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Venâncio, Henrique; Bianchi, Renata Alexandre; Lobato, Thaís Oliveira Santos; Sampaio, Marcus Vinicius; Santos, Jean Carlos

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ECOLOGY

Tritrophic interaction between the Mexican sunflower, the aphid *Aphis gossypii* and natural enemies in a greenhouse experiment

Henrique Venâncio[1*], Renata Alexandre Bianchi[2], Thaís Oliveira Santos Lobato[3], Marcus Vinicius Sampaio[4] and Jean Carlos Santos[5]

[1]Programa de Pós-Graduação em Entomologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-900, Vila Monte Alegre, Ribeirão Preto, São Paulo, Brazil. [2]Faculdade de Tecnologia de Franca, Universidade de Franca, Franca, São Paulo, Brazil. [3]Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil. [4]Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil. [5]Departamento de Ecologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, Sergipe, Brazil. *Author for correspondence. E-mail: henrivens@gmail.com

ABSTRACT. The establishment of invasive plants negatively affects natural environments. Invasive herbivores that attack weeds can be used as a form of biological control, but natural enemies of herbivores must be associated with this interaction to prevent the invasive phytophagous from become a local pest. We performed a greenhouse experiment to evaluate how the cotton aphid, *Aphis gossypii*, a ok and invasive herbivore, affects the performance of the weed *Tithonia diversifolia*, the Mexican sunflower. We also examined the relationship between the aphid and local natural enemies. Seedlings of *T. diversifolia* were divided in two groups: one infested by the aphid and another not infested. After 22 days, we assessed the relationship between aphid abundance and the presence of natural enemies (Coccinelidae and *Aphidius platensis*) on infested plants, and compared the vegetative performance of the two seedling groups. Both natural enemies were positively related to high aphid density on infested plants. Plants infested by the aphid presented foliar necrosis and senescence, and a reduction of around 50% in leaf number, foliar area, shoot length and shoot, root and total plant weight compared to non-infested plants. These results indicate potential biological control of Mexican sunflower seedlings by the cotton aphid, and control of this aphid by the studied natural enemies.

Keywords: *Tithonia diversifolia*; Cotton aphid; *Aphidius platensis*; Coccinelidae; Biological control.

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Introduction

The introduction of non-native species is one of the main causes of economic and biodiversity loss because it modifies local community structure and decreases crop productivity and establishment (Simberloff et al., 2013). Weed management is expensive due to high tolerance to many control methods and the absence of natural enemies in invaded environments (Bezemer, Harvey, & Cronin, 2014; van Kleunen, Dawson, & Maure, 2015; Cadotte, Campbell, Li, Sodhi, & Mandrak, 2018). Due to these impacts, and the challenges associated with eradication, many studies have explored alternative ways to suppress weeds in natural and anthropized areas.

Biological control is an efficient, low cost method of weed management when it is performed in accordance to standards for the risk of biological control agents, and includes minimal impacts to non-target species (Suckling & Sforza, 2014; Myers & Cory, 2017). Specialized herbivores are preferentially used as biological control agents of invasive plants to avoid impacts to non-target species (Myers & Cory, 2017). Nonetheless, weeds can be spontaneously attacked by invasive polyphagous herbivores already present in a natural area or near crops (Norris & Kogan, 2000). Such invasive species can contribute to reducing weed populations even in the absence of others efficient control agents (van Driesche, Hoddle, & Center, 2008; Tito & Torre-Mayorga, 2016).

Natural enemies in crops and natural environments are important for the management of insect pests (Norris & Kogan, 2000; Myers & Cory, 2017). The actions of these natural enemies can reduce the risk of weed-colonizing polyphagous insects spreading to crops (Symondson, Sunderland, & Greenstone, 2002;

Myers & Cory, 2017). Concomitantly, natural enemies can be applied in isolation or in association with other management techniques to decrease an herbivore population after the control of weeds (Tavares et al., 2011).

The Mexican sunflower [*Tithonia diversifolia* Hemsl. A. Gray (Asteraceae)] is a problematic weed for crops and natural areas in portions of Africa and Asia (Ayeni, Lordbanjou, & Majek, 1997; Wang, Sun, Cheng, & Yang, 2008), and although it occurs in Brazil (Ambrósio et al., 2008), there have been no reports of losses caused by this plant. The species is tolerant to many eradication methods (Muoghalu, 2008), and few herbivores are potential biological control agents (Simelane, Mawela, & Fourie, 2011; Mphephu, Olckers, & Simelane, 2017). However, the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), attacks *T. diversifolia* (Hall & Garraway, 2013), but the effects of this herbivore on the Mexican sunflower remain unknown. *Aphis gossypii* is a cosmopolitan polyphagous insect that attacks cultivated plants and weeds (Michelotto & Busoli, 2003). It has the greatest diversity of host plants and the widest geographic distribution among aphids, attacking almost 100 cultivated plant species worldwide, causing economic losses mainly to cotton and species of Cucurbitaceae (Blackman & Eastop, 2007).

In addition to its large range of host plants, the cotton aphid is preyed upon by many free-living insects and is host to parasitoids of the family Braconidae. (Ebert & Cartwright, 1997; Blackman & Eastop, 2007). Some groups of these natural enemies are commonly associated with *A. gossypii*: predators of the families Coccinelidae (Coleoptera), Chrysopidae (Neuroptera), Syrphidae and Cecidomyiidae (Diptera), and parasitoids of the genera *Aphelinus* Dalman (Hymenoptera: Aphelinidae) and *Lysiphlebus testaceipes* (Cresson) and the species *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae) (Bueno & Sampaio, 2009; Oliveira, Ferreira, & Sampaio, 2013; Brodeur et al., 2017; Hance, Kohandani-Tafresh, & Munaut, 2017). According to Tomanović, Petrovic, Mitrovic, and Kavallieratos (2014), *A. colemani* is a complex of three cryptic species, with the probable prevalence of *Aphidius platensis* (Brèthes) in South America. Many studies have shown that predators and parasitoids consume or parasitize large quantities of aphids quickly in commercial crops, thus preventing *A. gossypii* from becoming a local pest (van Steenis, 1992; van Steenis, 1995; Pérvez & Omkar, 2006; Vásquez, Orr, & Baker, 2006; Obrycki, Harwood, Kring, & O'Neil, 2009). Nonetheless, these relationships need to be studied in systems in which the direct or indirect effects of host plants on natural enemies are unknown.

We investigated the relationship between an infestation of *A. gossypii* on seedlings of *T. diversifolia* and the presence of natural enemies in a greenhouse experiment. More specifically, we evaluated the effects of infestation by this aphid on plant vegetative performance and abundance of natural enemies. According to the premises previously presented, since *A. gossypii* has potential for biological control of *T. diversifolia*, and can be controlled by predators, we expect that: (i) infestation by *A. gossypii* will result in low vegetative performance of *T. diversifolia* seedlings; and (ii) the abundance of natural enemies will be positively related to the abundance of *A. gossypii*.

Material and methods

Seed germination and study site

Individuals of *T. diversifolia* used in experiment were germinated from seeds collected during the summer of 2015 (between October and December) from a population located in an urban area of Uberlândia, state of Minas Gerais, Brazil (18°53'10" S, 48°13'56" W). Seeds were germinated in polyethylene pots (12 x 10 cm; 500mL) containing commercial organic substrate (Bioplant®), and maintained until the end of the study in a greenhouse located in Uberlândia (18°53'10" S, 48°15'36" W). Local illumination was provided by natural light, and the photoperiod was not controlled. Irrigation was performed homogeneously by an automatic system activated for five minutes four times daily. There were no plant species other than *T. diversifolia* in the greenhouse during the experiment.

Aphid infestation and plant groups

Individuals of *Aphis gossypii* spontaneously started colonization in the greenhouse two weeks after seeds were planted, at which time 168 seedlings had germinated and all had two or more pairs of expanded leaves. Only a small group of seedlings (~ 20 individuals) that were located next to each other had aphids (~ 10 aphids per individual) visible to the naked eye (*i.e.*, third instar or later stages). After this initial attack, we assessed all the leaves, shoots, soil and pots of the other plants with a handheld magnifying glass to isolate individuals that were not attacked by aphids. A total of 53 seedlings had no aphids present, which were reallocated to a stand 6-m away from the attacked plants. Since aphids of early instars could still be present in these apparently non-attacked plants, no effort was made to establish any type of physical isolation between the plants and the environment (*e.g.*, anti-aphid screen) so that natural enemies could settle on these plants.

Two weeks after colonization the number of aphids increased dramatically on the initially-attacked plants (~ 100 individuals per plant). All individuals of this group hosted apterous *A. gossypii* of many instars and winged individuals. Winged individuals of this species occur in high population densities, which facilitates dispersal to other plants (Ebert & Cartwright, 1997). Ladybug larvae (Coleoptera: Coccinellidae) and aphids parasitized by braconid wasps (*i.e.*, round form and straw yellow coloration) (Kavallieratos & Lykouressis, 2004) were present on the infested seedlings.

Due to the differences of the number of aphids between the two seedlings groups, we categorized *T. diversifolia* individuals with high densities of *A. gossypii* and natural enemies as the "infested group", and plants with no visible aphids as the "non-infested group". We kept these plant groups spatially separated until the end of experiment and did not perform any method to interfere with the attack of *A. gossypii* and other arthropods on the plants.

Approximately four weeks after the beginning of the treatments some infested seedlings started to exhibit signs of mortality (*i.e.*, presented only senescent leaves), at which time the experiment was terminated — 22 days after the initial attack of aphids.

Data collection

Thirty random seedlings of each of the two groups (60 seedlings in total) were used for collecting data on vegetative variables and the aphids, natural enemies and other insects present.

To determine arthropod abundance, including *A. gossypii* and its natural enemies, we collected the two most apical and expanded leaves of each seedling and placed them in Petri dishes, which were labeled and sealed with adhesive tape to prevent any arthropods from escaping. The Petri dishes were frozen for 48 hours at -15°C to kill any arthropods present without damaging morphological structures. Quantification and identification of arthropods was done using a stereomicroscope (Opton TIM-2B; optic zoom: 10–160x). Aphids and parasitoids were identified to species, while ladybugs were identified to the level of family.

We measured above and below ground vegetative structures of seedlings to compare how the two levels of aphid infestation affected the performance of *T. diversifolia*. We measured shoot length (cm) with a metric tape and counted the number of leaves for each seedling of each group. We scanned (600 dpi) the two previously-collected leaves and measured their area (mm^2) using ImageJ software (Schneider, Rasband, & Eliceiri, 2012); seedling leaf area was calculated as the mean of the areas of two leaves. We also dried the aerial and radicular portions of each seedling in a stove (50°C for 48 hours) to determine shoot and root dry weights (g), which were then used to determine the root-to-shoot ratio.

Statistical analysis

Normality of the data was assessed by the Kolmogorov-Smirnov test ($p < 0.05$) and, when necessary, residual homoscedasticity was analyzed graphically. Simple linear regression was applied to determine the relationship between aphid abundance (independent variable) and quantity of natural enemies (dependent variable). Since natural enemies of *T. diversifolia* were absent from the non-infested plants, the relationship between natural enemies and aphids was analyzed using only the infested group. We used the Student t-test for independent samples to compare foliar area, shoot length, shoot weight, root weight and total weight of infested and not infested groups. Due to the data being non-normal, we used the Mann-Whitney non-parametric test to compare the number of leaves and the root-to-shoot ratio between groups. All analyses were executed in SYSTAT 13 (2009).

Results and discussion

Vegetative performance

The high density of *A. gossypii* caused infested seedlings to present visually-apparent reduced vegetative performance relative to seedlings of the non-infested group (Figure 1a). Damage from the aphids also caused several necroses along apical leaf blades of infested plants, characterizing foliar senescence (Figure

1b). These impacts were also reflected in approximately 50% higher values for leaf number, leaf area, stem length and stem, root and total plant weight in non-infested than infested *T. diversifolia* (Table 1).

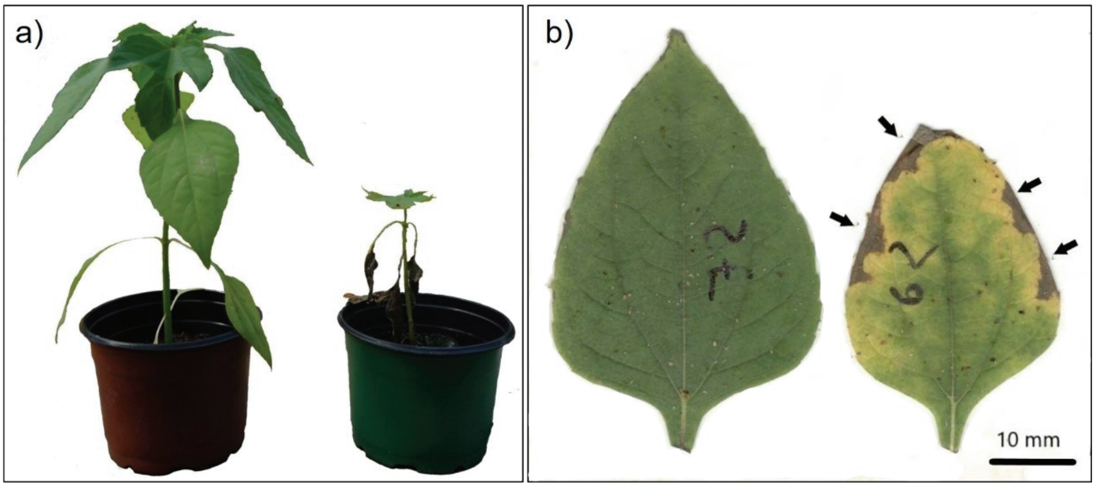

Figure 1. Vegetative characteristics of *Tithonia diversifolia* seedlings on different levels of infestation by *Aphis gossypii* in greenhouse. (a) Habitus of non-infested seedling (left) and infested seedling (right); and (b) leaf of non-infested individual (left) and leaf of infested seedling (right) with black arrows indicating marks of necrosis.

Table 1. Vegetative performance of *T. diversifolia* seedlings infested and non-infested by *Aphis gossypii*. Quantitative values are presented as mean ± standard error ($\bar{X}$±EP).

Variables	Non-infested (n = 30)	Infested (n = 30)	d.f.	Test value	*p*-value
Number of leaves	8.87 ± 0.21	5.3 ± 0.20	-	*U* = 22.50	<0.001
Leaf area (mm^2)	1484.15 ± 57.3	617.6 ± 24.90	58	*t* = 13.87	<0.001
Stem height (cm)	13.43 ± 0.43	8.4 ± 0.21	58	*t* = 10.46	<0.001
Shoot weight (g)	0.45 ± 0.03	0.22 ± 0.01	58	*t* = - 6.88	<0.001
Root weight (g)	0.24 ± 0.02	0.17 ± 0.01	58	*t* = 43.02	0.004
Total plant weight (g)	0.69 ± 0.05	0.39 ± 0.02	58	*t* = - 6.05	<0.001
Root-to-shoot ratio	0.56 ± 0.05	0.83 ± 0.06	-	*U* = 676	<0.001

The density of *A. gossypii* achieved in the present study is likely due to the high nutritional quality and ineffective defense of *T. diversifolia*. Many invasive plants allocate macronutrients to vegetative structures for growth and reproduction (Gioria & Osborne, 2014). Thus, the nutritional quality of the vegetative organs of this weed (e.g., carbohydrates and nitrogen) can increase developmental and reproductive rates of herbivores, thus leading to increased abundance of *A. gossypii* (Hosseini et al., 2010).

Aphis gossypii negatively affected vegetative performance of infested seedlings. When feeding, these aphids suck sap from the plant and inject saliva and other compounds into the hosts, thereby altering its physiological processes. As a result, aphid feeding is facilitated and the nutritional quality of the host plant is increased (Michaud, Zhang, & Bain, 2017). Aphids can remove large quantities of macronutrients, such as nitrogen, by sucking sap from host plants (Guerrieri & Digilio, 2008). In addition, they cause degradation of chlorophyll and increase the nitrogen content of leaves due to substances injected with saliva, which increases the nutritional quality of the plant for the aphid (reviewed by Michaud et al., 2017). Thereby, displacement and retention of these nutrients may occur in other vegetative parts of host plants (Goggin, 2007). Aphids can also inject substances in plants that reduce photo-assimilates by concentrating free amino acid content at the site of feeding (reviewed by Michaud et al., 2017). The nutritional manipulation of hosts by aphids can result in cell death (Goggin, 2007), which would explain the marks of necrosis observed on the leaves of *T. diversifolia* in the present study. Alternatively, individuals of this plant species can induce early leaf senescence as an induced defense to limit resource availability for *A. gossypii* (Goggin, 2007).

In relation to plant defense, Ambrósio et al. (2008) reported that leaves of *T. diversifolia* have glandular trichomes with secondary anti-herbivory compounds. Although similar structures inhibit aphids (Guerrieri & Digilio, 2008), they might not prevent the attack of the cotton aphid on some plants (Zarpas, Margaritopoulos, Stathi, & Tsitsipis, 2006; Leite, Picanço, Zanuncio, & Gusmão, 2007). Increased mortality of initial instars (Soglia, Bueno, & Sampaio, 2002) and decreased fecundity (Soglia, Bueno, Rodrigues, &

Sampaio, 2003) of *A. gossypii* were observed to be related to increased density of trichomes of another species of Asteraceae, *Dendranthema grandiflora* Tzvelev. Therefore, foliar trichomes and chemical defenses of *T. diversifolia* can reduce herbivory by this aphid, but are insufficient to prevent it. Future studies should evaluate which characteristics of the Mexican sunflower reduce *A. gossypii* infestation, since other environmental variables, such as temperature (Ebert & Cartwright, 1997; Soglia et al., 2003; Soglia et al., 2002; Zamani, Talebi, Fathipour, & Baniameri, 2006), also influence the population growth of this aphid.

We observed that infestation by *A. gossypii* can drastically limit the energy allocated by *T. diversifolia* seedlings to vegetative growth, resulting in low performance. It is worth mentioning, however, that vegetative growth of Mexican sunflower seedlings may have been limited in the present study by the size of the pots in which they were growing, thus enhancing the effects of the aphid attack on the growth variables of the analyzed seedlings. Furthermore, the environmental conditions of the greenhouse may have been favorable for population growth by polyphagous insects with high potential of population growth, such as *A. gossypii* (Brødsgaard & Albajes, 1999), thereby enhancing the effects of *A. gossypii* on the vegetative growth of *T. diversifolia*. Nevertheless, our results strongly evidence the effects of this aphid on the plant.

Aphids and natural enemies

A total of 28,141 individuals of *A. gossypii* were present on the collected leaves of infested *T. diversifolia*, with a mean of 938 ± 61 aphids per seedling (χ±EP, n = 30 seedlings). No aphids were found on seedlings of the non-infested group, which reinforces the discrepancy in aphid presence between the two groups of plants and justifies considering them as different levels of infestation.

No organisms other than *A. gossypii*, ladybugs (Coleoptera: Coccinellidae) and *A. gossypii* parasitized (mummified) by *A. platensis* (Braconidae: Aphidiinae) were present on the infested plants. Of these, there were 118 ladybug larvae and 440 individuals of *A. platensis* parasitizing aphids. The abundance of aphids was positively and significantly related to the number of ladybug larvae ($p < 0.001$) (Figure 2a) and mummified aphids ($p = 0.01$) (Figure 2b).

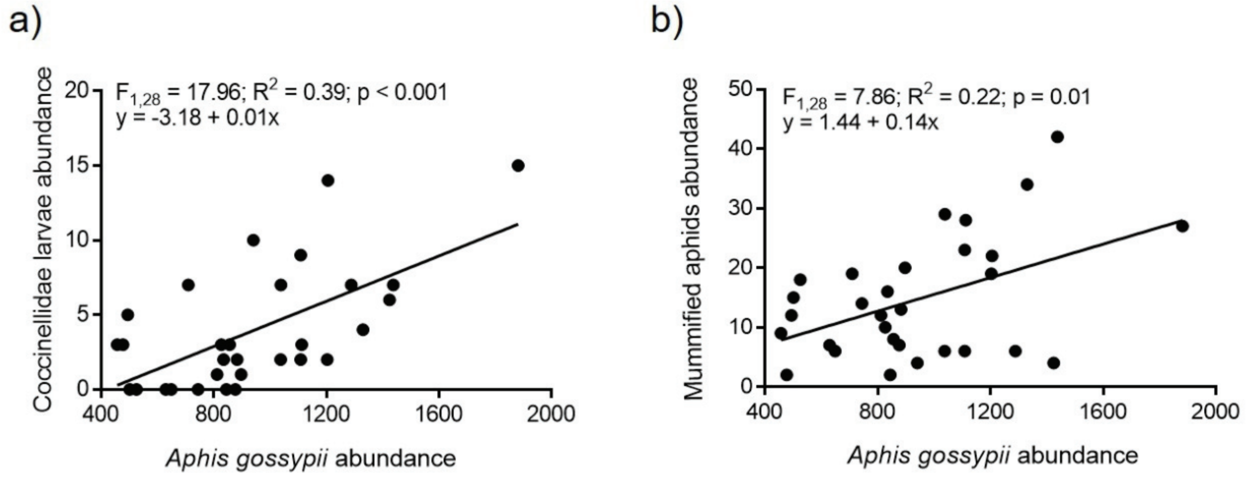

Figure 2. Abundance of (a) Coccinelidae larvae and (b) mummified aphids by *Aphidius platensis* in relation to the abundance of *Aphis gossyppi* infesting *Tithonia diversifolia* in a greenhouse.

Our results demonstrated that populations of *A. gossypii* can be regulated by ladybugs and *A. platensis*. The joint action of these two natural enemies results in high consumption of aphids in a short amount of time, thus characterizing them as good agents for biological control of the cotton aphid in plantations (Ebert & Cartwright, 1997; Kavallieratos, Stathas, & Tomanovic, 2004). In our study, however, the cotton aphid achieved a high population density, even in the presence of these natural enemies. Although the attack rate of the parasitoid *A. platensis* can be estimated by the number of mummies formed, the experiment was short (only 22 days), and consumption of aphids by the predatory ladybugs was not directly observed. These limitations prevented us from observing the actual efficacy of the biological control of the cotton aphid on *T. diversifolia*.

Conclusion

Our study showed that Mexican sunflower seedlings experienced limited vegetative growth due to the cotton aphid, which may contribute to controlling this invasive plant. At the same time, we showed that this aphid is itself potentially controlled by its natural enemies. The relationship between weed, invasive herbivore and natural enemies of the present study should be investigated in other environments with the use of protective measures and biological control safety protocols. Such investigations may verify the applicability of this relationship in places invaded by *T. diversifolia*, and where *A. gossypii* is not a threat to local plants.

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References

Ambrósio, S. R., Oki, Y., Heleno, V. C., Chaves, J. S., Nascimento, P. G., Lichston, J. E., ... Costa, F. B. (2008). Constituents of glandular trichomes of *Tithonia diversifolia*: relationships to herbivory and antifeedant activity. *Phytochemistry, 69*(10), 2052-2060. doi: 10.1016/j.phytochem.2008.03.019

Ayeni, A. O., Lordbanjou, D. T., & Majek, B. A. (1997). *Tithonia diversifolia* (Mexican sunflower) in south-western Nigeria: occurrence and growth habit. *Weed Research, 37*(6), 443-449. doi: 10.1046/j.1365-3180.1997.d01-72.x

Bezemer, T. M., Harvey, J. A., & Cronin, J. T. (2014). Response of native insect communities to invasive plants. *Annual Review of Entomology, 59*(1), 119-141. doi: 10.1146/annurev-ento-011613-162104

Blackman, R. L., & Eastop, V. F. (2007). Taxonomic issues. In H. F van Emden & R. Harrington (Eds.), *Aphids as crop pests* (p. 1-30). Wallingford, UK: CABI.

Brodeur, J., Hajek, A. E., Heimpel, G. E., Sloggett, J. J., Mackauer, M., Pell, J. K., & Völkl, V. (2017). Predators, parasitoids and pathogens. In H. F. van Emden & R. Harrington (Eds.), *Aphids as crop pests* (2a ed., p. 225-260). Boston, MA: CABI.

Brødsgaard, H. F., & Albajes, R. (1999). Insect and mite pests. In R. Albajes, M. L. Gullino, J. C. van Lenteren & Y. Elad (Eds.), *Integrated pest and disease management in greenhouse crops* (p. 48-60). Dordrecht, NL: Springer.

Bueno, V. H. P., & Sampaio, M. V. (2009). Desenvolvimento e multiplicação de parasitóides de pulgões. In V. H. P. Bueno (Ed.), *Controle biológico de pragas: produção massal e controle de qualidade* (2a ed., p. 117-168). Lavras, MG: UFLA.

Cadotte, M. W., Campbell, S. E., Li, S.-P., Sodhi, D. S., & Mandrak, N. E. (2018). Preadaptation and naturalization of nonnative species: Darwin's two fundamental insights into species invasion. *Annual Review of Plant Biology, 69*(1), 661-684. doi: 10.1146/annurev-arplant-042817-040339

Ebert, T. A., & Cartwright, B. (1997). Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). *Southwestern Entomologist, 22*(1), 116-153.

Gioria, M., & Osborne, B. A. (2014). Resource competition in plant invasions: emerging patterns and research needs. *Frontiers in Plant Science, 5*, 501. doi: 10.3389/fpls.2014.00501

Goggin, F. L. (2007). Plant–aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology, 10*(4), 399-408. doi: 10.1016/j.pbi.2007.06.004

Guerrieri, E., & Digilio, M. C. (2008). Aphid-plant interactions: a review. *Journal of Plant Interactions, 3*(4), 223-232. doi: 10.1080/17429140802567173

Hall, T A., & Garraway, E. (2013). The aphids (Hemiptera: Aphididae) of Jamaica, their hosts, predators, parasitoids and other associates. *Caribbean Journal of Science, 47*(2-3), 305-324. doi: 10.18475/cjos.v47i3.a19

Hance, T., Kohandani-Tafresh, F., & Munaut, F. (2017). Biological Control. In H. F van Emden & R. Harrington (Eds.), *Aphids as crop pests* (p. 448-492). Boston, MA: CABI.

Hosseini, M., Ashouri, A., Enkegaard, A., Goldansaz, S. H., Mahalati, M. N., & Hosseininaveh, V. (2010). Performance and population growth rate of the cotton aphid, and associated yield losses in cucumber, under different nitrogen fertilization regimes. *International Journal of Pest Management, 56*(2), 127-135. doi: 10.1080/09670870903248827

Kavallieratos, N. G., & Lykouressis, D. P. (2004). The coloration of *Aphis gossypii* mummies as a useful tool for Aphidiinae parasitoid identification (*Hymenoptera: Braconidae*). *Israel Journal of Entomology, 34*, 75-82.

Kavallieratos, N. G., Stathas, G. J., & Tomanovic, Z. (2004). Seasonal abundance of parasitoids (Hymenoptera: Braconidae, Aphidiinae) and predators (Coleoptera: Coccinellidae) of aphids infesting citrus in Greece. *Biologia – Section Zoology, 59*(2), 191-196.

Leite, G. L. D., Picanço, M., Zanuncio, J. C., & Gusmão, M. R. (2007). Factors affecting colonization and abundance of *Aphis gossypii* Glover (Hemiptera: Aphididae) on okra plantations. *Ciência e Agrotecnologia, 31*(2), 337-343. doi: 10.1590/S1413-70542007000200011

Michaud, J. P., Zhang, Y., & Bain, C. (2017). Feeding by *Melanaphis sacchari* (Hemiptera: Aphididae) facilitates use of sorghum by *Rhopalosiphum padi* (Hemiptera: Aphididae), but reciprocal effects are negative. *Environmental Entomology, 46*(2), 268-273. doi: 10.1093/ee/nvw167

Michelotto, M. D., & Busoli, A. C. (2003). Aspectos biológicos de *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) em três cultivares de algodoeiro e em três espécies de plantas daninhas. *Ciência Rural, 33*(6), 999-1004. doi: 10.1590/S0103-84782003000600002

Mphephu, T. E., Olckers, T., & Simelane, D. O. (2017). The tortoise beetle *Physonota maculiventris* (Chrysomelidae: Cassidinae) is suitable for release against the weedy Mexican sunflower *Tithonia diversifolia* (Asteraceae) in South Africa. *Biocontrol Science and Technology, 27*(4), 510-524. doi: 10.1080/09583157.2017.1317335

Muoghalu, J. I. (2008). Growth, reproduction and resource allocation of *Tithonia diversifolia* and *Tithonia rotundifolia*. *Weed Research, 48*(2), 157-162. doi: 10.1111/j.1365-3180.2007.00613.x

Myers, J. H., & Cory, J. S. (2017). Biological control agents: invasive species or valuable solutions? In M. Vilà & P. E. Hulme, (Eds.), *Impact of Biological Invasions on Ecosystem Services* (p. 191-202). Basel, SW: Springer.

Norris, R. F., & Kogan, M. (2000). Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Science, 48* (1), 94-158. doi: 10.1614/0043-1745(2000)048[0094:IBWAPA]2.0.CO;2

Obrycki, J. J., Harwood, J. D., Kring, T. J., & O'Neil, R. J. (2009). Aphidophagy by Coccinellidae: application of biological control in agroecosystems. *Biological Control, 51*(2), 244-254. doi: 10.1016/j.biocontrol.2009.05.009

Oliveira, R. S., Ferreira, S. E., & Sampaio, M.V. (2013). Parasitoides de pulgões na agricultura: produção massal e estratégias de aplicação em campo. In M. V. B. Figueiredo, D. M. P. Silva, J. N. Tabosa, J. Z. Brito, J. G. E. França, M. B. Wanderley, A. S. Santos-Filho, E. W. F. Gomes, G. M. B. Lopes, J. P. Oliveira, A. D. Santiago, F. G. Silva, M. I. N. Pacheco & C. C. F. Silva (Eds.), *Tecnologias potenciais para uma agricultura sustentável* (p. 303-317). Recife, PE: IPA.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671-675. doi: 10.1038/nmeth.2089

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., … Vilà, M. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution, 28*(1), 58-66. doi: 10.1016/j.tree.2012.07.013

Simelane, D. O., Mawela, K. V., & Fourie, A. (2011). Prospective agents for the biological control of *Tithonia rotundifolia* (Mill.) SF Blake and *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa. *African Entomology, 19*(2), 443-450. doi: 10.4001/003.019.0223

Soglia, M. C. M., Bueno, V. H. P., & Sampaio, M. V. (2002). Desenvolvimento e sobrevivência de *Aphis gossypii* Glover (Hemiptera: Aphididae) em diferentes temperaturas e cultivares comerciais de crisântemo. *Neotropical Entomology, 31*(2), 211-216. doi: 10.1590/S1519-566X2002000200006

Soglia, M. C. M., Bueno, V. H. P., Rodrigues, S. M. M., & Sampaio, M. V. (2003). Fecundidade e longevidade de *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) em diferentes temperaturas e cultivares comerciais de crisântemo (*Dendranthema grandiflora* Tzvelev). *Revista Brasileira de Entomologia, 47*(1), 49-54. doi: 10.1590/S0085-56262003000100007

Suckling, D. M., & Sforza, R. F. H. (2014). What magnitude are observed non-target impacts from weed biocontrol? *PLoS One, 9*(1), e84847. doi: 10.1371/journal.pone.0084847

Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology, 47*(1), 561-594. doi: 10.1146/annurev.ento.47.091201.145240

SYSTAT. (2009). *SYSTAT 13*. San Jose, CA: SYSTAT Software Inc.

Tavares, W. S., Cruz, I., Silva, R.B., Figueiredo, M. L. C., Ramalho, F. S., Serrão, J. E., & Zanuncio, J. C. (2011). Soil organisms associated to the weed suppressant *Crotalaria juncea* (Fabaceae) and its importance as a refuge for natural enemies. *Planta Daninha, 29*(3), 473-479. doi: 10.1590/S0100-83582011000300001

Tito, R., & de la Torre-Mayorga, F. (2016). Effects of an aphid pest on an invasive plant in the Peruvian Andes. *Plant Ecology, 217*(7), 817-823. doi: 10.1007/s11258-016-0596-7

Tomanović, Ž., Petrovic, A., Mitrovic, M., & Kavallieratos, N.G. (2014). Molecular and morphological variability within the *Aphidius colemani* group with redescription of *Aphidius platensis Brethes* (Hymenoptera: Braconidae: Aphidiinae). *Bulletin of Entomological Research, 104*(5), 552-565. doi: 10.1017/s0007485314000327

van Driesche, R., Hoddle, M., & Center, T. (2008). *Control of Pests and Weeds by Natural Enemies: An introduction to biological control*. Oxford, UK: Blackwell Publishing.

van Kleunen, M., Dawson, W., & Maurel, N. (2015). Characteristics of successful alien plants. *Molecular Ecology, 24*(9), 1954-1968. doi: 10.1111/mec.13013

van Steenis, Ir. M. J. (1992). Biological control of the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae): pre-introduction evaluation of natural enemies. *Journal of Applied Entomology, 114*(1-5), 362-380. doi: 10.1111/j.1439-0418.1992.tb01140.x

van Steenis, M. J. (1995). Evaluation of four aphidiine parasitoids for biological control of *Aphis gossypii*. *Entomologia Experimentalis et Applicata, 75*(2), 151-157. doi: 10.1111/j.1570-7458.1995.tb01921.x

Vásquez, G. M., Orr, D. B., & Baker, J. R. (2006). Efficacy assessment of *Aphidius colemani* (Hymenoptera: Braconidae) for suppression of *Aphis gossypii* (Homoptera: Aphididae) in greenhouse-grown *chrysanthemum*. *Journal of Economic Entomology, 99*(4), 1104-1111. doi: 10.1093/jee/99.4.1104

Wang, S.-H., Sun, W.-B., Cheng, X, & Yang, Y.-M. (2008). Reproductive characteristics of *Tithonia diversifolia* and its geographical spread in Yunnan province of South-West China. *Acta Ecologica Sinica, 28*(3), 1307-1313.

Zamani, A. A., Talebi, A. A., Fathipour, Y., & Baniameri, V. (2006). Effect of temperature on biology and population growth parameters of *Aphis gossypii* Glover (Hom., Aphididae) on greenhouse cucumber. *Journal of Applied Entomology, 130*(8), 453-460. doi: 10.1111/j.1439-0418.2006.01088.x

Zarpas, K. D., Margaritopoulos, J. T., Stathi, L., & Tsitsipis, J. A. (2006). Performance of cotton aphid *Aphis gossypii* (Hemiptera: Aphididae) lineages on cotton varieties. *International Journal of Pest Management, 52*(3), 225-232. doi: 10.1080/09670870600727305