant under natural field conditions, but there was no evidence of reproduction. The *A.*
437 *coronata* plants were harvested earlier (at 50-64 days) than the other nontarget plants (78-117
438 days) because they naturally senesced earlier, and it is not known if similar numbers of *A.*
439 *salsolae* occurred on the other nontarget plants at this time. The decrease in the proportion of
440 mite juveniles on *S. tragus* from 16% on day 42 (17 July) to 6% on day 114 (27 Sept.) and
441 increase in females (from 78 to 91%) may indicate an increasing proportion of diapausing
442 females late in the growing season (Valenzano et al., 2020).
443   The densities of live *A. salsolae* were much lower in the field experiment than in the
444 laboratory experiments for all plants tested ~~(Table 5)~~. In the field, mites are exposed to

445 additional causes of mortality, such as predators, pathogens, rain, extreme temperatures and
446 relative humidity, and possibly ultraviolet radiation (e.g., Goolsby et al., 2005; Oldfield, 2005;
447 Ozman and Goolsby, 2005; Moran et al., 2017), which would reduce population growth rate
448 compared to that under laboratory conditions. Plants grown outdoors are usually physically

449 tougher than those grown in greenhouses (Frye et al., 2007), which might also reduce the ability
450 of eriophyid mites to feed. Another difference between the laboratory and field is that mites
451 could disperse aerially only in the field because there was insufficient air movement in the
452 laboratory. We expected mites to disperse from unsuitable plants, which would also tend to
453 lower the densities in the field compared to the laboratory. Regarding *S. tragus*, small cuttings
454 which do not grow were used in the laboratory experiments whereas plants in the field grew
455 substantially during the course of the experiment. Thus, reproducing mites could disperse by
456 crawling within a plant as it grew, resulting in lower densities than would have occurred in the
457 laboratory on a cutting. Note that at the mean density of 23.2 mites per 10-cm cutting in the field
458 experiment, the total number of live mites per *S. tragus* plant was estimated to be between
459 113,000 and 831,000.
460   The ability of *A. salsolae* populations to persist for up to 49 days in the laboratory and up to
461 64 days in the field on at least some nontarget plants poses a challenge to assessing risk to
462 nontarget plant species. ~~In a laboratory no-choice experiment *Aceria solstitialis* de Lillo,~~
463 ~~Cristofaro and Kashefi, a prospective agent of yellow starthistle (*Centaurea solstitialis* L.),~~
464 ~~appeared to reproduce and persisted up to 60 days on some nontarget plants, including safflower~~
465 ~~(*Carthamus tinctorius* L.) and artichoke (*Cynara scolymus* L.), on which it did not persist in a~~
466 ~~field experiment (Stoeva et al., 2012). Thus, T~~there are at least two well-documented examples
467 of eriophyid mites persisting and reproducing on plants in the laboratory but not in the field
468 (Smith et al., 2010; Stoeva et al., 2012). Relatively little is known about the life expectancy
469 ~~ability~~ of eriophyid mites ~~to survive~~, although 4 to 5 weeks has been reported ~~considered by~~
470 ~~some to be the range~~ for protogynes (= nondiapausing females)~~;~~ (Channabasavanna and Nangia,
471 1984). ~~In a study of five eriophyid species in water droplets, mites survived for up to 1 to 11~~
472 ~~days at 25°C, depending on species and morph, and up to 1 to 7 weeks at 5°C (Valenzano et al.,~~
473 ~~2019). *Aceria tulipae* (Keifer) survived at least 80 days on potato dextrose agar (being tested as~~
474 ~~an artificial diet), but they did not start to oviposit until after they were transferred to wheat~~
475 ~~plants (del Rosario and Sill, 1964). Thus, it appears that E~~eriophyid mites have been shown to
476 survive for longer under cool conditions, ~~at cold than warm temperature,~~and even longer if they
477 can avoid desiccation, either due to high humidity or availability of water to imbibe, ~~and even~~
478 ~~longer~~ and if they can obtain some nutrition (del Rosario and Sill, 1964; Valenzano et al.,
479 2019~~refs~~). However, reproduction may occur only on the most suitable plants. Furthermore,
480 induction of galls is not necessarily a sign of successful reproduction (Craemer, 1995; McClay
481 and De Clerck-Floate, 2002). ~~For example, *Aceria malherbae* Nuzzaci, which was introduced to~~
482 ~~the USA and South Africa to control field bindweed, *Convolvulus arvensis* L. (Convolvulaceae),~~
483 ~~caused galling on 3 *Convolvulus* and 12 *Calystegia* species in laboratory and screen house~~
484 ~~studies (Clement et al., 1984; Rosenthal and Platts, 1990; Craemer, 1995). However, Craemer~~
485 ~~(1995) observed that although galling occurred on two nontarget species of *Convolvulus*, mites~~
486 ~~reproduced only on *C. arvensis*, suggesting that requirements for reproduction are more~~
487 ~~restrictive selective than for gall induction. Induction of galling without evidence of mite~~
488 ~~reproduction was also observed on *Calystegia sepium* (L.) R. Br. (McClay and De Clerck-Floate,~~

Commented [A14]: Review and no need to spell detail here. Next sentence suffices.

Commented [A15]: "Ability to survive" is ambiguous – it could be populations of the mites, hence "life expectancy" which applies to individuals.

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489 2002). In a field experiment, galling was observed on some nontarget *Convolvulus* and
490 *Calystegia* species in the summer that they were inoculated with *A. malherbae*; however, no
491 galling was observed on these plants the following year, suggesting failure of the mites to either
492 reproduce or to survive on them through the winter (which normally occurs underground on the
493 roots), whereas they could on *C. arvensis* (R.W. Hansen pers. comm. in Smith et al., 2010).

494   Assessment of the risk of a hazard involves estimating the amount of injury per attack and
495 multiplying it by the probability of attack (Lonsdale et al., 2001). However, for an arthropod
496 attacking a plant, the level of injury generally depends on the density of individuals (McClay and
497 Balciunas, 2005), unless a plant pathogen is also involved. So, in the case of assessing the risk
498 of a prospective biological control agent to harm a nontarget plant, it is important to determine if
499 the agent can multiply on the nontarget plant, because this is necessary in order to achieve
500 populations high enough to impact the plant on a sustained basis (Hinz et al., 2019). Although
501 *A. salsolae* could persist for up to 9 weeks on a nontarget plant (*A. coronata*) under field
502 conditions, and even reproduce slightly during 3 to 5 weeks under laboratory no-choice
503 conditions, it never attained populations anywhere close to those on *S. tragus*. The positive
504 control *S. tragus* plants in the field experiment had extensive galling. Although we did not
505 measure impact of the mite on *S. tragus* in the field experiment, a previous study measured a
506 reduction of 80% in aerial biomass and a reduction in seed production from 34.1 to 0 seeds per
507 10-cm branch tip (Smith et al., 2009) even though the final mite densities were 1/15th those of
508 the current field experiment. However, none of the nontarget plants showed any impact of the
509 mite in either the laboratory or field experiments. So, multiplying the low probability of
510 infestation by the low density on infested plants by the insignificant impact indicates negligible
511 risk to these nontarget plants.

512   Two eriophyid mites that could develop on and/or damage some nontarget plants in pre-
513 release studies have been previously released as biological control agents. *Aceria malherbae*,
514 mentioned above, was released in the USA in 1989 to control field bindweed weed, *Convolvulus*
515 *arvensis* L. (Convolvulaceae), and there have not been any reports of it attacking nontarget
516 species in the field under natural conditions (Smith et al., 2010). *Aculus hyperici* (Liro) was
517 introduced to Australia in 1991 to help control the weed St Johnswort, *Hypericum perforatum* L.
518 (Clusiaceae), despite indications from pre-release trials that this eriophyid mite could survive and
519 reproduce on at least four non-target species, including the Australian native *Hypericum*
520 *gramineum* Forst. (Cullen and Briese, 2001). Although *A. hyperici* subsequently infested *H.*
521 *gramineum* in the field, the mite had negligible impacts on all measured indices of growth and
522 reproduction, and it is not considered to harm the nontarget plant's population (Willis et al.,
523 2003). In both these examples, the mites have not produced significant impacts in the field on
524 nontarget plant species that are known to be within their physiological host range.

525   We conclude that *A. salsolae* is capable of very low reproduction on some nontarget plant
526 species, including *A. coronata*, *B. hyssopifolia*, *B. prostrata*, *K. scoparia* and *S. calceoliformis*
527 under some laboratory conditions, but that the mite is not likely to multiply on any of these
528 plants under field conditions. Furthermore, the mite does not appear to cause any significant

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529 harm to these nontarget plants when it does occur on them. *Atriplex coronata* and *S.*
530 *calceoliformis* are native annual species that overlap geographically with *S. tragus sensu lato*.
531 *Bassia hyssopifolia* and *B. prostrata* are alien to North America, although the latter has been
532 developed as a potential forage crop (Waldron et al., 2010). Given that these species are among
533 those most closely related to the target weed outside the genus *Salsola*, and thus should be the
534 most likely to be at risk based on the centrifugal phylogenetic hypothesis (Wapshere, 1974;
535 Briese, 2006; Berner et al., 2009.; Simberloff, 2012), and were the only ones that showed
536 persistence of live mites in laboratory studies, including those of previous host testing (Smith,
537 2005; Smith et al., 2009), this mite is not expected to pose a risk to any nontarget plants in the
538 contiguous USA.
539

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708

709 **TABLES**
710
711 Table 1. Size of potted test plants inoculated with *Aceria salsolae* in the laboratory host plant
712 suitability experiment (mean $\pm$ SE). *Salsola tragus* were cuttings held in water vials. Change in
713 height is final/initial.
714

Species	N	Initial height (cm)	Final height (cm)	Change in height
Atriplex coronata	9	8.22 $\pm$ 0.67	15.88 $\pm$ 3.71	1.84 $\pm$ 0.34
Atriplex truncata	9	8.72 $\pm$ 0.51	40.89 $\pm$ 1.86	4.86 $\pm$ 0.44
Bassia hyssopifolia	9	5.89 $\pm$ 0.37	44.06 $\pm$ 3.12	7.46 $\pm$ 0.18
Bassia prostrata	*12*	4.92 $\pm$ 0.50	8.71 $\pm$ 0.48	2.01 $\pm$ 0.24
Kochia scoparia	9	3.94 $\pm$ 0.24	16.83 $\pm$ 2.00	4.35 $\pm$ 0.50
Suaeda calceoliformis	9	4.67 $\pm$ 0.65	15.67 $\pm$ 2.80	3.71 $\pm$ 0.63
Salsola tragus	12	11.50 $\pm$ 0.56	11.75 $\pm$ 0.60	1.03 $\pm$ 0.02

715
716

717 Table 2. Number of plants tested for each species (N), duration of the field experiment,
718 percentage of plants infested (inoculated and uninoculated) at the end experiment, total number
719 of *Aceria salsolae* collected per 10-cm of cutting (mean ± SE), proportions of live and dead
720 mites collected, and proportions of juvenile, adult female and male among live *A. salsolae*
721 collected for each plant species tested.

722

Plant species [N]	Days post-inoculation mean[1] [*min - max*]	Plants inoculated [*uninoculated*] infested	Total number of *Aceria salsolae*/10 cm of cutting	Proportion of *Aceria salsolae* live [*juvenile, female, male*]	dead
Atriplex coronata [7]	55 [*50-64*]	71% [*0%*]	0.9 ± 0.3	33% [*0%, 67%, 33%*]	67%
Atriplex truncata [7]	99 [*90-110*]	0% [*0%*]	0.0 ± 0.0	0%	0%
Bassia hyssopifolia [10]	103 [*78-117*]	0% [*0%*]	0.0 ± 0.0	0%	0%
Suaeda calceoliformis [10]	104 [*81-117*]	0% [*0%*]	0.0 ± 0.0	0%	0%
Salsola tragus [10]	42 [*40-43*]	100%	2.0 ± 0.5	85% [*16%, 78%, 6%*]	15%
[10]	114 [*98-120*]	100%	26.8 ± 5.0	87% [*6%, 91%, 2%*]	13%

Commented [A20]:

Commented [A21]: S. tragus was moved from top of table to bottom.

723 [1] Mean was calculated excluding the outliers, which are 25 days post-inoculation for two *A. coronata* plants,
724 inoculated and uninoculated, respectively; 33 days post-inoculation for an *A. truncata* plant inoculated; 33
725 and 63 days post-inoculation for two *S. calceoliformis* plants, both inoculated, and 53 days post-
726 inoculation for a *S. calceoliformis* plant uninoculated.
727

728   Table 3. Parametric coefficients of negative binomial model ($[log(y) = intercept + b_1(x)]$,
729   where y = no.of live mites on 10-cm-long cuttings, x = plant species), and the corresponding
730   Incidence Rate Ratio (IRR), examining the effects of plant species on the density of live *Aceria*
731   *salsolae* in the field experiment.
732

	Estimate	SE	IRR	z-value	p-value
intercept[1]	3.10	0.19	-	15.95	< 2e-16
plant species[2]	-5.05	1.04	0.01	-4.85	= 1.25e-06

733   [1] intercept represents the effect of *Salsola tragus*
734   [2] plant species represents the effect of *Atriplex coronata* compared to that of *S. tragus*
735
736

Table 4. Parameters recorded to evaluate the potential impact of the possible presence of *Aceria salsolae* on plants (TEST = inoculated, CTRL(-) = not inoculated). **Bold** indicates the only two values which differed from each other within a plant species (t = -3.21, df = 11.97, p = 0.00758).

Plant species	Treat-ment	N	Volume (1000 cm³) mean	SE	Height (cm) mean	SE	Diameter Largest (cm) mean	SE	Smallest (cm) mean	SE	No. of secondary branches mean	SE
Atriplex coronata	TEST	7	1.6	0.4	24.6	2.7	12.9	1.3	8.71	1.1	9.1	2.3
	CTRL(-)	7	2.1	0.7	23.1	4.1	12.4	2.0	10.3	1.7	9.3	1.7
Atriplex truncata	TEST	7	505	109	75.7	8.4	109.0	12.3	98.6	11.2	31.7	3.0
	CTRL(-)	7	317	61	77.9	4.6	87.3	9.1	80.4	8.9	31.4	1.5
Bassia hyssopifolia	TEST	10	817	280	96.5	7.4	121.2	22.4	93.0	19.6	31.3	4.5
	CTRL(-)	10	950	320	111.6	14.0	113.8	16.6	100.0	14.9	44.9	5.1
Suaeda calceoliformis	TEST	10	49	11	**58.8**	**5.2**	41.5	5.0	32.7	3.4	17.4	1.1
	CTRL(-)	10	25	5	**40.9**	**2.1**	36.20	3.31	29.20	2.8	15.9	1.0

743 Table 5. Comparison of densities of live *Aceria salsolae* in laboratory no-choice and field
744 experiments (mean $\pm$ SE).
745
746

Plant species	Live mites/10 cm		
	Lab	Field	Decrease
Atriplex coronata	26.3 $\pm$ 13.1 *	0.27 + 0.13	99%
Atriplex truncata	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	100%
Bassia hyssopifolia	18.0 $\pm$ 5.5 *	0.00 $\pm$ 0.00	100%
Suaeda calceoliformis	48.6 + 11.4 *	0.00 $\pm$ 0.00	100%
Salsola tragus	789.6 + 139.2 *	23.2 + 4.4	97%

747 * Kruskal-Wallis test of Lab vs. Field, $p < 0.005$
748

749

FIGURE LEGENDS

751

Figure1. Maps of the two field garden plots set up for nontarget plants, inoculated with *Aceria salsolae* (test; on the right, grey circles) or not (negative control; on the left, white circles). Each letter corresponds to different plant species (a – *Atriplex coronata*; b – *Bassia hyssopifolia*; c – *Atriplex truncata*; d – *Suaeda calceoliformis*) and numbers refer to replicate for each species († – plants added on 21 June; ‡ – plants replaced on 25 June). White rectangles indicate pan traps.

Figure 2. Number of live *Aceria salsolae* on test plants at 3, 5 and 7 weeks after inoculation with 10 mites inside a containment laboratory (mean $\pm$ SE); model is exponential growth fit to *S. tragus* data.

Figure 3. Number of live *Aceria salsolae* per plant 5 weeks after inoculation with 15 mites inside a containment laboratory (mean $\pm$ SE)

Figure 4. Numbers of live (white bar) and dead (gray bar) *Aceria salsolae* per 10 cm of cutting of the species tested in the field experiment (mean $\pm$ SE).

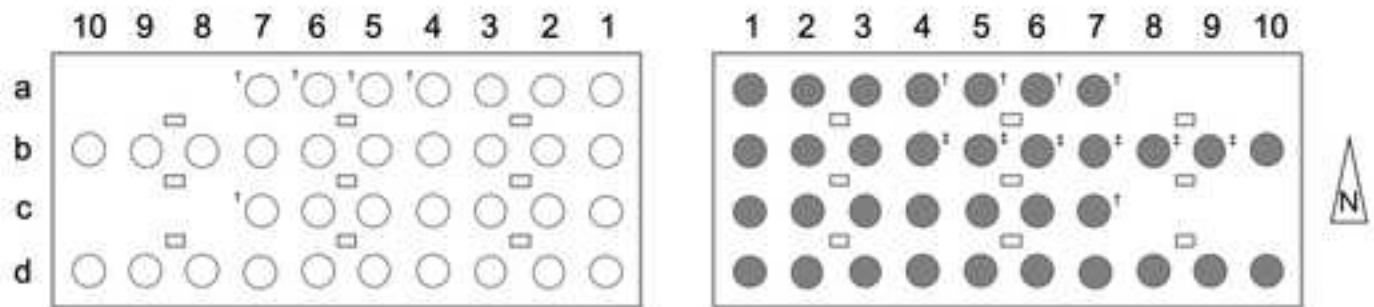Figure 5. Galls on inoculated *Salsola tragus* (red arrows) compared to normal growth (white arrow).

Figure 1

Click here to access/download;Figure;Slide1.jpeg ±

Fig. 1

Figure 2

Fig. 2

Figure 3

Fig. 3
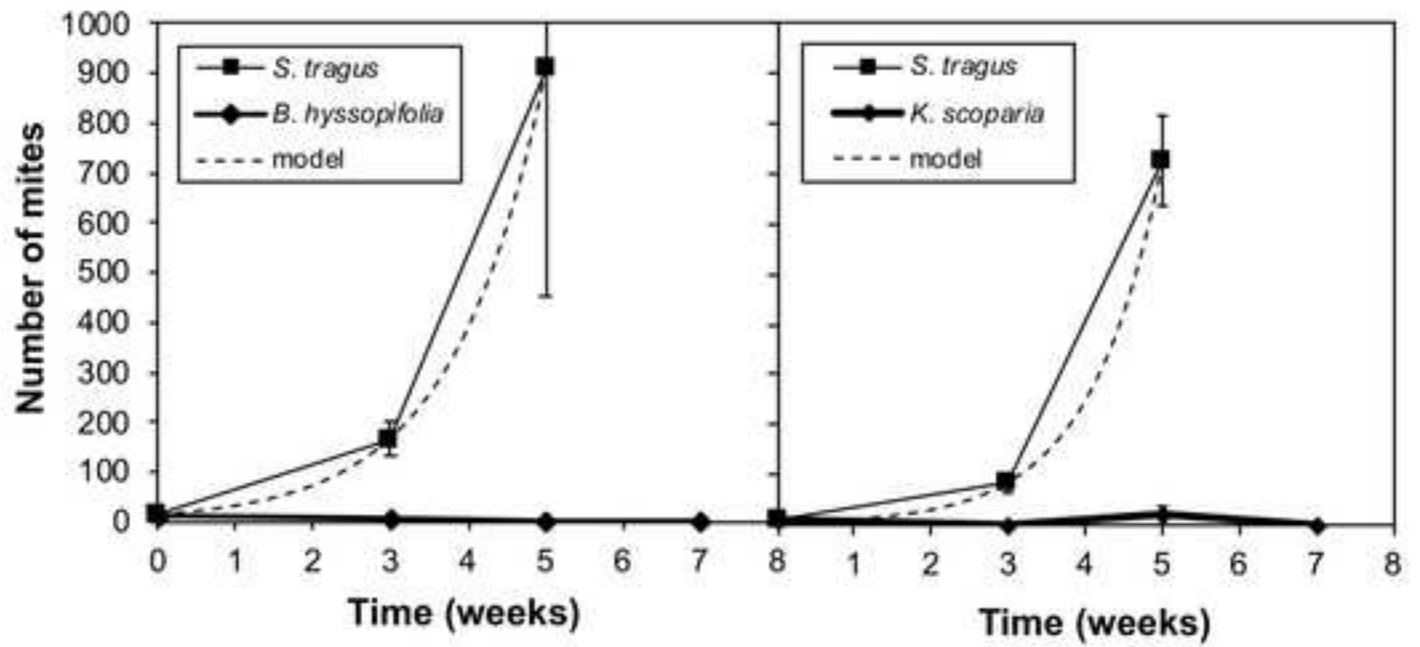

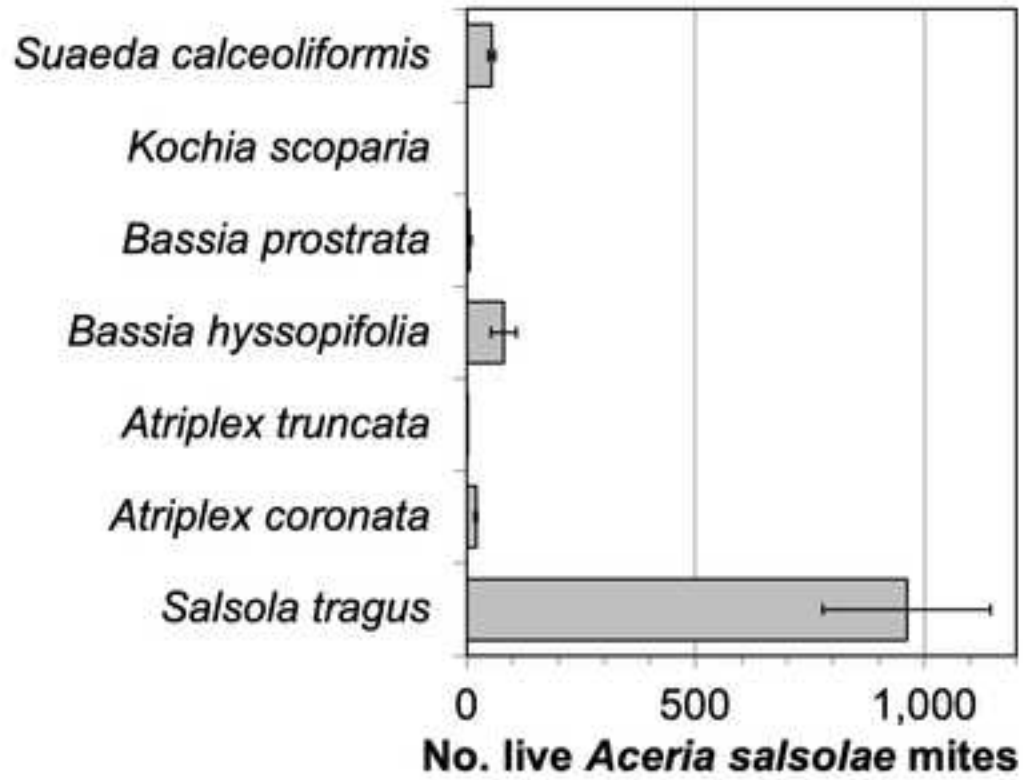No. live *Aceria salsolae* mites
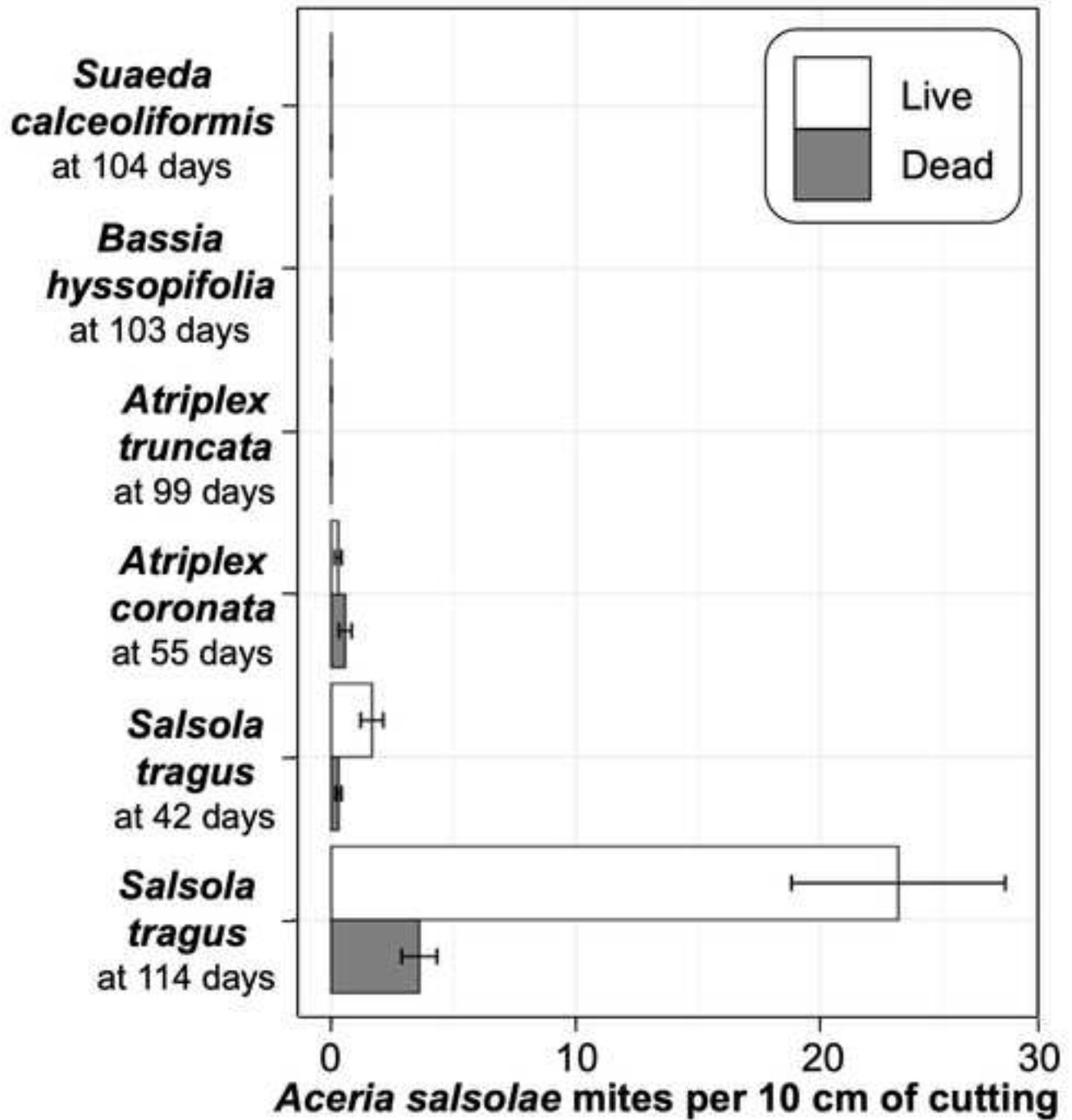
Figure 4

Fig. 4

Figure 5

Fig. 5

Author statement file

Francesca Marini, planned and conducted the field experiment, analyzed the results of the field experiment, co-wrote the paper.

Biljana Vidović, determined the indentification of mites from the field experiment.

Simone Lonis, conducted the field experiment, extracted and processed mites.

Maria Irene Wibawa, conducted the laboratory experiments, extracted and processed mites.

Enrico de Lillo, determined the indentification of mites from the laboratory experiments.

Javid Kashefi, collected mites for use in the laboratory and field experiments.

Massimo Cristofaro, supervised activities in Italy.

Lincoln Smith, conceptualized the project, designed and analyzed the results of the laboratory experiments, co-wrote the paper.