Short communication

Agricultural land management negatively affects pollination service in Pampean agro-ecosystems

H.J. Marrero[a,c,*], D. Medan[b,c], G.E. Zarlavsky[b], J.P. Torretta[b,c]

[a] *Instituto Argentino de Investigaciones de las Zonas Áridas, Mendoza, Argentina*
[b] *Cátedra de Botánica General, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina*
[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

A B S T R A C T

Agricultural land management modify environments in such a way that ecosystem functions are impaired. The establishment of exotic plants, favoured by agricultural land management, may alter the foraging behaviour of native floral visitors and consequently the pollination service they provide. To determine whether agricultural land management affects the pollination service provided by floral visitors for the most abundant plant species in a Pampean grassland ecosystem, we compared the pollination service of native and exotic entomophilous plants in landscape fragments with or without current agricultural usage. Both the quantity (number of conspecific pollen grains deposited on stigmas) and quality (proportion of heterospecific pollen grains deposited on stigmas) of the pollination service was estimated for three to nine entomophilous plants in each landscape fragment. Agricultural land management reduced the quality of the pollination service due to an increase in the deposition of heterospecific pollen on stigmas.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Agricultural land management modify environments by creating ecosystems reduced in functions and services (Sala et al., 2000). This environmental modification acts in different ways that are not mutually exclusive: (1) it may homogenize natural ecosystems, increasing their fragmentation and isolation (Krauss et al., 2010), (2) by decreasing species richness (Altieri, 1999), and (3) by modifying the functionality of the original ecosystem, since the abundance and richness of species that carry out ecosystem functions such as pollination, are decreased (Lentini et al., 2012). Specifically, agricultural land management can modify pollination service: (a) by creating habitats where mutualistic interactions (e.g. plant-pollinator) are more generalized (Marrero et al., 2014), (b) by increasing competition for the pollinator resources between co-flowering plants in the community (Ramírez, 2005), and (c) by favouring the establishment of exotic plant species, which may modify the interactions between the native plants and their floral visitors (Aizen et al., 2008; Morales and Traveset, 2009; Grass et al., 2013). However, the degree to which services and functions are affected in grassland agro-ecosystems is poorly understood.

Introduced exotic plants could alter pollination service by different mechanisms. Some exotic plants successfully compete for pollination with native species, as the former offer more floral resources (Chittka and Schürkens, 2001), or exhibit more units of floral attraction (flowers or inflorescences) (Morales and Traveset, 2009). The result is a reduction in seed set of the coexisting native species (Bjerknes et al., 2007; Woods et al., 2012). This may be due to a reduction in the visitation rate by pollinators, which decreases the deposition of conspecific pollen on the stigmas (Bjerknes et al., 2007), a loss of conspecific pollen arrival because of visits of pollinators to different plant species (Bjerknes et al., 2007), or an increase in the deposition of heterospecific pollen (Brown and Mitchell, 2001). Heterospecific pollen on the stigmas can affect seed formation due to different mechanisms (Morales and Traveset, 2008).

The aim of this study was to determine whether agricultural land management affects the pollination service provided by insects for the most abundant plant species, by comparing fragments recovered after a long time without any agricultural activities or with a conservation-aimed land management (hereafter called preserved fragments) with fragments under current agricultural usage (hereafter called agricultural fragments). We evaluated pollination service by analyzing the size and composition of the pollen loads deposited on stigmas by floral visitors. The tested hypothesis was: agricultural land management

* Corresponding author at: Instituto Argentino de Investigaciones de las Zonas Áridas, Mendoza, Argentina.
E-mail address: hugomarrero@gmail.com (H.J. Marrero).

provoke a decrease in the quality and quantity of pollination service. As compared to preserved fragments, in agricultural ones we expect to find: (a) a decrease in the quantity of conspecific pollen deposited on stigmas (i.e., a decrease in the magnitude of the pollination service), and (b) an increase in the quantity of heterospecific pollen deposited on stigmas (i.e., a decrease in the quality of the pollination service). Additionally, due to agricultural land management promote the establishment of exotic plant species (Marrero et al., 2014), hence in agricultural fragments (c) there will be a decrease in quality of pollination service of native plants compared with preserved fragments.

2. Material and methods

2.1. Study site

The study was carried out at three sites along a transect 700 km long located at 36°S in the provinces of Buenos Aires and La Pampa, Argentina. The site in the extreme east was in Estancia Las Chilcas (Pila, Prov. Buenos Aires; hereafter abbreviated as 'LC'). The intermediate site was in Estancia San Claudio (Carlos Casares, Prov. Buenos Aires; hereafter 'SC'). Lastly, the site at the extreme west end of transect was located in Estancia Anquilóo (Toay, Prov. de La Pampa; hereafter 'AN') (see Marrero et al., 2014 for a detailed description of the study sites). In SC and AN, four fragments of one hectare were selected, two agricultural and two preserved, while in LC three fragments of one hectare were selected, two agricultural and one preserved. Only one preserved area was studied in LC, due to the fact that no other landscape areas were found with which to adequately compare the agricultural fragments (see Marrero et al., 2014). The agricultural and preserved fragments were at least 500 m apart in order to guarantee their independence. Unlike the cattle production sites (LC and AN), in SC the field margins (4% of total area) are included in the cropping areas as they form part of the agroecosystem and are important biodiversity reservoirs (Torretta and Poggio, 2013).

The predominant land use in SC was agriculture, while in LC and AN it was livestock raising. The extension of the lots varied depending on the sites and the type of use. The farms with livestock were generally larger lots than farms with agriculture. In LC and SC, preserved fragments were enclosures with permanent fences which had not been cultivated or grazed for the last 20 years (SC) or for approximately the last 3 years (LC). In AN, preserved fragments were sites where cattle had been absent in the last 30 years from September until April of each year, and it was in that time window that sampling was carried out. In these fragments, farm managers had intentionally conserved the woody elements of the vegetation, including many entomophilous species (*Prosopis caldenia*, *Condalia microphylla* and *Geoffroea decorticans*, among others), although trampling by cattle may have affected the entomophilous herbaceous plants. It is important to emphasise that woodland clearance has traditionally been the greatest modification undertaken by human beings in this region, in order to make access for cattle easier and to have larger grazing areas available (González-Roglich et al., 2012).

In each site, between three and nine of the most abundant entomophilous plant species flowering between December 2010 and February 2011, were used as representative guild of the pollination network existing at each fragment. Generalist plants (i.e., those that formed part of core of the interaction network; 75% of the total plants studied that interacted with more than 20 species of floral visitors each) and specialist plants (the other 25% of the total plants studied that interacted with fewer than 10 species of floral visitors each) were selected in all fragments, on the basis of sampling data from previous years (Marrero et al., 2014). The mutualistic interaction networks generally show a nested structure, where the specialist species from one group (i.e., plants) interact more frequently with generalists from another group (i.e., floral visitors) (Vázquez and Aizen, 2004). The core of the network is the subgroup of generalist species from both groups that interact most frequently (Bascompte and Jordano, 2007). Furthermore, the exotic plants considered were generally abundant on the most agricultural fragments and were principally generalist species (i.e., highly visited).

2.2. Field sampling

To measure pollination service, between four and 20 individuals of each selected species (see Supplementary material, Appendix A) were marked in each fragment. The flowers of these individuals were chosen as the units of replication. Differences in the number of selected plant individuals per species reflected interspecific differences in the number of flowers per individual (e.g., plants of *Hirschfeldia incana* have on average 5.6 open flowers/inflorescence and many inflorescences per individual, while plants of *Nierenbergia aristata* only have on average 3.2 flowers per individual) (Marrero pers. obs.). All open flowers were removed from all selected plants, leaving only the flower buds that were expected to enter in anthesis the following day. In each individual, 25% of the buds of were covered with net bags to prevent visits from pollinators during anthesis (bagged flowers), whereas the rest were kept uncovered so they could be visited (open flowers, control). Two days later, the pistils of flowers were removed and taken to the laboratory in plastic containers. Styles and stigmas were removed under a stereomicroscope and were allowed to dry in a closed container. Particular effort was devoted to avoid accidental transfer of pollen between the stigmas during storage.

2.3. Laboratory work

For study the pollen loads, each stigma was macerated with a drop of NaOH:water 1:10 (w:v) for 24 h on a slide. Later, a small quantity of melted glycerol-gelatine (containing safranin to stain the grains) was added and the stigma was squashed lightly with a coverslip (Zarlavsky, 2014). Finally each preparation was analyzed under the microscope and the grains of conspecific and heterospecific pollen were counted. The identity of the grains was verified using reference preparations that had been prepared for the 141 species of entomophilous plants recorded previously (Marrero et al., 2014).

2.4. Pollination service

Values for the pollination service (measured as: magnitude = deposition of conspecific pollen; and quality = deposition of heterospecific pollen on the stigma) were obtained from 49 estimations of the service in the 11 fragments. The estimations were performed on 26 species of entomophilous plants from the 11 sampled fragments, and there were 49 estimations because some species were found in more than one fragment. Specifically, an estimation consisted in the comparison between the amount of pollen (heterospecific and conspecific) deposited on the stigmas in bagged and open flowers, for one plant species and in one study fragment.

2.5. Data analyses

For an initial approximation of the amount of pollen in different sites, we conducted an analysis of variance (ANOVA) of the pollen load in stigmas of native and exotic plants for our three sites. In order to test the magnitude of pollination service (prediction a), we performed generalized lineal mixed models (GLMMs). We used a

design of two fixed factors: treatment (agricultural and preserved) and type (open and bagged flowers), and two random factors: site (LC, SC and AN) and species (plants species present in each fragment used for the pollination service estimation), where each observational unit was one stigma. We used GLMMs with Gamma errors to model these response variables.

In order to test the quality of pollination service (prediction b) and to establish whether there were any significant differences between native and exotic species in preserved and agricultural fragments in the quantity of heterospecific pollen deposited on stigmas of the control flowers (prediction c), we performed GLMM. We used a design of two fixed factors: treatment (agricultural and preserved) and geographic origin of plants (exotic and native), and one random factor site (LC, SC and AN), where each observational unit was one stigma. We used GLMMs with Gamma errors to model these response variables.

All stigmas contained conspecific pollen loads and 85% of the stigmas contained heterospecific pollen loads. For the estimation of pollination quality, only stigmas that contained heterospecific grains were considered for the GLMMs (Appendix B). The use of stigmas without heterospecific grains in the analysis could generate underestimation, because these stigmas may not have received visits of floral visitors. To select the models that best fits the data, we used the Akaike information criterion (AIC) and selected the models with the lowest AIC. The GLMMs was analyzed with the R statistical program (R Development Core Team, 2013). We used the function glmer in lme4 library to conduct Gamma-errors GLMMs (R Development Core Team, 2013).

3. Results

In total, 1644 stigmas of 26 plant species (20 of them native) were analyzed, in which ca. 550,000 pollen grains were counted. Pooling both fragment types, stigmas of native plants had higher conspecific pollen loads than those of exotic species in LC (1100.22 vs. 138.31, $F = 6.48$, $p = 0.014$), but this relationship was not observed in SC (287.3 vs. 311.2, $F = 0.12$, $p = 0.728$) either AN (426.88 vs. 495.53, $F = 0.4$, $p = 0.5271$).

To estimate quantity of pollination service (prediction a), we performed four GLMMs that differed in the interactions among factors (Table 1). The model with the lowest AIC was the one that

included the fixed variable treatment (preserved and agricultural) (Table 1). Conspecific pollen loads did not significantly differ between bagged and open pollinated flowers in preserved and agricultural fragments (Chi2 = 2.4097, df = 1, $p = 0.1206$) (Appendix C).

In order to test prediction b and c we performed four GLMMs that differed in the interactions among factors. The model with the lowest AIC was the one that included the interaction between fixed variables geographical origin (exotic and native) and treatment (preserved and agricultural) (Table 2). Heterospecific pollen loads did significantly differ between bagged and open pollinated flowers in preserved and agricultural fragments, being higher in the agricultural ones (Table 3). Furthermore, our results provide support that the variations in pollination quality were better explained by heterospecific pollen on native plants stigmas in preserved fragments (Table 3). Native plants in preserved fragments were less affected than in agricultural fragments (Fig. 1).

4. Discussion

Our results support partially the hypothesis that agricultural land management modify pollination service, because our predictions were not equally fulfilled for quantity and quality (predictions a and b). The results show that agricultural land management were associated to the increase in deposition of heterospecific pollen, that native plants were comparatively more affected than exotic plants in the reception of heterospecific pollen, and that there was no parallel decrease in the deposition of conspecific pollen in the agricultural fragments.

The decrease in the quality of the pollination service in agricultural fragment may be related to frequent presence of exotic species of plants and pollinators in these sites (Marrero et al., 2014). Garibaldi et al. (2013) found that the honey bee (*Apis mellifera*), even when transporting large quantities of pollen, may be inefficient in the pollination of some crops. Morales and Traveset (2009) showed that, in presence of exotic plants, visits of pollinators to native plants are reduced and reproductive success of the latter tends to decrease. Moreover, the deposition of heterospecific pollen on stigmas is considered a strategy of competition (Bjerknes et al., 2007; Morales and Traveset, 2008) that could benefit exotic plants in agricultural fragments. We can not assume a reduction in seed-set production per fruit, although reduction can be produced by different mechanisms (see next paragraph). However, pollination service is reduced in agricultural fragments probably due to decreased in foraging quality.

Heterospecific pollen grains on the stigmas may lead to a decrease in the fitness of the plant (a) by the obstruction of the stigma, as the surface of the stigma is saturated with heterospecific pollen or the style is saturated with heterospecific pollen tubes; (b) because the stigma "closes" as the deposition of heterospecific pollen reduces stigmatic receptivity; (c) due to allelopatic inhibition of the conspecific pollen grains or tubes; and/or (d) by usurpation of the ovules, when heterospecific pollen tubes compete with conspecific pollen tubes for access to the ovules (Brown and Mitchell, 2001; Morales and Traveset, 2008 and

Table 1
GLMMs of pollination quantity. *Table 1*: shows the four generalized lineal mixed models (M1–M4) used to evaluate the quantity of pollination in preserved and agricultural fragments (treatment). We used a design of two fixed factors, treatment and type (bagged and open flowers), and two random factors site (LC, SC and AN) and SP (plant species). We choose the model with lowest ΔAIC (Akaike Information Criterion).

Models	df	ΔAIC
M1. Site + SP + Treatment + Type	6	137.79
M2. Site + SP + Treatment + Type + Treatment × Type	7	130.07
M3. Site + SP + Treatment + SP × Treatment + Type	5	175.49
M4. Site + SP + Type + SP × Type + Treatment	5	0

Table 2
GLMMs of pollination quality. *Table 2*: shows the four generalized lineal mixed models (M1–M4) used to evaluate the quality of pollination on exotic and native plants (geographic origin of plants species- GO) in restored and agricultural fragments (treatment). We used a design of two fixed factors, treatment and geographic origin of plants, and a random factor site (LC, SC and AN). We choose the model with lowest ΔAIC (Akaike Information Criterion).

Models	df	ΔAIC
M1. Site + Treatment + GO	5	76.78
M2. Site + Treatment + GO + Site × Treatment + Site × GO + Treatment × GO	8	2
M3. Site + GO	4	83.07
M4. Site + Treatment + GO + Site × GO + Treatment × GO	7	0

Table 3

GLMM outputs of pollination Quality. *Table 3*: shows the results of generalized linear mixed-effects models with Gamma errors of the numbers of heterospecific pollen deposited on the stigmas of exotic and native plants (geographic origin = GO) in agricultural and preserved fragments (treatment) at three sites in the Pampas region. Outputs related with model 4 in Table 2. There were two fixed factors (treatment and geographic origin) and a random factor (site). $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

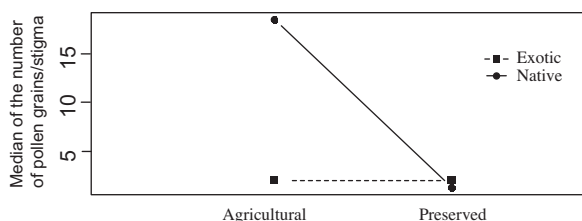| Fixed effect	Coeff.	SE	t
(Intercept)	0.9262	0.5259	1.761
Treatment	1.1784	0.1740	6.773***
GO	2.0753	0.7276	2.852**
Treatment × GO	-2.0050	0.2668	-7.516***

Fig. 1. Amount of heterospecific pollen deposited on the stigmas of native and exotic plants in agricultural and preserved fragments. The figure shows the interaction between the factors treatment (Agricultural and Preserved) and geographical origin of plants (Exotic and Native). The levels within our factors that best explain the variation in our data are Preserved and Native. See Table 3 for more information.

citations therein). We decided to use the number of heterospecific pollen in the stigmas as a measure of the quality of pollination service because few heterospecific grains could cause a significant difference in seed formation (Brown and Mitchell, 2001 and references therein), and because this measure may be more appropriate for the evaluation of pollination at community level (Ashman and Arceo-Gómez, 2013).

The presence of supergeneralist (and, at least for some plant species inefficient) pollinators like the ubiquitous *A. mellifera* (Garibaldi et al., 2013) could cause an increase in heterospecific pollen grains on stigmas. Vidal and Ramírez (2005) found that plant niches overlapped more in the presence of *A. mellifera,* and mentioned that this increase in overlapping might influence the deposition of heterospecific pollen on the flower stigmas. Santos et al. (2012) reported that honey bees monopolized the interactions within a community, and suggested that this might negatively affect the pollination service, as they are inefficient pollinators of native plants. However, it is premature to talk of negative effect of exotic pollinators in our study, because we should have data about the amount of heterospecific pollen grains deposited on stigmas by only exotic pollinators.

4.1. Conclusion

Agricultural land management in the Pampean region seems to be provoking a decrease in the quality of the pollination service available to entomophilous plants through an increase in the deposition of heterospecific pollen grains on stigmas. This decrease in the quality of the pollination service would mainly affect native plants species. However, agricultural land management lead to an increase in the abundance of flower/inflorescence of exotic species and these could affect pollen deposition on the stigmas. On the other hand, the distribution of exotic plant in the landscape and their association with exotic pollinators (e.g., *A. mellifera*), could

cause negative effects on plants pollination. Further studies should evaluate to what degree the quality of the pollination service, in particular the deposition of heterospecific pollen, affects plant fitness at the community level. There are reports of negative, neutral, and even positive effects, but the data are either restricted to isolated species or to small groups of species within a community (Morales and Traveset, 2008; and citations therein).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2015.10.024.

References

Aizen, M.A., Morales, C.L., Morales, J.M., 2008. Invasive mutualists erode native pollination webs. PLoS Biol. O6, e31.
Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosyst. Environ. 74, 19–31.
Ashman, T.L., Arceo-Gómez, G., 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. Am. J. Bot. 100, 1061–1070.
Bascompte, J., Jordano, P., 2007. Plant–animal mutualistic networks: the architecture of biodiversity. Annu. Rev. Ecol. Evol. Syst. 38, 567–593.
Bjerknes, A.L., Totland, Ø., Hegland, S.J., Nielsen, A., 2007. Do alien plant invasions really affect pollination success in native plant species? Biol. Conserv. 138, 1–12.
Brown, B.J., Mitchell, R.J., 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129, 43–49.
Chittka, L., Schürkens, S., 2001. Successful invasion of a floral market—an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411, 653.
Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhöffer, J.H., Freitas, B. M., Ghazoul, J., Greenleaf, S., Hipólito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K.M., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlöf, M., Seymour, C.L., Schüepp, C., Szentgyörgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N., Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey-bee abundance. Science 339, 1608–1611.
González-Roglich, M., Southworth, J., Branch, L.C., 2012. The role of private lands for conservation: land cover change analysis in the Caldenal savanna ecosystem, Argentina. Appl. Geogr. 34, 281–288.
Grass, I., Berens, D.G., Peter, F., Farwing, N., 2013. Additive effects of exotic plant abundance and land-use intensity on plant–pollinator interactions. Oecologia 173, 913–923.
Krauss, J., Bommarco, R., Guardiola, M., Heikkinen, R.K., Helm, A., Kuussaari, M., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., Pöyry, J., Raatikainen, K.M., Sang, A., Stefanescu, C., Teder, T., Zobel, M., Steffan-Dewenter, I., 2010. Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. Ecol. Lett. 13, 597–605.
Lentini, P.E., Martin, T.G., Gibbons, P., Fischer, J., Cunningham, S.A., 2012. Supporting wild pollinators in a temperate agricultural landscape: Maintaining mosaics of natural features and production. Biol. Conserv. 149, 84–92.
Marrero, H.J., Torretta, J.P., Medan, D., 2014. Effect of land use intensification on specialization in plant–floral visitor interaction networks in the Pampas of Argentina. Agric. Ecosyst. Environ. 188, 63–71.
Morales, C.L., Traveset, A., 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. Crit. Rev. Plant Sci. 27, 221–238.
Morales, C.L., Traveset, A., 2009. A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecol. Lett. 12, 716–728.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria http://www R-project.org/.

Ramírez, N., 2005. Temporal overlap of flowering species with the same pollinating agent class: the importance of habitats and life forms. Int. J. Bot. 1, 27–33.

Sala, O.E., Chapin III, S.F., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.

Santos, G.M.M., Aguiar, C.M.L., Genini, J., Martins, C.F., Zanella, F.C.V., Mello, M.A.R., 2012. Invasive africanized honeybees change the structure of native pollination networks in Brazil. Biol. Invasions 14, 2369–2378.

Torretta, J.P., Poggio, S.L., 2013. Species diversity of entomophilous plants and flower-visiting insects is sustained in the field margins of sunflower crops. J. Nat. Hist. 47, 139–165.

Vázquez, D.P., Aizen, M.A., 2004. Asymmetric specialization: a pervasive feature of plant-pollinator interactions. Ecology 85, 1251–1257.

Vidal, M.C., Ramírez, N., 2005. Especificidad y nicho de polinización de especies de plantas de un bosque deciduo secundario. Ecotropicos 18, 73–88.

Woods, T.M., Jonas, J.L., Ferguson, C.J., 2012. The invasive Lespedeza cuneata attracts more insect pollinators than native congeners in tallgrass prairie with variable impacts. Biol. Invasions 14, 1045–1059.

Zarlavsky, G.E., 2014. Histología vegetal: técnicas simples y complejas. 1° ed., Soc. Argen. Bot. Buenos Aires, Argentina, 198 pp.