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Authors
Petanidou, Theodora
Price, Mary V
Bronstein, Judith L
et al.

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Pollination and reproduction of an invasive plant inside and outside its ancestral range

Theodora Petanidou[a*], Mary V. Price[b], Nickolas M. Waser[b], Aphrodite Kantsa[a], Thomas Tscheulin[a], Rupesh Kariyat[c], Nickolas Krigas[d], Mark C. Mescher[e], Consuelo M. De Moraes[e], Judith L. Bronstein[f]

[a] Laboratory of Biogeography and Ecology, Department of Geography, University of the Aegean, University Hill, GR-81100 Mytilene, Greece

[b] School of Natural Resources and the Environment, University of Arizona, Tucson, AZ 85721, USA

[c] Department of Biology, University of Texas Rio Grande Valley, Edinburg, TX 78539, USA.

[d] Department of Ecology, School of Biology, Aristotle University of Thessaloniki, GR-54124, Thessaloniki, Greece

[e] Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland

[f] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

25   * To whom correspondence should be addressed. E-mail:

26   t.petanidou@aegean.gr

Highlights

• Weedy *Solanum elaeagnifolium* is invasive outside its ancestral North America range.

• We compared its sexual reproduction in Arizona, USA ("AZ") and Greece ("GR").

• Pollination in GR was by native bees that resemble ancestral AZ pollinators.

• GR plants invest more in flowers and ovules but do not produce more seeds.

• These results suggest promising avenues for further research.

37**Abstract**

38Comparing traits of invasive species within and beyond their ancestral

39range may improve our understanding of processes that promote

40aggressive spread. *Solanum elaeagnifolium* (silverleaf nightshade) is a

41noxious weed in its ancestral range in North America and is invasive on

42other continents. We compared investment in flowers and ovules,

43pollination success, and fruit and seed set in populations from Arizona,

44USA ("AZ") and Greece ("GR"). In both countries, the populations we

45sampled varied in size and types of present-day disturbance. Stature of

46plants increased with population size in AZ samples whereas GR plants

47were uniformly tall. Taller plants produced more flowers, and GR plants

48produced more flowers for a given stature and allocated more ovules per

49flower. Similar functional groups of native bees pollinated in AZ and GR

50populations, but visits to flowers decreased with population size and we

51observed no visits in the largest GR populations. As a result, plants in

52large GR populations were pollen-limited, and estimates of fecundity were

53lower on average in GR populations despite the larger allocation to flowers

54and ovules. These differences between plants in our AZ and GR

55populations suggest promising directions for further study. It would be

56useful to sample *S. elaeagnifolium* in Mediterranean climates within the

57ancestral range (e.g., in California, USA), to study asexual spread via

58rhizomes, and to use common gardens and genetic studies to explore the

59basis of variation in allocation patterns and of relationships between

60visitation and fruit set.

61

4   4

65 1. Introduction

66

67 The introduction of species beyond their ancestral range often causes 68 ecological damage, and aggressive spread of invasive species threatens 69 biodiversity worldwide (Elton 1958; Pimentel et al. 2001; Traveset and 70 Richardson 2006). Studies of species invasions suggest that they depend 71 both on attributes of the invaders and of the invaded systems, but much 72 about what promotes successful invasion remains a mystery (Richardson 73 and Pysek 2006; Tanentzap et al. 2010; van Kleunen et al. 2010).

74

75 Comparison across populations of invasive species within and beyond their 76 ancestral range may indicate ways in which individual traits have changed 77 during invasion, thus offering insight into factors that promote or 78 accompany geographic spread. Few studies have attempted such a 79 comparison for plants, and most of these have focused on success in 80 recruitment and on genetic variance in invading populations (De los 81 Santos et al. 2001; Lafuma and Maurice 2007; Mandák et al. 2009; Colautti 82 et al. 2011). Fewer have compared aspects of pollination and 83 reproduction (e.g., Caño et al. 2008, Li et al. 2012, Atlan et al. 2015), and 84 to our knowledge only three such were carried out in the wild (Stout et al. 85 2006; Petanidou et al. 2012; Montero-Castaño et al. 2014).

86

87 As a first step in exploring factors that might relate to the invasion success 88 of *Solanum elaeagnifolium*, a noxious weed, we examined components of 89 its present-day sexual reproduction in a sample of populations of different

6   6

90size and ecological context within and beyond the ancestral range.

91Determining a potential invader's ability to reproduce is critical, because

92propagule supply is essential for the founding and maintenance of

93populations (Barrett 2011). We measured (1) traits related to plants'

94initial investment of resources toward pistillate (female) sexual function

95(i.e., investment in flowers and ovules); (2) pollinator visits and pollination

96success; and (3) components of realized fecundity (i.e., fruit and seed

97production). The patterns that emerge indicate that plants sampled in the

98invaded range allocated more resources to flowers and ovules than those

99in the ancestral range, but received fewer visits from pollinators and did

100not produce more seeds. We suggest possible reasons for these results

101and some avenues for further research.

102

103**2. Materials and methods**

104

1052.1. Study species

106

107*Solanum elaeagnifolium* Cav. (silverleaf nightshade, Solanaceae) is a

108short-lived perennial herb with an ancestral range in the southwest to

109west-central USA and northern Mexico. By the 1970s the species had

110spread throughout the USA (Kearney et al. 1969; Munz 1974; Boyd et al.

1111984; Mekki 2007) and to all continents except Antarctica (Tscheulin et al.

1122009; Brunel et al. 2013). Plants often act as ruderals that colonize

113disturbed sites (USDA 2006; Tscheulin et al. 2008, 2009). The blue-to-lilac

114hermaphroditic flowers are nectarless and are pollinated mostly by bees

115that vibrate their wings to release pollen from the anthers ("buzz"

116pollination, Buchmann and Cane 1989). Plants appear to be

117predominantly self-compatible in Arizona (USA), within the ancestral

118range, and predominantly self-incompatible in Greece, part of the invaded

119range (Petanidou et al. 2012). Mature fruits are small, dry, globose berries

120that can contain >100 seeds (Tscheulin et al. 2009; Petanidou et al. 2012).

121Plants contain teratogenic compounds that are toxic to livestock (Baker et

122al. 1989; Keeler et al. 1990), lower the yield of many co-occurring crops

123(Boyd et al. 1984), and reduce the pollination success of native plants

124(Tscheulin et al. 2009; Vilà et al. 2009; Tscheulin and Petanidou 2013).

125

1262.2. Study populations

127

128We studied *S. elaeagnifolium* in southeastern Arizona, USA, and in Greece,

129where it was first recorded in 1927 (Krigas and Kokkini 2004). In each

130country, we sampled accessible populations that ranged in size and in the

131presence of other plant species, and that varied in type of disturbance

132they experience. In total we chose 27 populations (Table 1), 9 in

133southeastern Arizona ("AZ") near the town of Marana and the Santa Rita

134Mountains in Pima County, and near the towns of Willcox and San Simon in

135Cochise County; and 18 in Greece ("GR") on the Island of Lesvos in the

136northeastern Aegean Sea and near the city of Thessaloniki on the northern

137mainland.

138

139 To indicate different degrees of invasive spread we scored large

140 monospecific stands with >10,000 plants (see Fig. S1) as "2" on an ordinal

141 scale, monospecific stands with 2,000–10,000 plants as "1", and small

142 populations with < 2,000 plants intermixed with other non-crop species as

143 "0" (see Fig. S2). Because *S. elaeagnifolium* can propagate vegetatively

144 as well as sexually (Cooley and Smith 1971; Buchmann and Cane 1989;

145 Tscheulin et al. 2008, 2009) some "plants" were ramets of the same genet

146 (= clone). It should be kept in mind that when we refer here to "plants"

147 we may in some cases be describing different ramets of the same genet.

148 We also scored populations on roadsides, rangelands, and wastelands that

149 were exposed to periodic mowing or trampling as receiving "surface

150 disturbance", and those associated with agricultural fields that were

151 exposed to deeper soil turnover as being "tilled".

152

153 Population sizes and disturbance regimes differed between AZ and

154 GR samples (Table 1). In AZ we studied 3 small and 2 medium-sized

155 populations that experienced surface disturbance, and one small, 2

156 medium-sized, and one large that were tilled. In GR we studied 3

157 small, 2 medium-sized, and 3 large populations that experienced

158 surface disturbance and 9 large that were tilled. Thus there were

159 more large monospecific stands among GR populations, and more

160 monospecific stands among tilled populations.

161

162 2.3. Investment in flowers and ovules

163

164In each study population we tagged 29-60 plants at random. We

165estimated the number of flowers produced by each tagged plant based on

166the number of fruits it ultimately produced (see Section 2.5) as:

167

168# flowers per plant = # fruits per plant × (mean # OP flowers/mean # OP

169fruits)

 170

171where "#" signifies "number of" and "OP" refers to open-pollinated

172flowers and fruits from an experimental study of pollen limitation (see

173Section 2.4). As a further measure of investment in female function we

174collected ovaries from 1–2 randomly-chosen flowers on each tagged plant

175and counted ovules. To estimate total per-plant ovule numbers we

176multiplied mean per-flower ovule count for each population by estimated

177flowers per plant for each tagged plant in that population.

178

1792.4. Pollinator visits and pollination success

180

181We studied pollinator visits during the peak flowering period of *S.*

182*elaeagnifolium* (June–September) in 2006 in Arizona, 2006 and 2014 on

183Lesvos, and 2007 near Thessaloniki (Table 1). We surveyed each

184population several times (twice in Arizona and near Thessaloniki and four

185times in Lesvos, all 20-30 days apart). During each survey we spent one

186or two days in each population and took four 15-minute pollinator

187censuses on each day (total 60 or 120 minutes per population). During

188each census, we recorded the number of flower visits by each insect that

189entered a transect 25m long × 2m wide. At noon each day we counted all

190open flowers in the transect and from this calculated mean visits per

191flower per hour. All observations were made on sunny calm days during

192peak insect activity, between 0600 and 1300 h in AZ, and 0830 and 1500

193h in GR. Although all populations were surveyed more than once, we

194based analyses only on the survey that yielded the highest visitation to

195flowers. This approach allowed us to compare the local maximum of

196flowering and pollination across populations. The additional surveys of

197each population added to our sample of pollinators, as did an additional 15

198minutes spent after each census netting flower visitors outside of

199transects. Collected specimens were identified to species whenever

200possible and are deposited in the Melissotheque of the Aegean, Laboratory

201of Biogeography and Ecology, University of the Aegean (Petanidou et al.

2022013).

203

204As a measure of pollination success, we calculated a Pollen Limitation

205Index (PLI) for each population (Tscheulin and Petanidou 2013):

206

207PLI = 1 - [(average # seeds set after open pollination) / (average # of

208seeds set after pollen addition)]

209

210A value of 0 suggests that open pollination is maximally effective, whereas

2111 represents complete pollen limitation. We generated PLI values by

212marking 1-2 pairs of flower buds on each of the 29-60 tagged plants in

213each population, and pollinating one of each pair chosen at random (pollen

214addition, "PA") while leaving the other untreated (open pollinated, "OP").

215Pollen was collected in the morning from several donor plants 5–20 m from

216each recipient using a tuning fork or by shaking the anthers into a clean

217Petri dish, and was applied to stigmas with a clean toothpick (Tscheulin et

218al. 2009; Petanidou et al. 2012). We counted viable seeds in mature fruits

2195–6 weeks later, and calculated the mean number of seeds per fruit over

220all flowers receiving the same treatment in a population. In the few cases

221where PLI was negative the value was set to zero before analysis.

222

2232.5. Fruit and seed set

224

225At the end of the flowering season we measured plant height of each of

226the 29-60 tagged plants in each population to the nearest cm, as a proxy

227for overall size. At the same time we counted all fruits produced by each

228tagged plant, and considered the mean of these counts for each

229population as one component of average realized fecundity. Mean seeds

230per flower in the OP treatment served as another component, and when

231multiplied by the average estimate of flowers per plant for each population

232yielded a final component, the estimated mean total seeds per plant for

233that population.

 234

2352.6. Data analysis

236

237Some populations were destroyed or damaged before the study was

238completed; for these we analyzed only data collected before destruction

239(populations 11, 16, 24; Table 1) or from plants that escaped damage

240(populations 3, 7, 17). Seed set in GR population 11 was low even after

241pollen was added by hand. Because GR populations tend to be self-

242incompatible (Petanidou et al. 2012) this suggests low genetic diversity,

243and preliminary investigation supports this hypothesis (R. Kariyat et al.

244unpublished). We excluded GR population 11 from analyses of seed and

245fruit set and PLI, but included its fecundity values in Table 2 and figures.

246

247Our AZ and GR populations are samples from those geographic areas. In

248this sense the differences attributed to "country" in analyses cannot be

249taken to represent Arizona (or the USA) vs. Greece overall, nor the overall

250ancestral vs. invaded ranges. Nonetheless it is legitimate to compare how

251aspects of investment in female sexual function (i.e., flowers and ovules),

252pollination, and fecundity varied in our samples with population size score

253and type of disturbance, using ANOVA or ANCOVA. When multiple

254independent variables could be expected *a priori* to contribute to variation

255in a response variable, we took a model-selection approached based on

256minimizing AICc. Imbalance in our sample of AZ and GR populations did

257not permit estimation of country $\times$ population size $\times$ disturbance

258interactions. For whole-plant traits we included plant height as a covariate,

259since total flower, ovule, and fruit production generally scale with plant

260stature, and AZ and GR samples might differ in scaling relationships. For

261per-flower fecundity variables, we included pollinator visit rate and ovule

262number as covariates. To help evaluate any apparent nonlinear

263relationships between reproductive variables and population size we

264treated population size score as a continuous variable and included

265second-order as well as first-order terms. With the exception of plant

266height, analyses were based on grand means for populations, because we

267rarely obtained multiple values per plant for other measured variables, so

268that plants nested within populations served as the error term. In any

269event we are concerned here first and foremost with patterns of variation

270among populations. For plant height, we could use plants nested within

271populations as the error term and treat population nested within country

272as a random effect. We transformed variables as needed to normalize

273model residuals. All analyses were implemented in JMP Pro 11 (SAS

274Institute, Cary, North Carolina, USA). Table S1 summarizes models and

275variable transformations used in analyses.

276

277**3. Results**

278

2793.1. Investment in flowers and ovules

280

281In our AZ sample mean plant height increased from small to medium-sized

282to large populations (43.4 cm, 62.0 cm, 71.2 cm, respectively), whereas no

283such pattern appeared in GR populations (67.6 cm, 66.8 cm, 64.7 cm,

284respectively; Fig. 1; $F_{1,19.17} = 4.252$, P = 0.053 for the interaction between

285country and population size from ANOVA using ln-transformed height

286values). Similarly, tilled AZ populations supported taller plants on average

287than did populations experiencing surface disturbance (67.8 cm and 43.4

288cm, respectively), whereas this was not true in GR populations (65.1 cm

289and 65.4 cm, respectively; $F_{1,19.14} = 4.664$, P = 0.044 for the interaction

290between country and disturbance type).

291

292Estimated mean total flower production per plant was positively

293related to plant height ($F_{1,18} = 10.077$, P = 0.005 from ANOVA with

294ln-transformed values of flowers per plant). The slope of this

295relationship was not obviously different for AZ and GR populations

296(Fig. 2; $F_{1,18} = 0.251$, P = 0.623 for the interaction between country

297and height). Because AZ plants were smaller on average than GR

298plants, their estimated total flower production also appeared

299somewhat smaller (mean ± SE [N]: 309.5 ± 111.62 flowers [8] vs.

300403.0 ± 89.11 flowers [14]).

301

302Ovule number is a more precise proxy than flower number for initial

303female investment.   In both AZ and GR populations ovule number

304per flower increased nonlinearly to an apparent plateau with

305increasing population size (positive linear and negative second-

306order effects from polynomial ANCOVA with ln-transformed values of

307ovules per flower), but the plateau was somewhat lower in AZ than

308in GR populations (Fig. 3; $F_{1,17} = 4.666$, P = 0.045 for the interaction

309between country and population size). Plant height had a slight

310negative effect because several small populations had tall plants

311that produced flowers with few ovules (compare Figs.1, 3). Overall,

312AZ plants allocated fewer ovules per flower than GR plants (mean ±

313SE [N]: 55.3 ± 4.66 ovules [9] vs. 95.3 ± 5.75 [19]; $F_{1,17} = 102.488$,

15   15

314P < 0.0001). Since GR plants produced more flowers, they also

315allocated more to ovules on a per-plant basis.

316

3173.2. Pollinator visits and pollination success

318

319Flowers in all AZ populations and GR populations on Lesvos

320attracted up to six species of buzz-pollinating bees (Table 3). AZ

321and GR populations shared no species in common, but their bees

322belonged to equivalent functional groups and often to the same

323genera (e.g., *Xylocopa* and *Bombus*, Apidae; *Nomia* and *Pseudapis,*

324Halictidae). Several other insects, mainly small sweat bees (e.g.,

325*Halictus resurgens,* Halictidae), honeybees (*Apis mellifera*), and

326rarely small bees of the genus *Megachile*, visited flowers on Lesvos

327without buzzing, collecting pollen that was shed on the surface of

328anthers and the corolla after flowers were buzzed by other bees or

329shaken by winds (see Section 4.2). We assumed that non-buzzers

330did not release new pollen from anthers and had little impact on

331seed set, and so ignored them in visit rate estimates.

332

333Observed rates of flower visits by buzzing pollinators decreased

334nonlinearly with population size overall (Fig. 4; $F_{1,21} = 2.664$, P =

3350.118 and $F_{1,21} = 13.507$, P = 0.0014, respectively, from polynomial

336ANCOVA for first and second-order effects on square-root

337transformed values of visit rate). The decrease was less

338pronounced in AZ than GR populations ($F_{1,21} = 17.283$, P = 0.0004

339for the interaction between country and population size), but there
340was no clear difference in visit rates between AZ and GR (mean ±
341SE [N]: 0.070 ± 0.084 visits per flower per hour [9] vs. 0.113 ±
3420.061 [17]; $F_{1,21}$ = 1.70, P = 0.685). In the smallest populations,
343mean visit rates remained below one per flower per hour, except for
344GR population 10 on Lesvos (Table 2). In the 13 large GR
345populations near Thessaloniki (13, 14, 16-18, 20-27) we observed no
346visits at all, whereas this was not the case in the largest AZ
347population 7 (Table 2).

348

349PLI provides one gauge of the effectiveness of pollination; indeed it
350decreased overall with pollinator visit rate (Fig. 5; $F_{1,21}$ = 5.352, P =
3510.031 from ANCOVA using square-root-transformed values of visit
352rate) in a similar fashion in populations from AZ and GR ($F_{1,21}$ =
3530.341, P = 0.566 for the interaction between country and visit rate).
354Overall, PLI was lower in our AZ than GR populations (mean ± SE
355[N]: 0.447 ± 0.090 [9] vs. 0.878 ± 0.063 [17]; $F_{1,21}$ = 10.865, P =
3560.0034), but there was considerable scatter, with some GR
357populations (notably 10) having high PLI in spite of high visit rates,
358and others having lower visit rates but low PLI.

359

3603.3. Fruit and seed set

361

362Initial investment in flowers and ovules is expected to contribute to
363components of realized fecundity such as fruit and seed set.

17   17

364Indeed, of alternative models including ln (plant height), country,

365and pollinator visit rate, the best model indicated that total number

366of fruits produced by a plant was positively related to plant height

367($F_{1,17}$ = 11.211, P = 0.004 from ANOVA using ln-transformed values

368of fruits per plant), a relationship that did not obviously differ

369between AZ and GR populations (Fig. 6; $F_{1,17}$ = 0.808, P = 0.381 for

370the interaction between country and height). This result seems

371logical given that flower number increases with plant height (see

372Section 3.1). Nonetheless, even though AZ plants were on average

373slightly smaller than GR plants, fruit production was higher in AZ

374than GR populations (mean fruits per plant ± SE [N]: 80.400 ±

37517.196 [9] vs. 38.914 ± 13.490 [16]; $F_{1,17}$ = 12.243, P = 0.0027).

376Inferior pollination service in GR populations may have contributed

377to lower fecundity. Whereas pollinator visit rate was excluded from

378the best model described above (perhaps because it was an

379imperfect predictor of pollen limitation), the best model when we

380included PLI as a predictor of total fruits per plant chose PLI, ln

381(height), and PLI × ln (height), but excluded country. In this

382alternative model, ln (fruits per plant) increased with ln (height) as

383before ($F_{1,17}$ = 9.026, P = 0.008) and decreased with PLI ($F_{1,17}$ =

38414.008 P = 0.0016). There was no indication that PLI interacted

385with plant height.

386

387Although per-plant fruit production was more strongly related to PLI

388than to visit rate, the best-fit model for the number of seeds

389produced per marked, open-pollinated flower did include visit rate in

390addition to country. Mean seeds per flower increased with visit rate

391(Fig. 7; $F_{1,21}$ = 4.479, P = 0.046 from ANCOVA using square-root-

392transformed values for seeds per flower), and the increase was

393similar in AZ and GR populations ($F_{1,21}$ = 0.012, P = 0.914 for the

394interaction between country and visit rate). Open-pollinated flowers

395(OP treatment) in AZ populations produced more seeds than those

396in GR populations (mean seeds per flower ± SE [N]: 13.611 ± 4.568

397[9] vs. 4.888 ± 2.964 [16]; $F_{1,21}$ = 2.605, P = 0.121). In contrast,

398flowers that had received pollen by hand from distant donors in

399addition to open pollination (PA treatment) showed the opposite

400pattern. Those in AZ populations produced fewer seeds than those

401in GR populations (22.433 ± 6.872 [9] vs. 53.569 ± 4.706 [16]), as

402expected if one considers only mean differences in numbers of

403ovules per flower (see Section 3.1).

404

405**4. Discussion**

406

407This study was designed to compare aspects of reproduction of *S.*

408*elaeagnifolium* in replicate populations within and outside of the ancestral

409range, in order to shed light on factors that may affect invasion by this

410species. In what follows we first consider each aspect of reproduction and

411pollination that we studied, then conclude with implications for future

412studies.

413

414 4.1. Investment in flowers and ovules

415

416 Our GR populations allocated more than our AZ populations to female

417 sexual function (i.e., to flowers and ovules). Whereas plant stature in AZ

418 populations increased in larger populations and with deeper soil

419 disturbance, this was not evident for GR populations, where plants tended

420 to be as tall as the tallest AZ plants. As a result, GR plants were taller on

421 average, and produced more flowers. They also allocated on average

422 nearly twice as many ovules to each flower.

423

424 Williams et al. (2016) documented evolution of increased plant stature

425 across only 6 generations in a mesocosm invasion experiment. The tenure

426 of *S. elaeagnifolium* in Greece might suffice for similar changes, driven by

427 a positive correlation of height and seed dispersal distance (as Williams et

428 al. 2016 speculate for their system) or by selection for greater flower

429 number. Burd (1995) presented a model that predicts that increased

430 ovule number per flower can be selected when pollination service is more

431 variable. In this context, greater allocation to flowers and ovules in GR

432 populations might represent a "bet-hedging" response to greater variation

433 in the Greek pollination environment.

434

435 4.2. Pollinator visits and pollination success

436

437 Plants experienced high visit rates and good pollination success in some

438 Greek populations, such as those on Lesvos, due to the attention of native

439bees that resemble those in Arizona. Both AZ and GR populations also

440hosted similar guilds of non-buzzing flower visitors. Thus *S. elaeagnifolium*

441is successfully incorporated into networks of plant–pollinator interactions

442that lack its ancestral pollinators (see also Memmott and Waser 2002;

443Stout et al. 2006; Kaiser-Bunbury et al. 2009; Vilà et al. 2009). But

444pollination was far from assured in GR populations, especially those

445around Thessaloniki, even though Greece is considered a "bee paradise"

446(Petanidou and Ellis 1993, 1996; Petanidou and Lamborn 2005; Nielsen et

447al. 2011).   Use of agricultural chemicals, habitat loss, and industrial

448development may contribute to bee rarity around Thessaloniki, and

449frequent tilling may exclude ground-nesting bees (see also Williams and

450Kremen 2007). Due to limited flight range (Greenleaf et al. 2007), many

451bees cannot add large monospecific stands to a foraging itinerary that

452requires access to water and a progression of flowers sufficient for a

453complete life cycle. Thessaloniki exemplifies what we term an *invasive*

454*desert*—a monospecific stand that supports strikingly low ecological

455diversity (see Fig. S1).

456

457Although we recorded no buzzing pollinators in most Thessaloniki

458populations, flowers did set some seeds. Without vibration, it is difficult to

459extract pollen from the anthers of *S. elaeagnifolium*, but it remains

460possible that pollen grains are small enough (< 30µm; Luna-Cavazos and

461García-Moya 2002; Burkart et al. 2014) to be shaken out by local "etesian"

462winds on hot summer days. The presence of such pollen might explain

463visits by honey bees and other non-buzzing bees, which might cause some

464seed set, as appears to happen with hover flies visiting (and not buzzing)

465*Solanum dulcamara* (Waser et al. 2011). If this unexpected pollination

466occurs but is highly variable, its variation might contribute to greater

467sexual allocation by the bet-hedging mechanism discussed above.

468

4694.3. Fruit and seed set

470

471Although GR plants produced more flowers and ovules than AZ plants,

472they did not achieve greater fecundity in terms of fruit or seed set. This

473was true especially in large monospecific stands, which had large PLI

474values. These results suggest that GR populations received poorer

475pollination service, and perhaps also pollen of poorer "quality" (sensu

476Waser and Price 1983, p. 356; Aizen and Harder 2007). GR plants are less

477self-compatible than those in our AZ populations (see Petanidou et al.

4782012). Thus the availability of genetically-compatible pollen donors may

479be lower, especially if some populations are formed by extensive

480vegetative spread through rhizomes. We excluded one GR population

481from analyses because we suspected that low genetic variability caused

482pollen-supplemented flowers to have very low fecundity (see Section 2.6).

483

4844.4. Implications for future studies

485

486Our results suggest that increased investment in female sexual

487function accompanies dispersal of *S. elaeagnifolium* beyond the

488ancestral range as well as transition from small ruderal populations

489mixed with other species to large monospecific populations. This

490greater investment occurs in concert with reduced self-compatibility

491(Petanidou et al. 2012), and does not yield higher realized fecundity,

492at least in large GR populations, perhaps because such

493environments are unfavorable for pollinators and compatible mates

494are few.

495

496Nonetheless, *S. elaeagnifolium* is obviously successful as an invasive. Its

497aggressive spread appears to be favored by the same conditions in AZ and

498GR populations: deep soil disturbance over large areas, which may

499facilitate spread by rhizome fragments. While this does suggest some

500opportunities for control, such as replacement of tilling by mowing, many

501questions remain for further study.

502

503For closer comparison with Greece, a priority is to extend the study of

504pollination and reproduction to additional populations in more

505Mediterranean climates within the ancestral range, as opposed to the

506desert climates of Arizona. The species is found in Mediterranean-climate

507southern California (Munz 1974), South Africa (Boyd et al. 1984), and

508Australia (Zhu et al. 2013). Additional observations of pollinator visits in

509the largest GR populations (and elsewhere) also would be useful, in part to

510document temporal variation. Also valuable would be further investigation

511of the possibility of pollination via wind and non-buzzing visitors.

512

513 We have noted that low seed set and high PLI might be influenced by

514 paucity of compatible pollen even if pollinator visits are common. In our

515 experimental pollen additions we chose donors 5-20 m from recipient

516 flowers. Perhaps on this spatial scale plant ramets usually belong to

517 different genetic individuals, whereas at shorter scales, over which

518 pollinators transfer most pollen in dense populations (Waser 1982), ramets

519 often belong to the same genet and so mates tend to be incompatible.

520 This could be explored using genetic analysis to characterize the spatial

521 extent of clonal spread via rhizomes in populations of different sizes and

522 disturbance regimes (see Ellstrand and Roose 1987).

523

524 Our emphasis on sexual reproduction and aboveground allocation leaves

525 unanswered questions about belowground allocation to rhizomes. Analysis

526 of the extent of individual genets might be combined with measures of

527 vegetative spread via rhizomes in populations with different

528 characteristics. In a preliminary study (R. Kariyat et al. unpublished),

529 seeds performed poorly in many respects (e.g., in plant establishment)

530 compared to rhizome pieces collected from the same plants in large

531 Thessaloniki populations. Space filling by rhizomes (along with seeds)

532 may occur in tilled agricultural lands, where *S. elaeagnifolium* is

533 remarkably successful; seeds also might allow rapid colonization of new

534 suitable habitat fragments (see Williams et al. 2016).

535

536 Finally, any differences in the expression of sexual characteristics of

537 *S. elaeagnifolium* plants documented here, as functions of

538population size, disturbance regime, and country, might logically

539represent adaptive or neutral genetic differences that trace back to

540those individuals that founded Greek populations and those in

541Arizona landscapes that have been recently altered. Founder

542effects can be detected through reduced genetic diversity at neutral

543or quasi-neutral marker loci (Dlugosch and Parker 2008).

544Alternatively, any differences in character expression might

545represent adaptations arising during decades of habitation in

546Greece or in altered Arizona landscapes. Finally, they might be

547plastic responses to local conditions (Zhu et al. 2013). The classic

548approach (Langlet 1971) to distinguishing genetic and plastic

549mechanisms of phenotypic differentiation is to grow plants in

550common gardens, and to do this reciprocally between ancestral and

551invaded ranges.

552

553**Conflict of interest**

554The authors state that they have no conflict of interest.

555

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557

567

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569

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771**Table 1.** *Solanum elaeagnifolium* populations studied in Arizona (ancestral range) and Greece (invaded range). Criteria

772for evaluating type of disturbance and population size score are described in the text. Dates of observation of pollinators

773and of hand pollination to assess pollen limitation are given as days/month/year.

Population	Region	Coordinates	Habitat characteristics	Disturbance	Size (# of plants)	Size score	Dates of observation and hand pollination
Arizona							
1. Marana, Kai cotton	Pima	32° 26' 50"N, 111° 18' 12"W	within intensely cultivated cotton field	tilled	ca. 800	0	22-23, 25/07/2006
2. Marana, Kai fallow	Pima	32° 26' 55"N, 111° 18' 13"W	paved road verge	surface	>2000	1	22-23, 25/07/2006
3. Marana, Sanders cotton	Pima	32° 26' 45"N, 111° 14' 40"W	Unpaved road verge	surface	>2000	1	22-23, 25/07/2006
4. Marana, Sanders fallow	Pima	32° 28' 03"N, 111° 14' 03"W	Margins of intensely cultivated field	tilled	>2000	1	22-23, 25/07/2006
5. Santa Rita, lower	Pima	31° 46' 48N, 110° 53'16"W	paved road verge in a conservation area	surface	ca. 100	0	18-19/07/2006
6. Santa Rita, upper	Pima	31° 46' 12"N, 110° 53' 15"W	paved road verge in a conservation area	surface	ca. 500	0	18-19/07/2006
7. San Simon, huge	Cochise	32° 15' 05"N, 109° 11' 10"W	Entire surface of a fallow field	tilled	>10,000	2	05-06/08/2006
8. San Simon, Noland	Cochise	32° 15' 19"N, 109° 10' 35"W	Margins of intensely cultivated field	tilled	>2000	1	05-06/08/2006
9. Willcox, Moonlight	Cochise	32° 14' 19"N, 109° 46' 41"W	Unpaved road verge; wild	surface	ca. 800	0	26/08/2006
Greece							
10. Arisvi	Lesvos	39° 14' 08"N, 26° 13' 31"E	unpaved road verge; naturalized	surface	ca. 1500	0	4-5/07/2014
11. Eressos	Lesvos	39° 08' 24''N, 25° 55' 19"E	residential lawn; naturalized	surface	ca. 150	0	13/08/2006
12. Plomari	Lesvos	38° 58' 17''N, 26° 22' 57"E	paved road verge; naturalized	surface	ca. 100	0	7-8/07/2014
13. Anchialos, Cemetery	Thessaloniki	40° 43' 05"N, 22° 47' 07"E	dry waste land	surface	>10,000	2	24/07/2007
14. Anchialos, Farmaki	Thessaloniki	40° 43' 25"N, 22° 48' 31"E	cultivated field (barley, vetch)	tilled	>10,000	2	01/08/2007
15. Anchialos, Ioannou	Thessaloniki	40° 42' 19"N, 22° 47' 18"E	waste land/yard of a factory	surface	>2000	1	31/07/2007
16. Anchialos, Narlis	Thessaloniki	40° 43' 34"N, 22° 47' 57"E	cultivated field (barley, vetch)	tilled	>10,000	2	02/08/2007
17. Anchialos, Toumba	Thessaloniki	40° 43' 07"N, 22° 49' 05"E	waste land	surface	>10,000	2	02/08/2007
18. Anchialos, Vaggelis	Thessaloniki	40° 43' 40"N, 22° 48' 28"E	wheat field left fallow	tilled	>10,000	2	03/08/2007
19. Panorama	Thessaloniki	40° 35' 22"N, 23° 02' 38"E	semi-natural meadow in urbanized area	surface	>2000	1	04, 11/08/07
20. Raidestos, Atlanta	Thessaloniki	40° 32' 14"N, 23° 02' 49"E	wheat field	tilled	>10,000	2	13/07/2007

21. Raidestos, onion	Thessaloniki	40° 31' 05"N, 23° 03' 23"E	onion field	tilled	>10,000	2	30/07/2007
22. Raidestos, wheat	Thessaloniki	40° 30' 59"N, 23° 04' 25"E	wheat field	tilled	>10,000	2	11/07/2007
23. Raidestos, Wind	Thessaloniki	40° 30' 56"N, 23° 04' 21"E	Semi-natural Mediterranean scrub surface		>10,000	2	14/07/2007
24. Sindos, TEI	Thessaloniki	40° 39' 12"N, 22° 48' 50"E	wheat field	tilled	>10,000	2	19/07/2007
25. Sindos, cultivated	Thessaloniki	40° 41' 47"N, 22° 48' 35"E	waste land, partly cultivated	tillage	>10,000	2	15/07/2007
26. Triadi, Kosmidis	Thessaloniki	40° 32' 33"N, 23° 02' 37"E	wheat field left fallow	tillage	>10,000	2	20/07/2007
27. Triadi, Namco	Thessaloniki	40° 32' 57"N, 23° 02' 09"E	wheat field left fallow	tillage	>10,000	2	26/07/2007

774**Table 2.** Aspects of allocation to female sex function, pollination success, and realized fecundity in the study

775populations. The symbol # means "number of" and values are grand means for marked plants in each population ± SE

776(sample size). Sample size is number of plants for plant height and fruits per plant; otherwise number of flowers. Visit

777rate includes only visits by pollinators that buzzed flowers. OP = open-pollinated; PA = cross pollen added by hand; PLI =

778Pollen Limitation Index. * = study population destroyed or damaged during the study.

Study populations	plant height (cm)	# ovules flower^{-1}	# flowers per plant	# visits flower^{-1} h^{-1}	# seeds/ flower (OP)	# seeds/ flower (PA)	PLI	# fruits per plant
Arizona								
1. Marana, Kai cotton	69.0±2.6 (21)	32.3±1.96 (35)	713	0.046	0.7±0.64 (56)	1.8±0.65 (42)	0.61	38.2±9.77 (19)
2. Marana, Kai fallow	62.3±1.7 (29)	71.0±2.27 (73)	-	0.000	0 (75)	2.7±0.96 (73)	1	2.2±1.05 (18)
3. Marana, Sanders cotton	54.7±1.3 (41)	69.1±3.38 (30)	1205	0.027	0.8±0.60 (57)	2.4±1.21 (49)	0.67	30.0±6.13 (20)
4. Marana, Sanders fallow	67.4±3.6 (21)	68.7±3.07 (38)	387	0.014	13.4±2.18 (60)	36.9±3.12 (61)	0.64	203.1±20.77 (19)
5. Santa Rita, lower	29.9±1.1 (33)	41.8±3.80 (24)	44	0.034	8.7±1.50 (60)	6.6±0.88 (51)	0.00	33.7±3.01 (61)
6. Santa Rita, upper	38.9±1.0 (41)	43.5±1.84 (24)	46	0.252	16.4±1.73 (47)	14.9±1.67 (45)	0.00	35.0±3.90 (29)
7. San Simon, huge	71.2±2.8 (20)	53.6±1.74 (37)	371	0.044	37.6±2.92 (77)	39.5±2.93 (74)	0.05	163.8±37.13 (20)
8. San Simon, Noland	63.4±2.0 (21)	64.5±3.19 (37)	1636	0.015	33.0±3.32 (68)	46.2±3.94 (60)	0.29	120.4±22.84 (30)
9. Willcox, Moonlight	31.1±0.9 (54)	52.9±2.39 (31)	40	0.195	11.9±3.33 (41)	50.9±4.70 (29)	0.77	17.5±3.76 (32)
Greece								

10. Arisvi	73.0±1.5 (30)	49.5±1.53 (30)	253	1.130	8.7±1.81 (57)	24.1±2.86 (58)	0.64	102.0±16.59 (30)
11. Eressos*	-	49.9±6.63 (13)	-	0.620	48.7±4.30 (46)	60.7±5.13 (42)	0.20	-
12. Plomari	63.7±1.7 (30)	52.5±1.79 (30)	157	0.170	0.1±0.03 (59)	0.1±0.05 (59)	0	15.9±2.01 (30)
13. Anchialos, Cemetery	68.4±1.9 (29)	94.6±4.39 (60)	1234	0.000	2.1±1.94 (58)	4.7±1.40 (58)	0.55	41.1±5.40 (25)
14. Anchialos, Farmakis	71.1±1.9 (30)	108.6±4.86 (56)	394	0.000	1.7±1.13 (60)	71.9±4.86 (60)	0.98	59.1±7.82 (26)
15. Anchialos, Ioannou	68.4±2.0 (30)	106.9±3.91 (60)	315	0.000	1.4±1.20 (60)	68.6±5.31 (60)	0.98	52.5±7.22 (26)
16. Anchialos, Narlis*	-	111.5±6.16 (60)	-	-	-	-	-	-
17. Anchialos, Toumba*	-	77.78±3.77 (60)	-	0.000	0 (30)	64.6±7.93 (30)	1	-
18. Anchialos, Vaggelis	76.1±1.7 (30)	113.3±5.15 (60)	647	0.000	0.3±0.16 (60)	66.6±6.29 (60)	1	43.1±5.51 (29)
19. Panorama	65.1±2.4 (30)	104.7±7.39 (60)	317	0.008	0.9±0.35 (60)	56.9±10.59 (60)	0.98	52.9±6.99 (30)
20. Raidestos, Atlanta	54.3±2.5 (30)	99.8±5.03 (60)	103	0.000	2.9±1.58 (60)	51.0±5.60 (60)	0.94	19.0±3.85 (24)
21. Raidestos, onion	52.5±1.8 (30)	98.2±5.26 (61)	195	0.000	2.5±1.68 (60)	61.8±4.21 (60)	0.96	22.0±2.94 (31)
22. Raidestos, wheat	65.2±1.6 (30)	109.6±3.90 (60)	75	0.000	0.7±0.38 (60)	28.3±4.37 (60)	0.98	6.2±2.36 (25)
23. Raidestos, Wind	58.3±1.5 (31)	117.3±5.09 (60)	109	0.000	0.5±0.20 (62)	63.8±4.80 (62)	0.99	14.1±3.15 (25)
24. Sindos, TEI*	-	119.3±5.44 (72)	-	0.000	2.3±1.65 (70)	67.0±6.55 (70)	0.97	-
25. Sindos, cultivated	84.3±2.3 (37)	91.1±3.90 (73)	416	0.000	2.7±1.25 (72)	64.1±4.17 (72)	0.96	56.2±8.77 (33)
26. Triadi, Kosmidis	51.5±1.8 (30)	113.1±5.25 (60)	311	0.000	2.0±1.22 (58)	54.9±5.00 (58)	0.96	20.7±5.75 (15)
27. Triadi, Namco	63.4±1.4	114.1±4.61	1022	0.000	0.8±0.80	48.1±3.99	0.98	17.0±1.95

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(30)　　　(60)　　　　　　　　(60)　　　(60)　　　　　(29)

Table 3. Bee taxa recorded on *Solanum elaeagnifolium* flowers in our
781study populations, as indicated by "+". All taxa "buzzed" flowers, and
782thus are considered major pollinators, except those marked with *. Lesvos
783populations were regularly visited by non-buzzing honeybees that
784collected spilled pollen.

Population	Apidae									Andrenidae		Halictidae		
	Amegilla spp.	*Bombus terrestris*	*Exomalopsis*	*Melissodes* spp.	*Xylocopa californica* ssp.	*Xylocopa iris*	*Xylocopa violacea*	*Centris atripes, C. rodopis*	*Apis mellifera**	*Protandrena mexicanorum*	*Protoxaea*	*Pseudapis bispinosa* (= *Nomia bispinosa*)	*Nomia tetrazonata*	unidentified small bees*
Arizona														
1. Marana, Kai cotton	-	-	+	+	+	-	-	-	-	-	+	-	+	+
2. Marana, Kai fallow	-	-	+	+	+	-	-	-	-	-	+	-	+	+
3. Marana, Sanders cotton	-	-	+	+	+	-	-	-	-	-	+	-	+	+
4. Marana, Sanders fallow	-	-	+	+	+	-	-	-	-	-	+	-	+	+
5. Santa Rita, lower	-	-	-	-	+	-	-	+	-	+	+	-	+	+
6. Santa Rita, upper	-	-	-	-	+	-	-	+	-	+	+	-	+	+
7. San Simon, huge	-	-	-	-	-	-	-	-	-	-	+	-	-	+
8. San Simon, Noland	-	-	-	-	-	-	-	-	-	-	+	-	-	+
9. Willcox, Moonlight	-	-	+	-	+	-	-	-	-	-	-	-	-	+
Greece	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10. Arisvi	-	+	-	-	-	+	-	-	+	-	-	+	-	+
11. Eressos	+	+				+						+		+
12. Plomari	-	-	-	-	-	+	-	-	+	-	-	+	-	+
13. Anchialos, Cemetery	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14. Anchialos, Farmakis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15. Anchialos, Ioannou	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16. Anchialos, Narlis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17. Anchialos, Toumba	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18. Anchialos,	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Vaggelis													
19. Panorama	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20. Raidestos, Atlanta	-	-	-	-	-	41	-	-	-	-	-	-	-	-
21. Raidestos, onion	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22. Raidestos, wheat	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23. Raidestos, Wind	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24. Sindos, TEI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25. Sindos, cultivated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26. Triadi, Kosmidis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27. Triadi, Namco	-	-	-	-	-	-	-	-	-	-	-	-	-	-

785**Fig. 1.** Relationship between mean plant height and population

786size score. Plant stature increased with population size in our US

787(open circles) but not in our GR (filled circles) populations.

788**Fig. 2.** Estimated total flowers per plant as a function of plant

789stature. Flower number increased with plant height in both our US

790(open circles) and in our GR (filled circles) populations; US plants

791were smaller on average and produced fewer flowers on average.

792**Fig. 3.** Nonlinear increase in ovule number per flower with

793population size. In both our US (open circles) and in our GR (filled

794circles) populations, ovule number increased to an apparent

795plateau, which was lower in the US sample.

796

797Fig. 4. Nonlinear decrease in pollinator visits with size of our US

798(open circles) and GR (filled circles) populations. Observed visitation

799rates were higher in smaller populations, and highest in two

800populations on Lesvos; they declined to very low values or to zero in

801the largest populations, especially those around Thessaloniki.

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803

804**Fig. 5.** Pollen Limitation Index vs. pollinator visit rate. PLI

805decreased with pollinator visit rate in both our US (open circles) and

806in our GR (filled circles) populations, although the range of visit

807rates was far greater in GR.

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810**Fig. 6.** Total fruits per plant vs. plant stature. Fruit production 811increased close to linearly with plant height in both our US (open 812circles) and in our GR (filled circles) populations, but the overall 813mean was greater for US plants.

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815**Fig. 7.** Seeds per flower vs. pollinator visit rate. Mean seed set

816increased with visits in both our US (open circles) and in our GR

817(filled circles) populations. Flowers produced more seeds on

818average in US than in GR populations; it is not obvious from the

819figure that 15 GR populations are clustered near the origin (0,0), as

820Table 2 shows.

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AUTHOR CONTRIBUTIONS

TP designed the study with input from NW. TP and AK carried out fieldwork. MP and TT analysed the data. TP, NW, and MP wrote the manuscript. All authors edited the manuscript.

Table S1. Details of statistical models from which results reported in the text

are derived. "VARIABLE" = response variable; SOURCE = predictor

variable; "Country" = country of origin of the sample of study populations;

"Pop size" = population size score (0, 1, or 2) treated as a continuous

variable; "Disturbance" = type of soil disturbance (surface vs. tilled)

experienced by a population. Two alternative analyses are included for

fruits per plant, as described in the text. The analysis of seeds per flower

refers to marked flowers in the open-pollinated (OP) treatment.

VARIABLE	SOURCE	Df	F	P	COMMENTS
ln (Plant height)	Country	1,19.20	0.821	0.376	REML ANCOVA
Model R^2_{adj} = 0.724	Pop size	1,19.17	2.554	0.126	based on 775 individual
	Disturbance	1,19.14	4.875	0.040	values;
	Country × pop size	1,19.17	4.252	0.053	only fixed effect
	Country × disturbance	1,19.14	4.664	0.044	tests given
ln (Flowers/plant)	Country	1,18	0.034	0.879	ANCOVA
Model R^2_{adj} = 0.364	Plant height	1,18	10.077	0.005	based on population
	Country × plant height	1,18	0.251	0.623	means
ln (Ovules/flower)	Country	1,17	102.488	<0.0001	Polynomial
Model R^2_{adj} = 0.951	Plant height	1,17	9.537	0.007	ANCOVA
	Pop size	1,17	9.227	0.007	based on
	Pop size^2	1,17	62.645	<0.0001	population means
	Country × pop size	1,17	4.666	0.045	
√(Visits/flower/hour)	Country	1,21	0.170	0.685	Polynomial
Model R^2_{adj} = 0.786	Pop size	1,21	2.664	0.118	ANCOVA
	Pop size^2	1,21	13.507	0.0014	based on
	Country × pop size	1,21	17.283	0.0001	population means
PLI	Country	1,21	10.865	0.0034	ANCOVA
Model R^2_{adj} =	√(Visits/flower/	1,21	5.352	0.031	based on

0.490	hour)				
	Country × √(Visits/ flower/hour)	1,21	0.341	0.566	population means
ln (Fruits/plant) Model R^2_{adj} = 0.409	Country	1,17	12.243	0.0027	ANCOVA
	ln (Plant height)	1,17	11.211	0.0038	based on population
	Country × ln (plant height)	1,17	0.808	0.381	means
ln (Fruits/plant) Model R^2_{adj} = 0.449	PLI	1,17	14.008	0.0016	ANCOVA
	ln (Plant height)	1,17	9.026	0.008	based on
	PLI × ln (plant	1,17	0.148	0.706	population
√(Seeds/flower) Model R^2_{adj} = 0.338	Country	1,21	2.605	0.121	ANCOVA
	√Visits/flower/hour	1,21	4.479	0.046	based on population
	Country × √(Visits/ flower/hour)	1,21	0.012	0.914	means

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831**Fig. S1.** An *invasive desert* near Anchialos, in the western metropolitan

832area of Thessaloniki—a large area taken over by a very aggressive

833monospecific stand of *S. elaeagnifolium* (image: T. Petanidou).

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842**Fig. S2.** A smaller, less aggressive population of *S. elaeagnifolium* in the

843countryside near Willcox, Arizona (image: T. Petanidou).

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