Plant species variation in bottom-up effects across three trophic levels: A test of traits and mechanisms

Running title: **Plant trait-mediated indirect effects**

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ABSTRACT

1. An increasing number of studies have addressed the mechanisms by which plant inter-specific variation influence interactions at higher trophic levels, but little is known about the underlying plant traits driving these dynamics.

2. Here we investigated the effects of host plant species on herbivore-parasitoid interactions and the underlying traits driving such effects. For this, we measured the abundance of seed-eating bruchids and their parasitoids across seven sympatric populations of the bean species *Phaseolus coccineus* and *P. vulgaris* in Central Mexico. To investigate the mechanisms underlying differences between bean species in bruchid-parasitoid interactions, we carried out two laboratory experiments to test whether bruchid and parasitoid performance differed between plant species. We also measured seed size and phenolic compounds to investigate if seed traits mediate bruchid-parasitoid interactions by influencing herbivore susceptibility or resistance to parasitoids.

3. Field surveys revealed that the rate of parasitoid recruitment to bruchids was significantly higher on *P. vulgaris* than on *P. coccineus*. Subsequent laboratory bioassays indicated that bruchids developed more slowly and exhibited lower fitness on *P. vulgaris* seeds than on *P. coccineus* seeds. Accordingly, we found that bean species differed in seed size, with *P. vulgaris* having smaller (less nutritious) seeds, which explains why bruchid development was slower on this plant species.

4. These results provide a mechanism for why bruchids exhibited higher parasitism rates on seeds of *P. vulgaris* in the field which could be due to Slow-Growth/High-Mortality effects, a smaller physical refuge provided by the seed, or both factors. The roles of these mechanisms remain inconclusive without further study.

Keywords: *bruchids, parasitoids,* Phaseolus, *phenolic compounds, seed size, trait-mediated indirect effects*

INTRODUCTION

The effects of plant phenotypic variation on consumers can be of environmental and/or genetic origin, and within the latter category effects may occur due to variation within (i.e. among genotypes) or among species. The effects of plant intra- and inter-specific variation on higher trophic levels have been documented most extensively within the context of plant-herbivore interactions, where plant genotype or species differences in defences and nutritional quality influence herbivore behaviour, abundance, and diversity (e.g. Karban, 1992; Hare, 2002; Helms et al., 2004; Mooney & Singer, 2012). In addition, studies have also documented effects of plant intra- and inter-specific variation on higher trophic levels and entire food webs, with genotypes or species varying in community structure of associated arthropod faunas (Helms et al., 2004; Johnson & Agrawal, 2005; Whitham et al., 2006; Harvey et al., 2011; Mooney & Singer, 2012) and in the strength of consumptive interactions (Mooney & Agrawal, 2008; Abdala-Roberts et al., 2012).

Plant genetic or species effects on higher trophic levels may be direct or indirect (Hare, 2002). Within the latter category, there are two mechanisms by which these bottom-up effects are transmitted indirectly to higher trophic levels (e.g. herbivores and their enemies). First, plant trait variation can influence herbivore abundance, which in turn influences herbivore enemy abundance (“density-mediated indirect effect”; Mooney & Singer, 2012). Under this scenario, there are no changes in per capita interaction rates (i.e. strength or function of interactions; Wootton, 1994). Alternatively, plant genetic effects may indirectly influence herbivore enemies by altering herbivore traits (“trait-mediated indirect effect”; Mooney & Singer, 2012), leading to changes in the function of herbivore-enemy interactions (interaction modification, sensu Wootton, 1994). For example, the Slow-Growth/High Mortality hypothesis poses that herbivore development on a poor quality host plant (species or genotype) will be slower than on a high quality host, exposing the herbivore to heightened predation risk and thus greater attack rates by natural enemies (Benrey & Denno, 1997; Moran & Hamilton, 1980; Williams, 1999). Alternatively, herbivores feeding on plants with high levels of chemical defences might sequester these chemicals which then serve as defence against their natural enemies, thus weakening the strength of natural enemy top-down effects (Moran & Thompson, 2001; Singer et al., 2014). In addition, there are a number of other mechanisms by which intra- or inter-specific variation in plant traits may modify herbivore-enemy interactions (reviewed by Hare 2002; Mooney and Singer 2012). Some of the most common ones are differences in the strength of plant volatile emissions (i.e. host location cue used by herbivore enemies) (Turlings et al., 1990; Dicke & van Loon, 2000), as well as variation in the degree to which host plants provide physical refuge for herbivores (e.g. Price 1988; Andow, 1991), all of which may independently or in combination drive plant trait-mediated effects on associated interactions.

While there is a growing number of studies evaluating the effects of plant intra- and inter-specific variation on higher trophic levels (reviewed by Hare, 2002; Mooney & Singer, 2012; Chen et al., 2015), comparatively fewer have examined the mechanisms underlying such effects (Abdala-Roberts & Mooney, 2013). In particular, separating density-mediated effects from interaction modifications is a key distinction in order to predict ecological and evolutionary consequences of plant phenotypic variation on higher trophic levels (Abrams, 1982; Mooney & Singer, 2012). For instance, changes in the strength of species interactions (i.e. interaction modifications), rather than density-mediated effects, represent a primary source of evolutionary change due to alteration of selection on species traits (Miller & Travis, 1996; Inouye & Stinchcombe, 2001; Abdala-Roberts & Mooney, 2015). A recent example within the context of plant intra-specific variation documented that milkweed (*Asclepias syriaca*) genotype modified ant-aphid interactions, influencing the number of ants attracted per aphid (Mooney & Agrawal, 2008). In addition, another recent study focusing on plant species-level effects by Singer et al. (2012) reported density-dependent recruitment of insectivorous birds to caterpillars across eight tree species, with bird predation rates increasing with host tree quality, and where high quality host trees had higher caterpillar densities. Finally, although an increasing number of studies have broadly documented the mechanisms by which plant intra- and inter-specific are transmitted to higher trophic levels, the underlying plant traits driving such effects are not always addressed (Mooney & Singer, 2012).

Here we investigated the effects of host plant species on consumptive interactions at higher trophic levels (herbivores and their enemies), as well as the underlying mechanisms driving such effects. To this end, we carried out a field study where we measured herbivory by seed-eating beetles (hereafter “bruchids”) and attack by their parasitoids across seven sympatric populations of two bean species of the genus *Phaseolus* (*P. coccineus* and *P. vulgaris*, Fabaceae) in Central Mexico. To evaluate the traits that mediate plant species’ effect on bruchids and parasitoids performance, we next carried out two laboratory experiments and measured seed traits related to plant resistance, including seed size, and seed chemical defences (i.e. phenolic compounds). By addressing these above, our work builds towards a more complete understanding of the mechanisms and underlying traits by which plant inter-specific variation cascades-up to influence higher trophic levels.

MATERIAL AND METHODS

Study system

We studied two bean species of the genus *Phaseolus* (Fabales: Fabaceae) in Central Mexico: *P. coccineus* and *P. vulgaris* (Fig. SM1). The center of origin of the genus is within Mexico, where the greatest diversity of wild and domesticated beans is found (Delgado-Salinas et al., 2006). *Phaseolus vulgaris* is naturally distributed at elevations ranging from 1300 to 1900 m, in tropical deciduous forests. Its flowers are white, pink, or purple, and give way to pods that are 8–20 cm long, pods are green, yellow, black, or purple and usually contain 4–6 multicolored seeds (Gentry, 1969; Freytag & Debouck, 2002). The flowers of *P. coccineus* are red and they give way to dark green pods which 9-13 cm long, pods usually contain 3-5 seeds which are black, white, cream or brown (Freytag & Debouck, 2002). *Phaseolus coccineus* is naturally distributed at higher elevations (>1400 m) in pine-oak forests, but populations of the two species exhibit extensive overlap, especially in Central Mexico (Freytag & Debouck, 2002). *Phaseolus coccineus* is largely allogamous and perennial, while *P. vulgaris* is autogamous and annual, nevertheless the reproductive phenology of both species largely overlap, fruiting from December to April (Gentry, 1969; Freytag & Debouck, 2002). Both species are among the five within the genus that been domesticated (Delgado-Salinas et al., 2006). *Phaseolus coccineus* is cultivated mostly at the center of its wild form’s distribution, and is mostly consumed locally, although its production has spread to South America and Europe (Delgado-Salinas, 1988). *Phaseolus vulgaris* is widely cultivated and is nowadays considered one of the most important legumes worldwide for direct human consumption (Broughton et al., 2003).

In Central Mexico, seeds of both species exhibit high rates of attack by three species of bruchid beetles (Coleoptera: Bruchidae), namely: *Acanthoscelides obtectus*, *A. obvelatus*, and *Zabrotes subfasciatus* (Fig. SM1) (Alvarez et al., 2005; Zaugg et al., 2013). These species are specialized on the genus *Phaseolus* and co-occur on the same host plants. *Acanthoscelides* species are distributed worldwide, and although the two species of this genus studied here exhibit very few morphological differences, *A. obvelatus* is univoltine whereas *A. obtectus* is multivoltine (Alvarez et al., 2005). Both species lay their eggs on seeds within dry pods in the field or directly on the stored (harvested) beans. *Zabrotes subfasciatus* is multivoltine and restricted to Mexico and Central America (Zaugg et al., 2013) where it is also an important pest of stored beans as well as several other crop species. Females enter the mature pods through a hole in the pod and glue their eggs onto the seed coat (Campan & Benrey, 2006). Larvae feed inside the seeds and after four larval instars they pupate and emerge as adults (Campan & Benrey, 2006). More than one larva (3-4) of *Acanthoscelides* sp. and *Z. subfasciatus* can be typically found per pod (X. Moreira, personal observation).

In Central Mexico, the most common natural enemies of these bruchids are three parasitic wasps of the genus *Horismenus* (*H. missouriensis*, *H. butcheri*, and *H. depressus*; Hymenoptera: Eulophidae) and *Stenocorse bruchivora* (Hymenoptera: Braconidae) (Fig. SM1) (Hansson et al., 2004; Bonet, 2008; Zaugg et al., 2013). These solitary ectoparasitoids wasps are specialized on bean-feeding bruchid beetles (Aebi et al., 2008; Zaugg et al., 2013), and attack third and fourth instar larvae (Campan & Benrey, 2004). Other parasitoid wasps also found at our study sites are *Dinarmus basalis*, *Eupelmus* sp., *Chryseida* sp., *Microdontomerus* sp., but these are usually present in much lower abundances.

Field experiment: Effects of host plant species on seed herbivores, parasitoids and their interactions

Seeds of both *P. vulgaris* and *P. coccineus* were collected at seven sympatric populations in Central Mexico from December 2007 to March 2008 (Table SM1). The populations were located at roadsides, rural habitats, and close to cultivated crops at elevations ranging from 1,791 to 2,039 m (Table SM1). At the end of the growing season (March), once pods were dry, at each site we collected all the mature bean pods present (approximately 50–100 per plant) from three to a maximum of 80 randomly selected individuals depending on the population size. In total we sampled pods from 221 individuals, 70 were *P. coccineus* and 151 were *P. vulgaris*.

Once collected, pods from each population were shelled; seeds were placed in ventilated plastic containers, and incubated under controlled conditions (26 ºC day temperature, 14 hrs daylight, 70% R.h.). Each container was inspected daily for emerging bruchids and parasitoids. After emergence, all bruchids and parasitoids were recorded and identified to species or family level. Emerging insects were collected and individually stored in vials with 70% ethanol.

The effects of host plant species (*P. coccineus* vs. *P. vulgaris*) on bruchid and parasitoid abundance (mean number of individuals per plant) were analysed using linear mixed models (PROC MIXED in SAS 9.2, SAS Institute, Cary, NC) (Littell et al., 2006). The models included the effect of plant species treated as a fixed factor, and the effect of plant population treated as random because populations were randomly chosen and representative of sympatric populations in Central Mexico. To account for differences in the number of seeds sampled per plant (which would likely influence bruchid recruitment), we included the number of seeds per plant as a covariate in the bruchid abundance model. As bruchid abundance influences parasitoid recruitment, we included the number of bruchids as a covariate in the parasitoid abundance model. Normality was achieved by log-transforming original variables. We provide least square means ± S. E. as descriptive statistics.

To investigate whether host plant species influenced bruchid-parasitoid interactions, we departed from the above model of parasitoid abundance and used a linear mixed model (PROC MIXED in SAS 9.2) that tested for the effects of plant species (fixed), number of bruchids, and their interaction on parasitoid abundance. As above, we included the effect of population treated as random. The interaction term tests for a difference between bean species in the slope of the relationship between bruchid and parasitoid abundance which is indicative of trait-mediated interactions (Abdala-Roberts et al., 2012; Moreira et al., 2012), i.e. changes in the per capita effects of parasitoids on bruchids. Because we cannot match parasitoid and bruchid species (there were different species of both insects in the same seeds), all analyses were based upon pooled abundances across bruchid and parasitoid species.

Laboratory experiments: Effects of host plant species on bruchid and parasitoid fitness correlates

To test for host plant species effects on bruchid and parasitoid fitness correlates, we reared insects on undamaged seeds of each bean species collected from four randomly chosen populations (Cuernavaca, San Jose de los Laureles, Malinalco and Tepoztlan, Table SM1).

For the bruchid performance experiment, we used *Z. subfasciatus* (multivoltine species easily reared in the laboratory) as the study model (Campan & Benrey, 2004). In February of 2011, we collected several adult individuals of this bruchid species from our wild bean populations and reared them on cultivated seeds of red kidney beans in climate chambers (27 ºC and 70% Relative humidity) at the University of Neuchâtel (Switzerland). Since 2012 we have been maintaining a population of this species, and every year we add new individuals collected from the field to add genetic variability. We filled eight plastic containers (height: 15 cm, diameter: 11 cm), with 50 undamaged seeds (one container per population) of both bean species (two bean species × four populations = eight containers). We then added 50 male and 50 female freshly emerged adult bruchids to each container. After three days of mating and subsequent oviposition on beans of each focal species, we randomly selected 25 bruchid-infested seeds per population per species and used a paintbrush to remove all but three newly laid eggs from the surface of each seed. Newly laid eggs (one day old) are milky white, and become transparent before the larvae hatch. We then placed five bruchid-infested seeds per population in small plastic containers (height: 2 cm, diameter: 2.5 cm) (two bean species × four populations × five containers = 40 containers) in the climate chamber (27 ºC and 70% R.h.) and inspected them daily for newly emerged bruchids. On average, adult bruchids emerged 28 days later. Immediately after emergence, each individual was deep-frozen at -80 ºC and oven-dried for 48 h at 65 ºC to constant weight. We recorded (i) the dry weight of each individual as a proxy of body size, (ii) developmental time of each individual (measured as the number of days until emergence), (iii) percentage of males (measured as the number of males divided by the total number of emerged bruchids) and (iv) survival (measured as the number of emerged bruchids divided by the number of eggs on the coat, which always three) (Campan & Benrey, 2004; Zaugg et al., 2013). For a closely related bruchid species (*Callosobruchus maculatus*, Coleoptera: Bruchidae), it has been shown that female eggs are allocated to high-quality hosts and male eggs to low-quality hosts so a higher percentage of emerging males is directly related to lower bruchid fitness (Spitzen & van Huis, 2005).

For the parasitoid performance experiment we used *S. bruchivora* as the study model (Campan & Benrey, 2004). We collected parasitoids from bruchid-infested seeds of wild bean populations in Mexico and reared them on red kidney bruchid-infested beans in climate chambers (27 ºC and 70% R.h.) at the University of Neuchâtel. We carried out this experiment at the same time as the bruchid performance experiment. As above, we selected bruchid-infested seeds and removed all but three eggs from the coat of each seed. After seed selection, we filled small plastic containers (height: 2 cm, diameter: 2.5 cm) with five of these seeds (six containers per species and population, two bean species × four populations × six replicates = 48 containers) and placed these containers in a climate chamber (27 ºC and 70% R.h.). Once bruchid larvae had reached the third or fourth instar inside the seed and can be parasitized (ca. 17 days after oviposition), we placed two inexperienced wasps (one male and one female) inside each plastic container (Campan & Benrey, 2004). We allowed the females to parasitize for a period of three days (females lay one egg on each bruchid larva). During this time, we provided a drop of honey to enhance oogenesis (Schmale et al., 2001). Adult parasitoids emerged approximately 17 days later. Immediately after emergence, each parasitoid specimen was deep-frozen at -28 ºC and oven-dried for 48 hrs at 65 ºC to constant weight. We recorded (i) the dry weight and tibia length of each individual (using an ocular micrometer) as proxies of body size, (ii) percentage of males (measured as the number of males divided by the total number of emerged parasitoids), and (iii) success of parasitism (measured as the number of emerged parasitoids divided by [number of emerged bruchids + number of parasitoids]) (Campan & Benrey, 2004; Zaugg et al., 2013). We did not measure parasitoid developmental time because it was not possible to record the exact day when bruchid larvae were parasitized. As would be expected, our laboratory experiment was not free of limitations relative to field conditions (e.g. parasitoids were only given three days to parasitize their hosts, were confined in a small space with their hosts and were lacking some important host-plant cues), making a direct link between field and lab results more difficult. However, success of parasitism was similar and consistent with those observed in a previous field study with the same plant, bruchid and parasitoid species and in the same plant populations (Zaugg et al., 2013), and levels of bruchid parasitism in the lab fell within the range of parasitism rates observed in the field (Zaugg et al., 2013). These findings provide strong indication that some important aspects of the biology of bruchid-parasitoid interactions were preserved under laboratory conditions.

The effects of host plant species (*P. coccineus* vs. *P. vulgaris*) on bruchid (weight and developmental time) and parasitoid (weight and tibia length) fitness correlates were analysed using linear mixed models (PROC MIXED in SAS) (Littell et al., 2006). The main effect of host plant species was treated as a fixed factor whereas the effects of plant population and container (to account for repeated measures recorded on the same experimental unit) were treated as random factors. Because female body size is larger for both bruchids and parasitoid, we separately analysed the weight, tibia length, and developmental time for males and females. For survival and male percentage of bruchids and success and male percentage of parasitoids (one value per container) we used linear mixed models (PROC MIXED in SAS) (Littell et al., 2006) with the host plant species as a fixed factor and plant population as a random factor. Normality was achieved by log-transforming original variables, except for tibia length of female parasitoids which was analysed with a generalized linear mixed model in PROC GLIMMIX using a Poisson distribution (log link function), as these data were non-normal after transformation. We use least square means ± S. E. as descriptive statistics.

Seed traits: Plant species differences in seed size and chemical defences

We measured the size of 54 randomly chosen seeds per plant species per population (same as above used for the bruchid and parasitoid performance bioassays; Table SM1). In total, we measured 432 seeds corresponding to 54 seeds × four bean populations × two bean species.

Using the same four populations, we also quantified the concentration of phenolic compounds in seeds of five randomly selected individuals per population, per species (we used a pool of 10 seeds per individual for phenolic extractions). In total, we quantified phenolic compounds from seeds of 40 individuals corresponding to four bean populations × two bean species × five individuals. Phenolic compounds are widely recognized as herbivore feeding deterrents across many plant taxa (Salminen & Karonen, 2011; Mithöfer & Boland, 2012). Extraction of phenolic compounds was carried out by using 300 mg of plant tissue with aqueous methanol (1:1 vol:vol) in an ultrasonic bath for 15 min, followed by centrifugation and subsequent dilution of the methanolic extract (Moreira et al., 2014). Samples were analysed by HPLC using a Grace C18 reversed phase column (3µm, 150×4.6 mm, Grace Davison Discovery Science, Columbia, MD, USA) and an YL9100 instrument

(YL Instrument Co., the Republic of Korea), with diode array detection. The 15 μl injection was eluted at a constant flow of 0.7 ml/min with a gradient of acetonitrile and 0.25% phosphoric acid in water as follows: from 80% to 50% water in 5 minutes, then form 50% to 30% in five minutes, and kept at 30% for 7 minutes, and a final step from 30% to 5% in 4 minutes, followed by 5 minutes of equilibration time. Peaks were detected by a diode array detector at 270 nm (for hydrolysable tannnins), 320 nm (for ferulic acid derivates), 370 nm (for flavonoids), and 500 nm (for anthocyanins). Absorbance spectra were recorded from 200 to 900 nm. Peaks showing a characteristic absorption band of phenolics (Mabry et al., 1970) were recorded. Concentrations were calculated a standard curve that related peak areas to known ferulic acid, quercetin (for phenolics), and cyaniding (for anthocyanins) concentrations using 270 nm absorbance.

To test for bean species differences in seed size and phenolic compounds, we used linear mixed models (PROC MIXED in SAS) (Littell et al., 2006) testing for an effect of host plant species (fixed) and including plant population as a random factor. We report least square means ± S. E. as descriptive statistics.

RESULTS

Field study: Effects of host plant species on seed herbivores, parasitoids and their interactions

The overall field sampling yielded a total of 143,203 seeds across all sympatric wild bean populations (28,820 in *P. coccineus* and 114,383 in *P. vulgaris*). From these, we recorded 41,415 emerging arthropods, of which 32,411 were bruchid beetles and 9,004 were parasitic wasps.

Total bruchid abundance varied greatly among populations (within bean species). In particular, the mean number of bruchids per plant ranged from 21.60 ± 88.24 to 235.13 ± 49.33 in *P. coccineus* (11-fold variation), and from 73.11 ± 65.77 to 323.67 ± 113.93 in *P.*

vulgaris (four-fold variation). Despite such extensive variation, host plant species did not have a significant effect on total bruchid abundance (Fig. 1a). Likewise, parasitoid abundance also varied extensively among populations, within bean species. In particular, the mean number of parasitoids per plant ranged from 2.00 ± 39.24 to 38.17 ± 32.04 in *P. coccineus* (19-fold variation) and from 11.25 ± 39.24 to 235.00 ± 45.31 in *P. vulgaris* (21-fold variation). In this case, we found a significant effect of plant species on total parasitoid abundance, with *P. vulgaris* seeds having 2.2-fold more parasitoids than *P. coccineus* seeds (Fig. 1b). In addition, the mechanistic model for parasitoid abundance revealed a significant plant species × bruchid abundance interaction, i.e. bruchid-parasitoid interactions differed between bean species. This interaction occurred because the rate of parasitoid recruitment per bruchid was 2.1-fold greater for *P. vulgaris* than for *P. coccineus* ($F_{1,210} = 10.85$, $P = 0.001$; Fig. 2), indicating that trait differences between bean species mediated the strength of parasitoid-bruchid interactions.

<u>Laboratory experiments: Effects of host plant species on bruchid and parasitoid fitness correlates</u>

Overall, bruchids reared on *P. coccineus* seeds performed better than those reared on *P. vulgaris* seeds (Fig. 3). Firstly, the percentage of emerged bruchids per egg on *P. coccineus* seeds was 59% higher than in *P. vulgaris* seeds (Fig. 3a). Secondly, the dry weight of male and female bruchids on *P. coccineus* seeds was 21% and 11% higher, respectively, than on *P. vulgaris* seeds (Fig. 3d, 3f). Finally, developmental time of female bruchids on *P. coccineus* seeds was significantly shorter (by one day) than on *P. vulgaris* seeds (Fig. 3e). Plant species did not have a significant effect on the percentage of males that emerged from the seeds (Fig. 3b).

There was some evidence (albeit weaker relative to bruchids) that host plant species influenced parasitoid performance. Specifically, the tibia length of male parasitoids (proxy of body size) reared on bruchid-infested *P. coccineus* seeds was 13% greater compared with males reared on seeds of *P. vulgaris* (Fig. 4d). However, plant species had no significant effects on success of parasitism (Fig. 4a), percentage of parasitoid males (Fig. 4b), male and female weight (Fig. 4c, 4e), or female tibia length (Fig. 4f).

Seed traits: Effects of host plant species on seed size and chemical defences

We found significant differences in seed size between plant species, with *P. coccineus* seeds being 40% larger than *P. vulgaris* seeds (Fig. 5a). We also found significant differences for total seed phenolics among plant species (Fig. 5b), with the concentration of total phenolics in *P. coccineus* seeds being 26% higher than in seeds of *P. vulgaris* (Fig. 5b). Analyses by phenolic compound class indicated that the concentration of flavonoids in seeds of *P. coccineus* was 38% higher than in seeds of *P. vulgaris* (337.2 ± 35.7 vs. 243.7 ± 35.4 µg g^{-1}, $F_{1,34} = 11.09$, $P = 0.002$). We found no significant differences between species for ferrulic acid derivates (i.e. lignins) (59.0 ± 6.9 µg g^{-1} in *P. coccineus* vs. 50.8 ± 6.9 µg g^{-1} in *P. vulgaris*; $F_{1,34} = 0.86$, $P = 0.361$) or anthocyanins (258.1 ± 15.5 µg g^{-1} in *P. coccineus* vs. 225.8 ± 15.5 µg g^{-1} in *P. vulgaris*; $F_{1,35} = 2.16$, $P = 0.151$), suggesting that flavonoids drove the overall difference in concentration of phenolics between species.

DISCUSSION

The mechanisms by which plant bottom-up effects influence higher trophic levels have been increasingly documented over last decade (e.g. Helms et al., 2004; Johnson & Agrawal, 2005; Mooney & Agrawal, 2008; Mooney & Singer, 2012; Singer et al., 2012; Petry et al., 2013). A common pathway for plant phenotypic variation to influence higher trophic levels is through density-mediated effects where plant traits influence higher trophic levels through linear food chains (e.g. sequential changes in abundance across trophic levels) but consumer interactions are not modified (Mooney & Singer, 2012). However, there is also evidence that plant species or genotypes mediate consumer interactions indirectly via changes in consumer traits. For example, plant genetic types (e.g. species or genotypes) can indirectly influence natural enemies by affecting herbivore quality, susceptibility, and host location with this in turn driving changes in the strength of natural enemy top-down effects on herbivores (Turlings et al., 1990; Werner & Peacor, 2003; Helms et al., 2004; Mooney & Agrawal, 2008; Gols et al., 2009; Mooney & Singer, 2012; Singer et al., 2012). For example, Mooney & Agrawal (2008) showed that genotypes of the common milkweed indirectly influenced aphid-ant interactions by changing the quality and quantity of the aphids' honeydew. In this study, we provide similar evidence for effects of plant-species level variation on herbivore-enemy interactions (i.e. plant trait-mediated indirect effects) and further address the plant traits that are likely mediating such dynamics. In particular, our results show how phenotypic differences between *P. vulgaris* and *P. coccineus* resulted in changes in the function of bruchid-parasitoid interactions. We found that the rate of parasitoid recruitment to bruchids was higher on *P. vulgaris* seeds than on *P. coccineus* seeds. In addition, laboratory experiments showed that bruchids exhibited lower fitness (i.e. lower survival, reduced growth, and longer developmental times, albeit for females only in the latter case) on *P. vulgaris* seeds than on *P. coccineus* seeds. Overall, these results provide support for the Slow-Growth/High-Mortality hypothesis, which predicts that herbivore development on low-quality host plants should be slower, increasing the risk of herbivore mortality from natural enemies (Moran & Hamilton, 1980; Clancy & Price, 1987; Benrey & Denno, 1997).

Although an increasing number of studies have identified the mechanistic pathways underlying plant bottom-up effects on consumers, in most cases there is no information on the specific plant traits driving these dynamics (Hare, 2002). Our results provide strong evidence of seed traits potentially mediating plant inter-specific variation for bruchid-parasitoid interactions. Specifically, we found that *P. vulgaris* had smaller seeds than *P. coccineus*, which likely resulted in lower resource availability (and/or quality). Accordingly, previous studies have shown that seed predators and frugivores prefer larger seeds and fruits, presumably because they offer greater resource availability and/or quality (Janzen, 1971; Gómez & Zamora, 1994; Herrera, 2000). Potentially as a result of this difference, bruchid developmental time was longer on *P. vulgaris* seeds than on *P. coccineus* seeds with this in turn leading, at least in part, to stronger top-down effects by parasitoids on the former bean species (Fig. 2). In agreement with these findings, previous work in this system has shown that *Z. subfasciatus* beetles developed more slowly and produced smaller offspring when feeding on smaller and less nutritious seeds of wild *P. coccineus* and *P. vulgaris* populations compared with their cultivated counterparts which have larger and more nutritious seeds (Benrey et al., 1998; Campan & Benrey, 2006). Additionally, previous study has found that larger seeds of a closely related *Phaseolus* species (*P. lunatus*) contained higher protein contents than smaller ones (Hernández-Cumplido et al., unpublished data).

Despite evidence presented here in support of the Slow-Growth/High-Mortality hypothesis, we recognize that other unmeasured mechanisms (e.g. emission of plant induced volatiles; Turlings et al., 1990) might be also driving herbivore-parasitoid interactions in our study system. In particular, we speculate that parasitoids could have preferred to oviposit on *P. vulgaris* seeds which are smaller than *P. coccineus* seeds, because of easier access of the parasitoid ovipositors to the bruchid larvae inside (Sivinsky et al., 1997; Wang et al., 2009; Chen et al., 2015). Likewise, other seed traits such as coat thickness cannot be discarded as predictors of parasitoid attack. Based on this, our findings support the argument that seed traits, in particular size, are likely important in mediating variation in bruchid-parasitoid interactions among species of *Phaseolus*. Finally, it is important to note that differences in bruchid parasitism between bean species might be partly due to changes in bruchid species composition (Schmitz et al., 2004; Mooney & Singer, 2012). Therefore, further work is necessary to improve our understanding on differences between bruchid species in their susceptibility to parasitoids, as well as if the parasitoid species studied are equivalent with respect to host use.

Interestingly, despite exhibiting greater size, and thus presumably higher resource availability, *P. coccineus* seeds also contained higher concentrations of phenolic compounds (especially flavonoids) than *P. vulgaris* seeds. Although higher concentrations of chemical defences are typically associated with reduced seed quality and would thus lead to lower bruchid performance on *P. coccineus*, there are at least two likely reasons why this was not the case. Firstly, bruchids feeding on *P. coccineus* seeds could have sequestered secondary metabolites, a phenomenon that is frequently exhibited by specialist insect herbivores (Opitz & Müller, 2009; Boeckler et al., 2011; Mason et al., 2014), and the sequestered compounds are in turn used by the herbivore as chemical defences against natural enemies (Boeckler et al., 2011). However, parasitoid fitness did not vary between bean species for any of the measured traits and for male tibia length the mean value was even greater for *P. coccineus* (suggesting higher fitness on this plant species), which argues that this mechanism was not at work. Alternatively, studies have shown that specialist insect herbivores can tolerate and even benefit from the consumption of highly defended plant tissues, through mechanisms of detoxification (Bernays, 1998; Mason et al., 2014). Under this scenario, parasitoid fitness would not be expected to vary between plant species, as observed in this study. In support of this interpretation, previous work has shown that *Z. subfasciatus* is able to tolerate high concentrations of cyanogenic compounds present in *P. lunatus* (lima bean) without exhibiting detectable reductions in fitness correlates (Shlichta et al., 2014).

In conclusion, we show that bottom-up effects of host plant species on higher trophic levels are driven by trait-mediated indirect effects. In particular, differences in seed quality between plant species presumably modified herbivore susceptibility and thus the strength of natural enemy top-down effects. We argue that further studies are needed to separate density-mediated from interaction modification effects as well as determine the underlying traits by which intra- and inter-specific variation influence herbivore-enemy interactions. In doing so, we will build a more robust body of theory explaining how plant intra- and inter-specific variation shapes associated consumer communities. Additionally, we suggest that future work should investigate the presence of opposing patterns of selection on traits that provide a direct fitness benefit to the plant but may also decrease fitness under a multi-trophic setting (Herrera et al., 2002; Agren et al., 2013). In our system, larger seeds will directly contribute to greater offspring survival and overall fitness but at the same time may also suffer from greater herbivore attack and weaker herbivore suppression by natural enemies (due to faster herbivore development).

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AUTHOR CONTRIBUTIONS

XM formulated the idea of the manuscript. XM and BB designed the experiments. XM, SGK, JHC performed the experiments. SR analyzed phenolic compounds. XM analyzed the data. XM and LAR wrote the manuscript; and other authors provided editorial advice.

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FIGURE LEGENDS

Figure 1. Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on the mean number of (a) bruchid beetles and (b) bruchid parasitoids per plant across seven sympatric populations of these bean species located in Central Mexico. Bars are least square means ± s.e.m. (N = 70 for *P. coccineus* and N = 151 for *P. vulgaris*). F-values, degrees of freedom and associated significance levels (*P*) are shown.

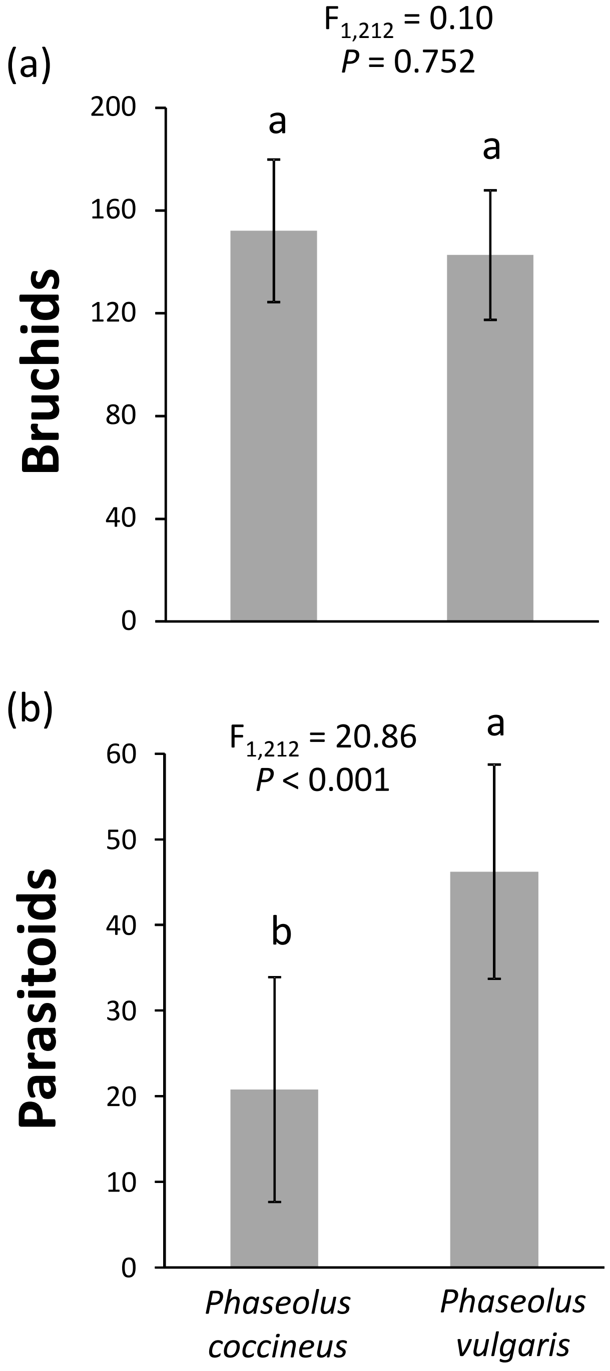
Figure 2. Relationship between the mean number of parasitoids and bruchids per plant associated with (a) *Phaseolus coccineus* and (b) *P. vulgaris* across seven sympatric populations of both species located in Central Mexico. Each point represents an individual plant (N = 70 for *P. coccineus* and N = 151 for *P. vulgaris*). F-values, P-values, degrees of freedom, and predicted relationships are based upon simple linear regressions performed separately for each bean species. The slope of the relationship differed between host plant species ($F_{1,210}$ = 10.85, P = 0.001), with the per capita rate of parasitoid recruitment being significantly greater (2.1-fold) for *P. vulgaris*.

Figure 3. Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) survival of *Zabrotes subfasciatus* bruchids (percentage of emerged bruchids per number of glued eggs on the seed coat), (b) percentage of bruchid males (number of males divided by total individuals), (c) (e) developmental time (days until adult emergence) of male and female bruchids and (d, f) dry weight (in mg) of male and female bruchids in a laboratory experiment. Seeds were selected from four sympatric populations of both species located in Central Mexico. Bars are least square means ± s.e.m. (N = 20). F-values, degrees of freedom

and associated significance levels (*P*) are shown. Different letters indicate significant ($P < 0.05$) differences between host plant species.

Figure 4. Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) success of parasitism of the parasitoid *Stenocorse bruchivora* (percentage of emerged parasitoids per number of glued bruchid eggs on the seed coat), (b) percentage of parasitoid males (number of males divided by total individuals), (c) (e) dry weight (in mg) of male and female parasitoids and (d, f) tibia length (ocular units) of male and female parasitoids in a laboratory experiment. Seeds were selected from four sympatric populations of both species located in Central Mexico. Bars are least square means ± s.e.m. (N = 24). F-values, degrees of freedom and associated significance levels (*P*) are shown. Different letters indicate significant ($P < 0.05$) differences between host plant species.

Figure 5. Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) size (in cm) and (b) total phenolics (in µg g^{-1} d.w.) of seeds of each species. Seeds were selected from four sympatric populations of both species located in Central Mexico. Bars are least square means ± s.e.m. (N = 216 for seed size and N = 20 for total phenolics). F-values, degrees of freedom and associated significance levels (*P*) are shown.



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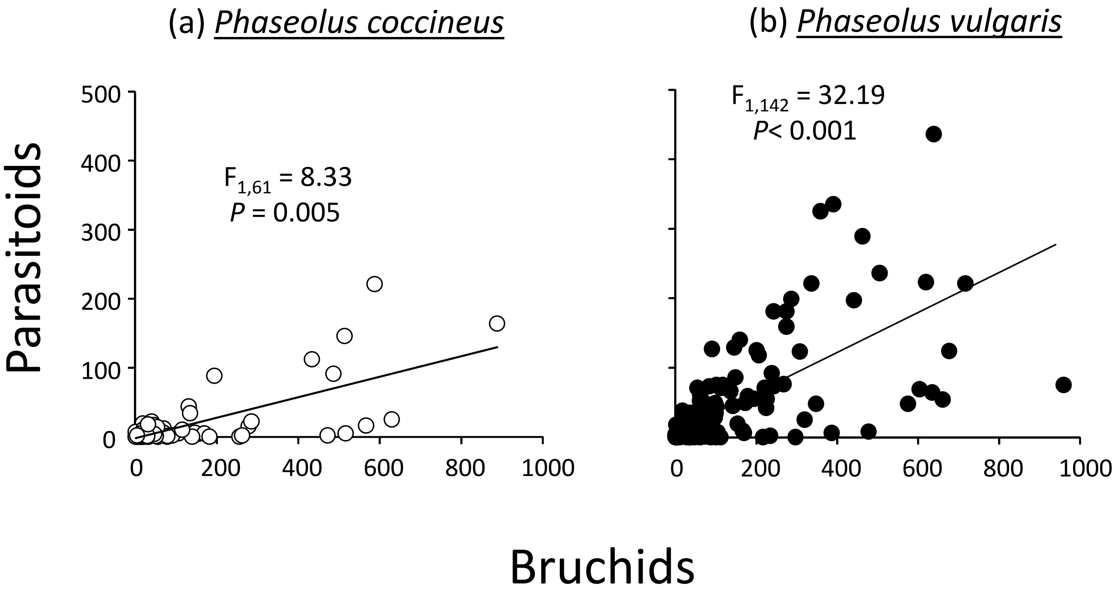

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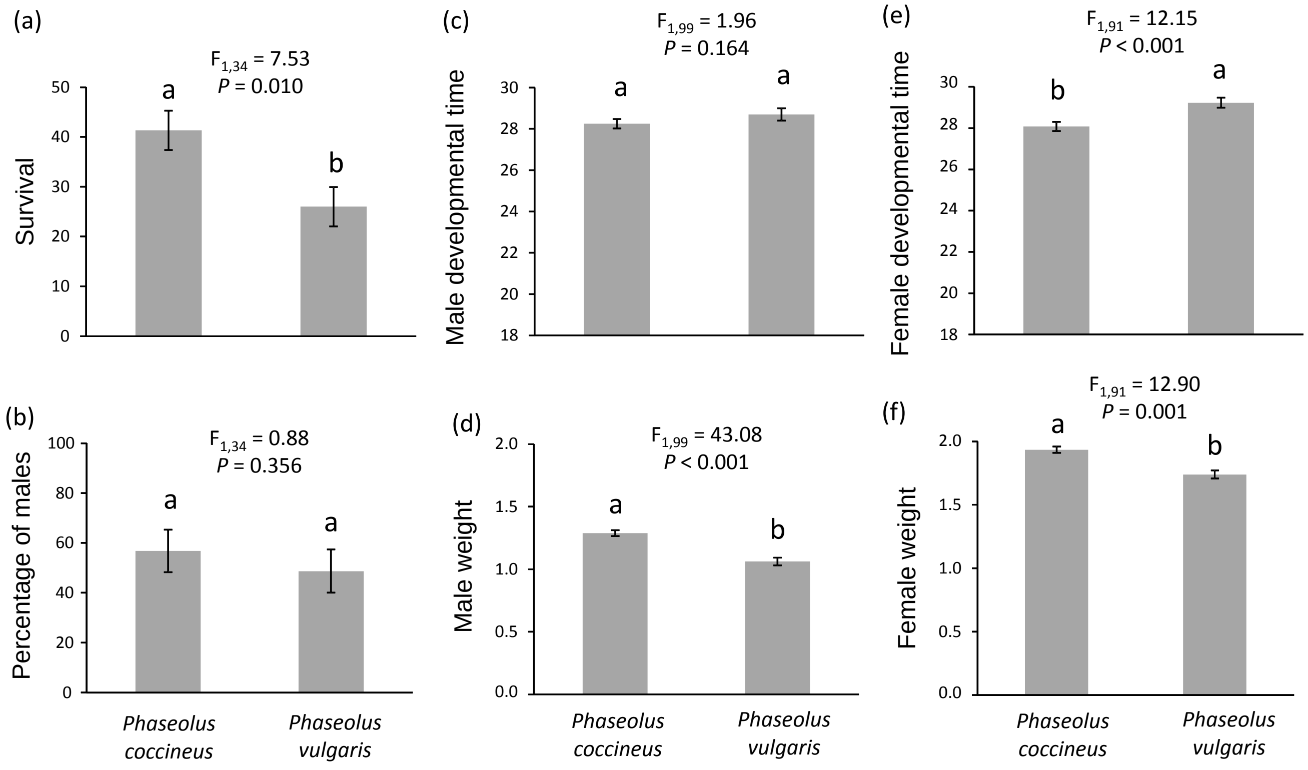

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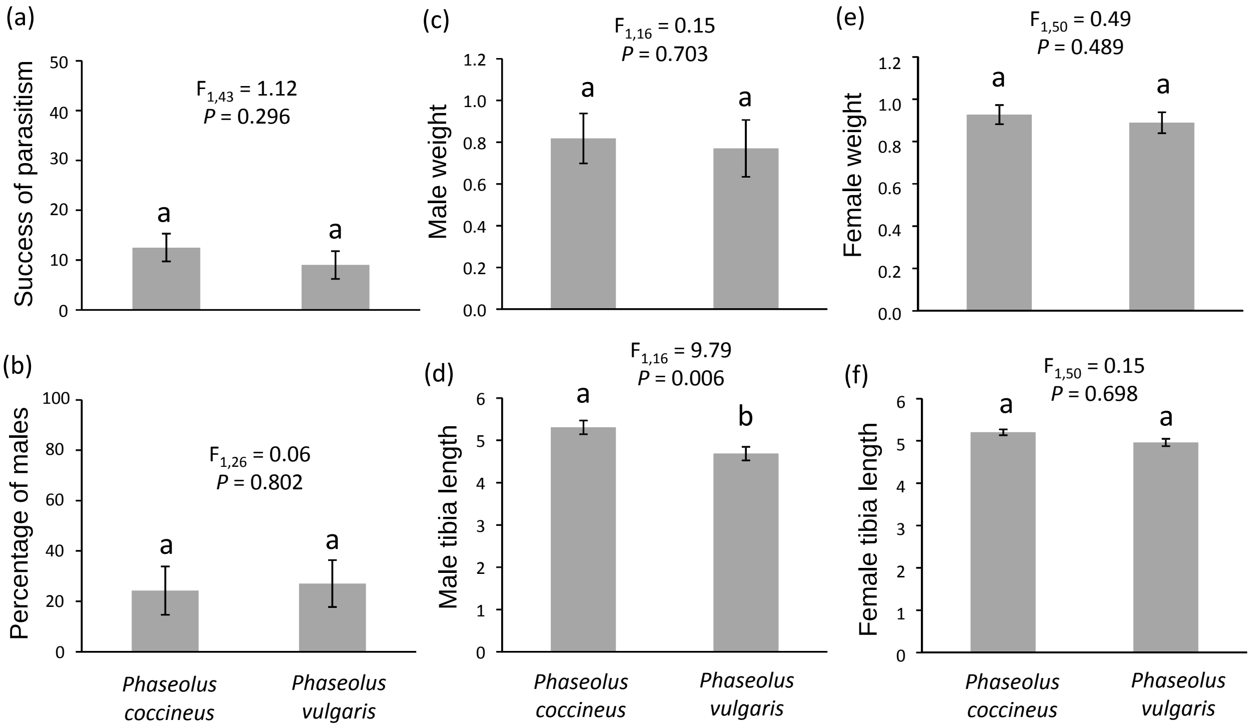

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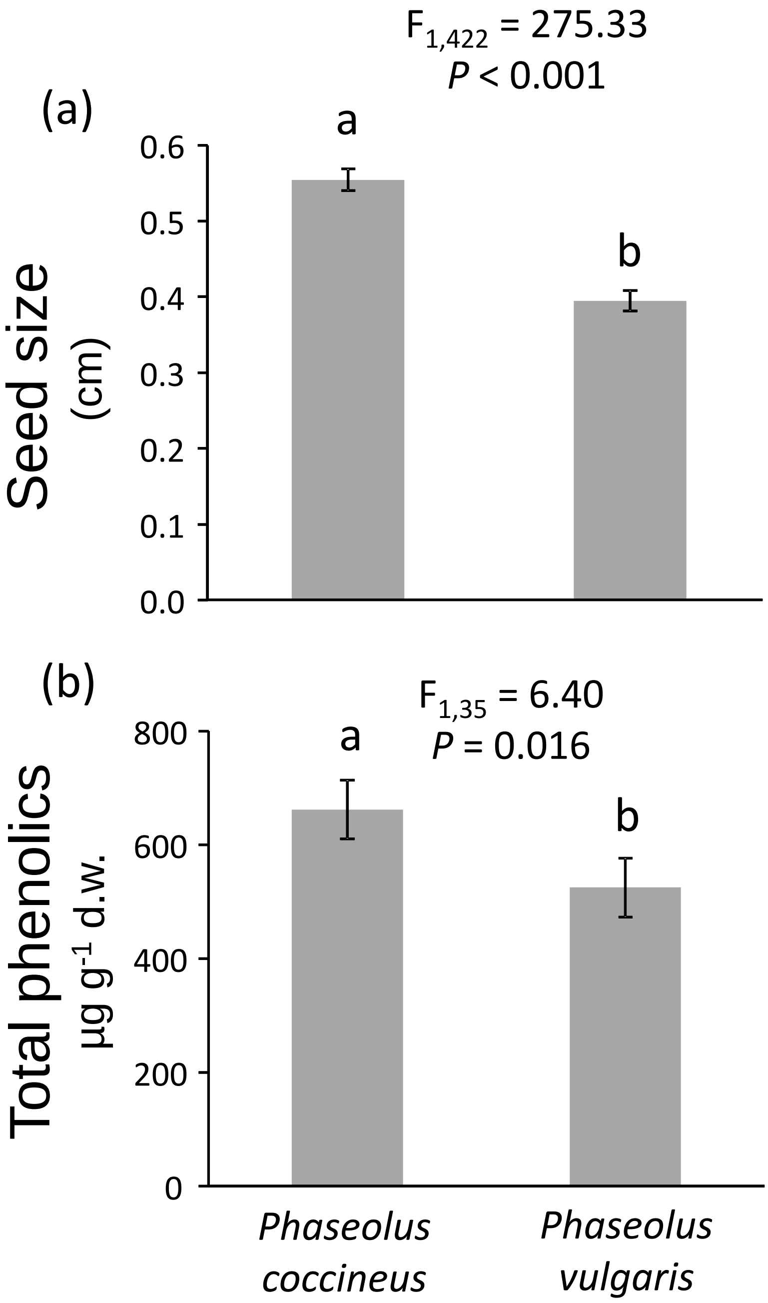

Figure 5. Moreira et al