

# Effects of arbuscular mycorrhizal fungi on above-ground tri-trophic interactions are contingent upon plant genetic effects of cross type in the perennial herb *Ruellia nudiflora*

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**Funding information**  
Consejo Nacional de Ciencia y Tecnología

Handling Editor: Brian Silliman

## Abstract

- Recent work has improved our understanding of the linkages between above- and below-ground interactions mediated by plants. However, relatively few of the studies conducted thus far have focused on multi-trophic interactions (i.e. beyond two trophic levels) and the influence of plant genetic intraspecific variation on these dynamics has rarely been addressed.
- We tested the effect of arbuscular mycorrhizal fungi (AMF) on above-ground tri-trophic interactions associated with the canopy of the perennial herb *Ruellia nudiflora*, and further determined whether genetic effects due to cross type (i.e. whether a plant originated from self- or cross-pollination) influenced these interactions.
- We propagated plants originating from self- or cross-pollination, and within each category inoculated half of the plants with AMF. We subsequently established a common garden where plants were exposed to naturally occurring seed-eating caterpillars and their parasitoids. We measured plant growth, fruit output, calculated the proportion of attacked fruits by the caterpillar and the proportion of parasitized caterpillars, and also estimated the proportion of “rescued” seeds by parasitoids representing an indirect positive effect of the third trophic level on the plant by reducing caterpillar consumption.
- Arbuscular mycorrhizal fungi drove 18% and 15% increases in plant growth and fruit output respectively, and drove a 25% reduction in caterpillar fruit attack, but did not influence parasitism or parasitoid seed “rescue.” In contrast, cross type did not influence growth, fruit number, herbivore attack, parasitism or seed rescue. More importantly, however, we found a significant AMF by cross type interaction on caterpillar attack, where AMF significantly reduced fruit attack (by 30%) in progeny from cross-pollination but did not influence herbivory in progeny from self-pollination.
- Synthesis.** Results indicate that effects of arbuscular mycorrhizal fungi on above-ground interactions are contingent upon plant intraspecific variation originating from cross type, which is likely a common source of variation in associated interactions for plants with mixed mating systems. Further studies examining plant-mediated below- and above-ground interactions should consider the influence of specific sources of plant genetic variation, as well as address the consequences of such dynamics for interactions beyond two trophic levels.

## KEY WORDS

above-ground effects, mixed mating system, plant genetic variation, selfing, tri-trophic interactions

## 1 | INTRODUCTION

Much of our understanding of species interactions has originated from studies addressing the ecological and evolutionary consequences of pairwise mutualistic or antagonistic interactions (Herrera & Pellmyr, 2002; Marquis, 1992; Parra-Tabla & Herrera, 2010; Thompson, 1999). However, these interactions are embedded within a complex community matrix where third-party species may alter focal pairwise interactions and lead to indirect effects and unexpected outcomes (Abdala-Roberts & Mooney, 2014; Strauss & Irwin, 2004). One example of non-additive dynamics stemming from multi-species interactions are plant-mediated interactions among insects, which are widespread because of the close dependency of insect communities on plant individual-, population- and community-level variation (Denno & Kaplan, 2007; Ohgushi, 2005). Much of this work has focused on understanding the ways in which plants mediate interactions among insect herbivores via induced plant responses to herbivore feeding (Ohgushi, 2005; Strauss & Irwin, 2004). Effects on herbivores can in turn have extended consequences on their natural enemies and ultimately shape entire multi-trophic communities (Campos-Navarrete, Munguía-Rosas, Abdala-Roberts, Quinto-Cánovas, & Parra-Tabla, 2015; Dicke & Vet, 1999; Poelman et al., 2009; Utsumi, Ando, Roininen, Takahashi, & Ohgushi, 2013). Recent studies have also shown that plants mediate interactions between below- and above-ground consumer communities, and that such effects are transmitted by plants in both directions (Pozo et al., 2013; Rasmann & Agrawal, 2008; Van Dam & Heil, 2011). To date, however, most studies on plant-mediated interactions have been bi-trophic in nature, ignoring more complex interactions occurring across three or more trophic levels (e.g. Abdala-Roberts & Mooney, 2014; Gange, Brown, & Aplin, 2003; Hempel et al., 2009). Recent work has shown that addressing plant-mediated interactions under a multi-trophic setting adds much needed ecological realism and moves us a step closer to understanding complex interactions and the community-level consequences of these dynamics (Barber, Kiers, Theis, Hazzard, & Adler, 2013; Bennett, Millar, Gedrovics, & Karley, 2016).

Arbuscular mycorrhizal fungi (AMF) are extremely common in natural systems and are associated with roots of 76% of terrestrial plant species world-wide (Brundrett, Bouger, Bernie, Grove, & Malajczuk, 1996). They usually act as plant mutualists and enhance plant growth and reproduction (Koricheva, Gange, & Jones, 2009), and by altering the plant's phenotype influence interactions with neighbouring plants or consumers (e.g. Bezemer & Van Dam, 2005; Van der Putten, Vet, Harvey, & Wäckers, 2001). For example, AMF root colonization has been shown to alter plant quality (defences or nutrients) and such effects frequently lead to reductions in herbivory (e.g. Bennett et al., 2016; Hoffmann, Vierheilig, Peneder, & Schausberger, 2011; Li et al.,

2016; Pozo et al., 2013). However, plant-mediated interactions involving AMF (as well as other mutualistic microbes, e.g. N-fixing bacteria) and canopy-associated consumers have usually considered dynamics occurring at two trophic levels, ignoring the extended consequences on predators and parasitoids (but see Gange et al., 2003; Hoffmann et al., 2011). A consideration of tri-trophic interactions is necessary to achieve a community-based understanding of plant-mediation of above- and below-ground communities of microbes and insects.

Another important aspect to consider in studying plant-mediation of species interactions is the influence of plant intraspecific variation (reviewed by Bailey et al., 2009; Crutsinger, 2016; Whitham et al., 2006). Over the last decade, studies have demonstrated that plant intraspecific variation plays an important part in shaping the outcome of plant-consumer interactions (Carmona & Johnson, 2016; Crutsinger, 2016; Mooney & Agrawal, 2008; Whitham et al., 2006). Most studies to date have addressed such effects by establishing common gardens composed of plants from different clones or maternal lines randomly selected from one or multiple populations (Crutsinger, 2016). However, random selection of genotypes may not always be a realistic way of evaluating how plant genetics influences interactions, and there are likely specific sources of intraspecific variation that are important but have not been explicitly addressed. One of these sources of plant genetic variation originates from whether offspring is the product of self- vs. cross-pollination ("cross type," hereafter). An extreme case of selfing is inbreeding depression, which has well-known negative effects on plant growth and reproduction that in turn affect associated consumers (reviewed by Carr & Eubanks, 2014). For example, studies have found that progeny derived from multiple generations of selfing are less defended against herbivores and less attractive to pollinators (e.g. Bello-Bedoy & Núñez-Farfán, 2010; Ivey & Carr, 2005), and establish weaker interactions with AMF (Botham, Collin, & Ashman, 2009; Collin & Ashman, 2010; Varga & Kytöviita, 2014). However, few studies on the ecological effects of plant intraspecific variation, particularly that originated from cross type, have considered effects involving both below- and above-ground interactions (but see Bennett et al., 2016; Li et al., 2016) or interactions extending beyond two trophic levels. Addressing the influence of plant genetic variation originating from cross type on multi-trophic above- and below-ground interactions can provide a better understanding of how plants mediate complex interactions between microbes and insects and the community-wide consequences of such effects. These plant genetic effects are likely widespread provided that mixed mating systems are present in more than 40% of angiosperm species (Goodwillie, Kalisz, & Eckert, 2005), and therefore deserve more attention within a community context.

Here we evaluate the effects of AMF on above-ground tri-trophic interactions associated with the perennial herb *Ruellia nudiflora*

Engelm. & Gray Urb. (Acanthaceae), and further test for the contingency of such effects upon plant cross type. *Ruellia nudiflora* is a cleistogamous species with a mixed mating system and therefore the production of autogamous and exogamous progeny is common in populations of this plant (Munguía-Rosas, Parra-Tabla, Ollerton, & Cervera, 2012). In addition, its roots are frequently colonized by several species of AMF that enhance plant growth and survival (Ramos-Zapata, Campos-Navarrete, Parra-Tabla, Abdala-Roberts, & Navarro-Alberto, 2010). These conditions provide an ideal scenario to test for the bottom-up effects of AMF on above-ground interactions and whether plant cross type determines the outcome of these dynamics. To address this, we established a common garden experiment with progeny from self- or cross-pollination with and without AMF, and measured the individual and interactive effects of AMF and cross type on fruit attack by a specialist seed-eating caterpillar as well as attack by parasitic wasps attacking this herbivore. Specifically, we sought to answer the following: (1) Is there an effect of AMF on plant-caterpillar and caterpillar-parasitoid interactions? (2) Are such effects contingent upon the cross type (self- vs. cross-pollination) that originated the offspring, such that linkages between below- and above-ground interactions cannot be fully understood without the consideration of this source of plant genetic variation? We predicted that AMF would reduce fruit attack by the seed predator (see Hoffmann et al., 2011; Pozo et al., 2013) and this would in turn reduce parasitism (see Gange et al., 2003; Pozo et al., 2013), but that such effects would be stronger for progeny from cross-pollination because plants originating from this cross type typically exhibit stronger interactions with AMF than offspring from self-pollination (Botham et al., 2009; Collin & Ashman, 2010). By addressing these questions, the present study builds towards a better understanding of how plant genetics determines the linkage between above- and below-ground interactions between microbes and insects, and moves a step closer to addressing the community-wide consequences of such effects by addressing interactions beyond two trophic levels.

## 2 | MATERIALS AND METHODS

### 2.1 | Study species

*Ruellia nudiflora* is distributed from southern Texas (USA) to northern Honduras (Abdala-Roberts, Moreira, Rasmann, Parra-Tabla, & Mooney, 2016; Ortegón-Campos, Parra-Tabla, Abdala-Roberts, & Herrera, 2009), and it is usually found growing in open and disturbed sites (Munguía-Rosas et al., 2012). Most leaves grow near ground level, forming a sort of rosette at the base of the plant. This species produces cleistogamous (CL) flowers, which do not open and undergo obligate self-pollination as well as chasmogamous (CH) flowers, which have open corollas, are visited by pollinators, and undergo cross-pollination. CH flowers also frequently exhibit automatic self-pollination upon corolla dehiscence (Abdala-Roberts, Marrufo-Zapata, Arceo-Gómez, & Parra-Tabla, 2014). Fruits are dry and dehiscent capsules, and seeds are dispersed ballistically (Munguía-Rosas et al., 2012). In the Yucatan Peninsula (Mexico), *R. nudiflora* is associated

with AMF (e.g. *Acaulospora* and *Glomus*), which increase plant growth and survival (Parra-Tabla, Munguía-Rosas, Campos-Navarrete, & Ramos-Zapata, 2016; Ramos-Zapata et al., 2010). In addition, both types of fruits are attacked by larvae of a seed-eating moth (*Tripudia paraplesia*, Noctuidae; Pogue, 2009) which specializes on the genus *Ruellia* (Abdala-Roberts et al., 2016). The entire larval stage occurs within a single fruit, only one larva is found per attacked fruit, and a single, unparasitized larva consumes all seeds within a fruit (Abdala-Roberts & Mooney, 2014). In turn, caterpillars are attacked by several species of parasitic wasps belonging to *Braconidae*, *Ichneumonidae* and *Pteromalidae*, which reduce or stop seed consumption by the caterpillar. As a result, attacked fruits where the caterpillar was parasitized usually have remaining seeds that are not consumed ("rescued" seeds hereafter) by the herbivore which represent a precise measure of the indirect (positive) effect of parasitoids on plant fitness (Abdala-Roberts & Mooney, 2014; Abdala-Roberts, Parra-Tabla, Salinas-Peña, Díaz-Castelazo, & Delfín-González, 2010; Cuautle & Parra-Tabla, 2014).

### 2.2 | Seed germination and genetic families

In late July 2015, we selected 10 adult plants ("maternal plants" hereafter) from a population located at the Campus de Ciencias Biológicas y Agropecuarias of the Universidad Autónoma de Yucatán, México (CCBA - UADY; 20°51'59.49"N, 89°37'31.36"W). These maternal plants exhibited similar vegetative (e.g. size, number of leaves) and reproductive (e.g. number of flowers, fruits) characteristics, and distance between plants was at least 10 m to maximize within-population variation (Abdala-Roberts & Mooney, 2014). Plants were carefully unearthed and planted in 2-L pots inside a nursery. To generate the two types of progeny based on cross type (self- vs. cross-pollination), for each plant we manually pollinated 10 CH flowers: five flowers were pollinated with pollen of the same flower and five flowers were pollinated with a random mixture of pollen from multiple parents. We did not use CL fruits to generate progeny from self-pollination because CL and CH fruits vary in traits such as seed number, seed germination rate and seedling vigour (Munguía-Rosas, Campos-Navarrete, & Parra-Tabla, 2013; Munguía-Rosas, Parra-Tabla, & Montiel, 2013). This variation might result in differences in plant performance (and associated interactions) relative to cross-CH progeny that are not due to cross type, but rather to differences in resource allocation between fruit types (i.e. we only used CH fruits to maintain fruit type constant and avoid confounding effects of fruit type and cross type).

### 2.3 | AMF treatment and experimental design

To propagate AMF, in August 2014 we collected soil and root samples from the same site where the maternal plants were found. First, we sterilized soil samples and then mixed roots and sterilized soil in a proportion of 1:1. AMF were propagated using these soil samples based on the mixed crop technique using different plant species as trap plants (e.g. *Zea mays*, *Sorghum vulgare* and *Phaseolus vulgaris*) during a whole year previous to inoculation (Brundrett et al., 1996).

Arbuscular mycorrhizal fungi spores present in the inoculum were obtained using the extraction technique of Gerdemann and Noholson (1963). We mounted semipermanent spore preparations using polyvinyl alcohol and Melzer solution for observation under an optic microscope. Taxonomic identification of AMF spores was conducted using the International Culture Collection of (Vesicular) AMF and the website of the University of West Virginia (<http://www.agro.ar.szczezin.pl/~jblaszkowski/Introduction.html>). Based on these resources, the AMF species identified in the inoculum were: *Claroideoglomus claroides*, *Funneliformis geosporum*, *Ambispora gerdemannii*, *Glomus mosseae*, *Glomus caesaris*, *Acaulospora scrobiculata*, *Acaulospora laevis* and *Acaulospora morrowiae*.

We placed 50 ml of the AMF inoculum (mixture of AMF species mentioned above) into each 1-L pot (bags of 1 kg capacity) with sterile soil where half the seeds of each parent plant (AMF+) would be individually planted, whereas the other half of the pots where control seeds would be planted were not inoculated (AMF-). In November 2015, once seedlings had four true leaves, we moved them to a common garden located in an open area designated for field experiments at the CCBA-UADY (20°51'58.42"N, 89°37'20.62"W). Plants were individually sowed in each pot and plant positions were randomly allocated throughout the common to guarantee that treatments were interspersed and every plant had the same probability of being visited by seed predators and parasitoids. Plants were placed at a density of four plants per m<sup>2</sup>, which is within the typical range of intraspecific plant density in *R. nudiflora* populations (Vargas-Mendoza, Ortegón-Campos, Marrufo, Herrera, & Parra-Tabla, 2015). The experiment therefore represented a two by two factorial design with AMF inoculation (fixed, two levels: AMF+ and AMF-) and cross type (fixed, two levels: self- and cross-pollination) as main effects. In total, we used 379 plants in the experiment, of which 190 corresponded to the self-pollination treatment (101 AMF+ and 89 AMF+) and 189 to the cross-pollination treatment (103 AMF+ and 86 AMF-). All plants were watered every 2 days throughout the experiment (July 2015–March 2016).

## 2.4 | Response variables

### 2.4.1 | *Ruellia nudiflora* growth and reproduction

In early December 2015, we estimated plant cover as a proxy of plant size, calculated as the product of the largest diameter from leaf tip to tip by the diameter perpendicular to the former (Ortegón-Campos et al., 2009). In addition, from December 2015 to March 2016, twice a week we collected all mature fruits per plant. More than 90% of the fruits produced were CL, a typical pattern of first year plants of *R. nudiflora* which rely heavily on CL reproduction (Munguía-Rosas et al., 2012).

### 2.4.2 | Seed predation and parasitism

Fruits were dissected in the laboratory and examined under a stereoscopic microscope to determine if they were attacked by the

caterpillar and if so, whether the caterpillar was parasitized (Abdala-Roberts et al., 2010; Ortegón-Campos et al., 2009). Herbivore attack is easily identified based on the presence of the larva, frass and/or an exit hole which the caterpillar makes to leave the fruit prior to pupating (Abdala-Roberts et al., 2010). Likewise, parasitoid attack is also easily identified based on the presence of the adult parasitoid (before it exits the fruit) or cocoons (prior to adult emergence). For the statistical analyses, we calculated the proportion of caterpillar-attacked fruits (number of fruits attacked by caterpillar/total number of fruits produced) and the proportion of parasitized caterpillars (number of fruits attacked by caterpillar with a parasitoid/number of fruits attacked by caterpillar) for each plant. We also counted the number of seeds remaining in fruits with parasitized caterpillars and calculated the proportion of "rescued" seeds as: remaining seeds in fruits with parasitized caterpillars/potential number of seeds produced in fruits attacked by the herbivore (Abdala-Roberts & Mooney, 2014). To estimate the potential number of seeds produced in attacked fruits, we used the average number of seeds per fruit (specific to each genetic family) from fruits not attacked by the herbivore. Fruit size is correlated with seed number and previous work has shown that there is no difference in size between attacked fruits and fruits not attacked by the caterpillar. This indicates that using the mean number of seeds per fruit from fruits that were not attacked is a reasonable estimate of the hypothetical or potential seed number in attacked fruits had they not been attacked and their seeds consumed (Abdala-Roberts et al., 2014). We estimated the proportion of saved seeds per fruit and then averaged these proportions across fruits per plant for statistical analysis.

## 2.5 | Statistical analyses

We tested for the effects of AMF, cross type, and their interaction on plant cover, fruit number, the proportion of attacked fruits, the proportion of parasitized caterpillars and the proportion of rescued seeds by parasitoids using general and generalized linear mixed models in R 3.3.0 (R Core Team, 2014). To assess the influence of plant size on seed predator attack, we ran the model for the proportion of attacked fruits including plant cover as a covariate. In each of these models, we also included maternal plant as a random factor to account for residual variation among maternal families not due to cross type (i.e. residual genetic variation or maternal effects). For each model, we chose the most adequate error distribution and link function based on previous inspection of the data and the type of data in each case (e.g. counts, proportions); for plant cover we used a normal distribution (identity as link), for fruit number we used a Poisson distribution (log link), and for the proportion of attacked fruits, parasitized caterpillars, and rescued seeds we used a binomial distribution (logit link). For plant cover, we used the nlme package for normally distributed data; for fruit number, herbivore attack and parasitism, we used the matrix and LME4 packages, respectively; and for the proportion of rescued seeds, we used the MASS and glmmADMB packages to account for inflation of zeros. Whenever the AMF by cross type interaction was significant, we performed

**TABLE 1** Results from generalized linear mixed models testing for the effects of arbuscular mycorrhizal fungi (AMF, with and without), and cross type (CT, self- vs. cross-pollination) on *Ruellia nudiflora* growth (cover, per cm<sup>2</sup>), reproduction (fruit number), above-ground interactions (proportion of attacked fruits by a seed-eating caterpillar and proportion of caterpillars parasitized by wasps), and indirect effects of parasitic wasps on seed output (proportion of “rescued” seeds, see Section 2). Significant ( $p < .05$ ) results are in bold

Response variable/ factor	AMF		Cross type		AMF x CT		
	Statistics	F/Z-value	p	F/Z-value	p	F/Z-value	p
Growth		$F_{366} = 12.32$	.0005	$F_{366} = 1.20$	.27	$F_{366} = 1.91$	.16
Fruit number		$Z_{374} = -2.60$	.009	$Z_{374} = -0.46$	.64	$Z_{374} = 0.01$	.98
Proportion of attacked fruits		$Z_{374} = -2.09$	.03	$Z_{374} = 0.39$	.69	$Z_{374} = 2.29$	.02
Proportion of parasitized caterpillars		$Z_{374} = 0.88$	.3	$Z_{374} = -1.06$	.28	$Z_{374} = 0.76$	.44
Proportion of “rescued” seeds		$Z_{374} = 0.04$	.96	$Z_{374} = 0.90$	.36	$Z_{374} = -0.60$	.55

pairwise comparisons of treatment level means for a given factor within each level of the other factor using the multcomp and lsmeans options. In all cases, we present least-square means and SE as descriptive statistics.

### 3 | RESULTS

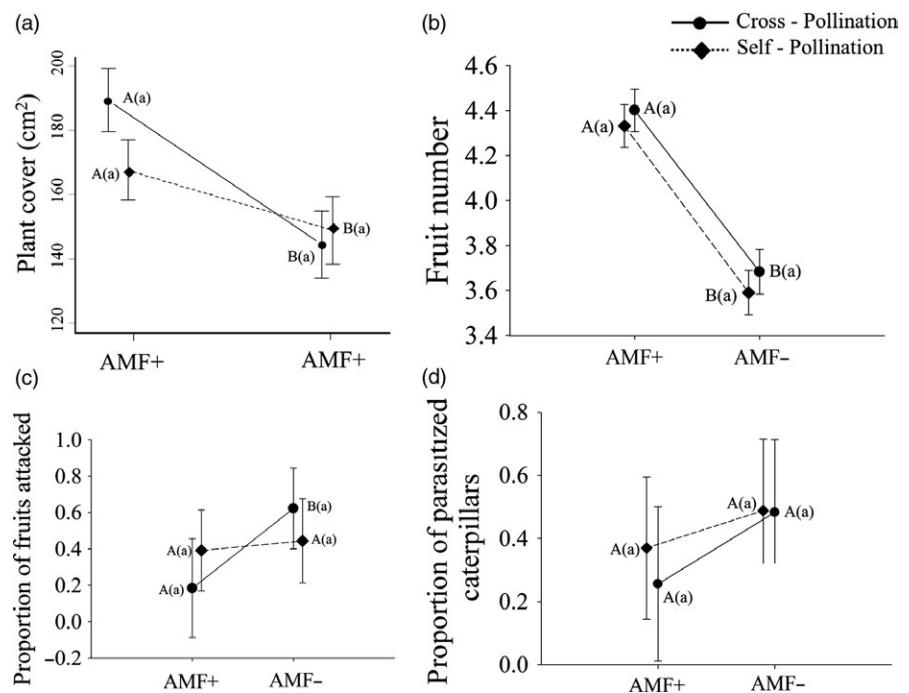
#### 3.1 | Effects of cross type and AMF on plant growth and reproduction

We found significant effects of AMF on plant growth and fruit output, but no effects of cross type or the interaction on either variable (Table 1; Figure 1a,b). Plants inoculated with AMF exhibited an 18% increase in plant cover (AMF+ =  $178.66 \pm 6.82$  cm<sup>2</sup>; AMF- =  $146.31 \pm 7.4$  per cm<sup>2</sup>), as well as a 15% increase in fruit

output (AMF+:  $4.46 \pm 1.09$  fruits; AMF-:  $3.73 \pm 1.09$  fruits) relative to plants that were not inoculated (Table 1).

#### 3.2 | Effects of cross type and AMF on seed predation and parasitism

On average,  $39.18 \pm 1.77\%$  of fruits collected during the experiment were attacked by the seed predator. Results from the statistical model indicated a significant effect of AMF on fruit attack, where the proportion of attacked fruits was 50% lower for inoculated plants relative to controls (AMF+:  $0.26 \pm 0.17$ ; AMF-:  $0.52 \pm 0.15$ ). However, we also found a significant interaction between AMF and cross type (Table 1), where AMF caused a significant (25%) reduction in the proportion of attacked fruits in plants from cross-pollination but did not significantly alter fruit attack in plants from self-pollination (Figure 1c). The effect of plant size on herbivore attack was not significant (Table S1).



**FIGURE 1** Effects of cross type (CT, self- vs. cross-pollination) and arbuscular mycorrhizal fungi (AMF+ = with mycorrhizae, AMF- = without mycorrhizae) on *Ruellia nudiflora* (a) plant cover, (b) fruit number, (c) the proportion of attacked fruits by a seed-eating caterpillar and (d) the proportion of caterpillars parasitized by wasps. Values are backtransformed least-square means ( $\pm$ SE). Upper case letters indicate significant differences between AMF environments within each cross type, whereas lower case letters indicate significant differences between cross types within each AMF environment ( $p < .05$  in both cases, based upon multiple comparison tests). Overlapping means and SE were slightly offset for purposes of visual clarity

Despite observing a relatively high incidence of parasitism during the experiment,  $38.42 \pm 1.82\%$  of the seed-eating caterpillars were attacked by parasitic wasps. Results from the statistical model indicated no effects of cross type, AMF or their interaction on the proportion of parasitized caterpillars (Table 1; Figure 1d). In addition, we found that on average  $16.93 \pm 1.75\%$  of seeds in attacked fruits were rescued from seed predator consumption, but results from the statistical analyses indicated that there were no individual or interactive effects of AMF and cross type on parasitoid indirect effects on seed output (Table 1).

## 4 | DISCUSSION

Our results indicated significant effects of AMF on plant traits and plant–seed predator (but not seed predator–parasitoid) interactions, where plants inoculated AMF exhibited a lower proportion of attacked fruits compared to plants without AMF. In contrast, there was no overall effect of cross type on above-ground plant–seed predator or seed predator–parasitoid interactions associated with *R. nudiflora*. More importantly, we found interactive effects of AMF and cross type on plant–seed predator interactions, whereby AMF reduced the proportion of attacked fruits for plants from cross-pollination but did not influence fruit attack for plants from self-pollination. This finding shows that the linkages between this below-ground mutualism and above-ground plant–insect interactions are contingent upon *R. nudiflora* intraspecific variation, in this case the source of pollen received by the mother plant that produced the offspring. Interestingly, the individual effect of AMF and the interactive effects of AMF and cross type on seed predation did not influence the third trophic level, as the proportion of parasitized caterpillars remained largely unchanged across levels of both factors. Overall, these results provide a more complete view of the connections among plant genetics and above- and below-ground plant-associated interactions, and bring together theory on ecological effects of plant–insect community ecology and plant community genetics. Results further emphasize the importance of testing for the effects of specific sources of plant intraspecific variation, particularly genetic variation due to cross type in plants with mixed mating systems and its role in controlling the outcome of plant-mediated interactions.

We found positive effects of AMF on plant growth and reproduction, which agrees with previous work with *R. nudiflora* (Ramos-Zapata et al., 2010). In contrast, we found no effect of cross type on plant traits, which is consistent with prior studies with this species showing weak differences in growth and reproduction between that progeny derived from a single generation of selfing relative to progeny from cross-pollination (Munguía-Rosas, Campos-Navarrete, et al., 2013; Munguía-Rosas, Parra-Tabla, et al., 2013). In combination, these results suggest that a single generation of selfing is not enough to produce detectable effects on fitness correlates in this species. In addition, previous work with *R. nudiflora* has reported low to moderate levels of inbreeding depression on flower output, but only under limiting conditions (e.g. low light availability; Munguía-Rosas, Campos-Navarrete,

et al., 2013; Munguía-Rosas, Parra-Tabla, et al., 2013). Theory predicts that high rates of selfing in cleistogamous species such as *R. nudiflora* purges deleterious alleles and this reduces the negative effects of inbreeding depression on plant fitness (Culley & Klooster, 2007; Oakley & Winn, 2008), such that a high frequency of selfing in this species presumably does not produce detectable effects on plant fitness or influence traits associated with insects. However, more work is needed to test for the abiotic context-dependency of selfing effects on associated interactions in this herb.

Our findings for above-ground interactions indicated that AMF drove an overall reduction in the proportion of attacked fruits by the specialist seed-eating caterpillar, which is in agreement with previous studies reporting on AMF-mediated increased herbivore resistance (e.g. Bennett et al., 2016; Li et al., 2016). Although we ignore the specific mechanisms or plant traits underlying this effect, we note that both plant size and fruit number were greater in plants with AMF and these phenotypic changes could have influenced herbivore attack. However, there was no effect of plant cover on herbivore attack, suggesting that this trait did not mediate AMF effects on the herbivore. Alternatively, differences in fruit output could influence attack rates as this herbivore exhibits a negative density-dependent (saturating) functional response with increasing fruit number (Abdala-Roberts & Mooney 2013). Nonetheless, differences between treatments in mean fruit output were relatively small and variation in fruit crop size for individual plants in this species must be greater to detect seed predator satiation, suggesting this trait was likely not responsible for the effect of AMF on herbivory. We speculate that AMF-driven alterations in other plant traits such as physical, chemical defences (including volatile emissions) in fruits or other plant reproductive tissues could have been responsible for the observed effect of AMF on fruit attack. In contrast, cross type did not influence plant growth, reproduction or presumably any other traits of potential importance to plant–seed predator interactions, and this in turn led to similar levels of herbivore attack for offspring of both cross types.

Although cross type had no overall effect on herbivory, we did find that this source of genetic variation conditioned effects of AMF on plant–seed predator interactions. Indeed, the most notable result from this study was probably the presence of interactive effects of AMF and cross type on plant–seed predator interactions, where AMF caused a substantial (30%) reduction in the proportion of attacked fruits for progeny from cross-pollination but did not alter fruit attack on plants from self-pollination. As such, this is one of the few studies to demonstrate the influence of plant genetics on linkages between below- and above-ground interactions associated with shared host plants, and further demonstrates such effects for a specific source of plant genetic variation. In a related study, Li et al. (2016) found negative effects between above- and below-ground insect herbivores in China and such effects were contingent on whether genotypes were from native populations or from populations planted outside the species distribution range (e.g. from USA, where this species is invasive). Although we did not measure plant traits potentially driving interactive effects of AMF and cross type in *R. nudiflora*, results suggest that AMF induced phenotypic changes in plants from cross-pollination but

not in those from self-pollination, and this in turn influenced fruit attack only in the former group of plants. In this sense, a prior study with *R. nudiflora* found that plants derived from obligated self-pollination exhibited weaker interactions with AMF (Parra-Tabla et al. 2015; and see Collin & Ashman, 2010), which would explain why this mutualism exerted stronger effects on seed predation in plants from cross-pollination. We therefore speculate that plants from cross-pollination had higher levels of AMF colonization, and that this led to greater investment in plant defences and increased herbivore resistance relative to plants from self-pollination. Previous studies with other plant species have shown that AMF induce plant chemical defences and volatile emissions that influence insect feeding or behaviour, and in some cause an increase in resistance against insect herbivores (Barber et al., 2013; Gange & West, 1994; Tao, Ahmad, Roode, & Hunter, 2016). Accordingly, future work measuring chemical defences in *R. nudiflora* is a necessary step forward in trying to understand the mechanisms and traits by which AMF influences seed predation, as well as the contingency of such effects upon plant genetic variation originated from self- vs. cross-pollination.

Despite finding a main effect of AMF and interactive effects of AMF and cross type on plant–seed predator interactions, these effects did not influence seed predator–parasitoid interactions or the indirect (positive) effects of parasitoids on plant seed output measured as the proportion of “rescued” seeds. These findings agree with our previous work with *R. nudiflora* showing that phenotypic effects of plant genetic variation and soil nutrient availability influence plant–seed predator interactions but not seed predator–parasitoid interactions (Abdala-Roberts & Mooney, 2014, 2015). In addition, results also agree with another study showing that plant traits such as inflorescence size and number, and number of fruits influence seed predator recruitment but not parasitoids (Cuautle & Parra-Tabla, 2014). Accordingly, studies in other systems have found similar results indicating that herbivores are usually more strongly affected by plant phenotypic variation than predators or parasitoids, in part because the effects of plant variation become weaker as they are transmitted up the food chain (Abdala-Roberts & Mooney, 2014; Bailey et al., 2009; Johnson & Agrawal, 2005; Schädler, Brandl, & Kempel, 2010). This is to be expected as herbivores directly depend upon plant abundance or traits associated with nutritional quality, whereas predators are in many cases indirectly influenced by plants via effects on herbivores (Johnson, 2008). We do note, however, that previous work in this system has shown that indirect effects of parasitoids on *R. nudiflora* seed output are influenced by soil nutrient availability (Abdala-Roberts et al., 2014), calling for further work testing whether the observed effects of cross type and AMF on parasitoids are dependent upon the abiotic context.

#### 4.1 | Conclusions and perspective on future work

Overall, this study emphasizes the role of plant intraspecific variation as a key determinant of the outcome of plant-mediated interactions between above- and below-ground communities associated with shared host plants. We further demonstrate the role of cross type as a potentially widespread source of plant intraspecific variation

in multi-trophic interactions, particularly given the high prevalence of mixed mating systems in flowering plants. Community genetics work has ignored this source of plant intraspecific variation (or variation in specific traits), and work on ecological effects of inbreeding has been highly reductionist by focusing on pairwise interactions, overlooking more complex interactions and community-level consequences. Further work merging these research areas is needed to examine the connections between plant offspring pollen source (as mediated by pollinators and plant mating systems) and above- and below-ground communities, as well as potential bottom-up and top-down feedbacks among trophic levels arising from such dynamics.

#### ACKNOWLEDGEMENTS

Comments and suggestions by two anonymous reviewers and by Miguel Munguía-Rosas and Jorge Navarro helped to improve the manuscript. We also thank Elizabeth Herrera-Parra, Teresa Quijano-Medina, Rigel Silveira and Unath León who provided assistance in the field and laboratory. B.M.-A. was supported by a CONACyT Ph.D. scholarship.

#### AUTHORS' CONTRIBUTIONS

B.M.-A., V.P.-T. and J.R.-Z. conceived and designed the experiments; B.M.-A. performed the experiments; B.M.-A., V.P.-T., and L.A.-R. analysed the data; B.M.-A., V.P.-T., L.A.-R., and J.R.-Z. wrote the manuscript.

#### DATA ACCESSIBILITY

Data files used for statistical analyses performed in this study are available at Dryad Digital Repository <https://doi.org/10.5061/dryad.1r59s> (Mejia-Alva, Ramos-Zapata, Abdala-Roberts, & Parra-Tabla, 2017).

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**How to cite this article:** Mejia-Alva B, Ramos-Zapata J, Abdala-Roberts L, Parra-Tabla V. Effects of arbuscular mycorrhizal fungi on above-ground tri-trophic interactions are contingent upon plant genetic effects of cross type in the perennial herb *Ruellia nudiflora*. *J Ecol*. 2017;00:1–9.  
<https://doi.org/10.1111/1365-2745.12859>