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### *Myiarchus* flycatchers are the primary seed dispersers of *Bursera longipes* in a Mexican dry forest: An overlooked coevolutionary process?

R. Carlos Almazán-Núñez, Luis E. Eguiarte, María del Coro Arizmendi, Pablo Corcuera

We evaluated the seed dispersal of *Bursera longipes* by birds along a successional gradient of tropical dry forest (TDF) in southwestern Mexico. B. longipes is an endemic tree to the TDF in the Balsas basin. The relative abundance of frugivorous birds, their frequency of visits to *B. longipes* and the number of removed fruits were recorded at three study sites with different stages of forest succession (early, intermediate and mature) characterized by distinct floristic and structural elements. Flycatchers of the Myiarchus and Tyrannus genera removed the majority of fruits at each site. Overall, visits to B. longipes were less frequent at the early successional site. Birds that function as legitimate dispersers by consuming whole seeds and regurgitating or defecating intact seeds in the process also remove the pseudoaril from seeds, thereby facilitating the germination process. The highest germination percentages were recorded for seeds that passed through the digestive tract of two migratory flycatchers: *M. cinerascens* and *M. nutingii*. Perch plants, mainly composed of legumes (e.g., Eysenhardtia polystachya, Acacia cochliacantha, Calliandra eryophylla, Mimosa polyantha), serve also as nurse plants since the number of young individuals recruited from *B. longipes* was higher under these than expected by chance. This study shows that Myiarchus flycatchers are the most efficient seed dispersers of *B. longipes* across all successional stages. This suggests a close mutualistic relationship derived from adaptive processes and local specializations throughout the distribution of both taxa, as supported by the geographic mosaic theory of coevolution.

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2	forest: An overlooked coevolutionary process?
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24 Abstract. We evaluated the seed dispersal of *Bursera longipes* by birds along a successional gradient of tropical dry forest (TDF) in southwestern Mexico. B. longipes is an endemic tree to 25 the TDF in the Balsas basin. The relative abundance of frugivorous birds, their frequency of 26 27 visits to B. longipes and the number of removed fruits were recorded at three study sites with different stages of forest succession (early, intermediate and mature) characterized by distinct 28 29 floristic and structural elements. Flycatchers of the *Myiarchus* and *Tyrannus* genera removed the majority of fruits at each site. Overall, visits to B. longipes were less frequent at the early 30 successional site. Birds that function as legitimate dispersers by consuming whole seeds and 31 32 regurgitating or defecating intact seeds in the process also remove the pseudoaril from seeds, thereby facilitating the germination process. The highest germination percentages were recorded 33 34 for seeds that passed through the digestive tract of two migratory flycatchers: M. cinerascens and 35 M. nutingii. Perch plants, mainly composed of legumes (e.g., Eysenhardtia polystachya, Acacia cochliacantha, Calliandra eryophylla, Mimosa polyantha), serve also as nurse plants since the 36 number of young individuals recruited from *B. longipes* was higher under these than expected by 37 chance. This study shows that *Myiarchus* flycatchers are the most efficient seed dispersers of *B*. 38 *longipes* across all successional stages. This suggests a close mutualistic relationship derived 39 40 from adaptive processes and local specializations throughout the distribution of both taxa, as supported by the geographic mosaic theory of coevolution. 41

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43 Key words: germination, establishment, flycatchers, nurse plants, restoration, coevolution.

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#### 47 Introduction

Biotic seed dispersal plays a central role in the spatial dynamics of plant populations (Spiegel & Nathan, 2007; Schupp, Jordano & Gómez, 2010; Forget et al., 2011; Jordano et al., 2011). Dispersion may encourage the establishment of plants since intraspecific competition is generally lower in sites distant from the parent plant (Godínez-Alvarez, Valiente-Banuet & Rojas-Martínez, 2002; Schupp, Milleron & Russo, 2002). Moreover, the incidence of pathogens and predators is usually reduced at new sites (Jordano et al., 2011, Obeso, Martínez & García, 2011).

55 In arid and semi-arid environments, biotic dispersal, germination and seedling establishment are critical phases of plant life cycles (Steenbergh & Lowe, 1969; Valiente-Banuet 56 et al., 1991; Godínez-Alvarez & Valiente-Banuet, 1998; Orozco-Almanza et al., 2003; Padilla & 57 58 Pugnaire, 2006). During the dry season, for example, seedlings face adverse factors, such as dry soil, direct sunlight and extreme temperatures (Godínez-Alvarez & Valiente-Banuet, 1998). 59 However, dispersers aid in finding better conditions by depositing seeds in preferable 60 microhabitats (i.e., under nurse plants) that promote germination and seedling survival (Pérez-61 Villafaña & Valiente-Banuet, 2009). 62

Inadequate agricultural practices have reduced the undisturbed area of tropical dry forests (TDF) in Mesoamerica to fragments at various successional stages (Quesada et al., 2009). Since the soil seed bank may be considerably reduced in disturbed fragments (Uhl, 1987; Martins & Engel, 2007), seed dispersal can play an important role in the recruitment of plants and hence contribute towards the composition and density of woody plants and the eventual restoration of these forests (Hammond, 1995; Peña-Claros & De Boo, 2002).

69 The vegetation of the Balsas basin in southwestern Mexico consists mainly of TDF (Rzedowski, 1978), and the dominant arboreal elements belong to the genus Bursera, 70 Burseraceae (Rzedowski, Medina & Calderón de Rzedowski, 2005; Almazán-Núnez et al., 71 2012), whose fruits are a source of food for resident and migratory frugivorous and insectivorous 72 birds during the dry season (Ortiz-Pulido & Rico-Gray, 2006; Ramos-Ordoñez & Arizmendi, 73 74 2011). Furthermore, the distribution of these birds in different successional stages of TDF is related to the presence of these trees (Almazán-Núñez et al., 2015). Although the role of 75 insectivorous birds, such as flycatchers and vireos, as consumers and dispersers of Bursera spp. 76 77 has been previously described (Greenberg, Foster & Márquez-Valdelamar, 1995; Hammond, 1995; Ortiz-Pulido & Rico-Gray, 2006; Ramos-Ordoñez & Arizmendi, 2011), there are few 78 79 detailed reports on this subject. For instance, Ramos–Ordoñez & Arizmendi (2011) analyzed the 80 seed dispersal of *B. morelensis*, while Ortiz-Pulido & Rico-Gray (2006) studied the same process in *B. fagaroides*, but no additional studies have been carried out in TDF to evaluate and compare 81 biotic seed dispersal between sites with different degrees of disturbance (Hammond, 1995; 82 Quesada et al., 2009). 83

Furthermore, the majority of studies on frugivory and seed dispersal have been limited to 84 85 single locations (Ortiz-Pulido & Rico-Gray, 2006; Ramos-Ordoñez & Arizmendi, 2011), but patterns that are elusive at local scales may emerge in regional studies that cover areas with varying 86 levels of disturbance or recovery (Carlo, Aukema & Morales, 2007). Frugivory and seed 87 88 dispersal can also differ across a geographic mosaic, as interacting species may not necessarily have the same distribution (Thompson, 2005). That is, according to the mosaic of coevolution 89 90 ideas, differential patterns of species distribution result in local specializations and interactions 91 of varying degrees (Nuismer, 2006).

Moreover, studies that seek to understand patterns of biotic seed dispersal and seedling establishment in TDF at different levels of perturbation or seral stages, form the scientific basis for applied studies focusing on management and conservation strategies in these forests. In the TDF, native plants, particularly those from the Burseraceae family, are important in forest regeneration and can help to revert fragmentation (Ramos-Ordoñez, Arizmendi & Márquez-Guzmán, 2012).

The genus Bursera is a distinctive component of TDF in Mesoamerica, including ca. 107 98 species (De-Nova et al., 2012). Its distribution spans from northern Mexico to the northern 99 100 region of South America (Becerra et al., 2012). The diversification of this genus has been related 101 to the southward expansion of TDF in response to the elevation of the Sierra Madre del Sur and the Mexican Volcanic Belt (De-Nova et al., 2012). Bursera evolutionary history indicates that 102 103 much of the biological richness of the Mesoamerican TDF derived from increased rates of speciation within habitats from the early Miocene to the Pliocene, due to marked arid regimes 104 (Becerra, 2005; Dick & Wright, 2005). This scenario matches other hypotheses proposing that 105 these lineages were mostly restricted to dry environments of Mexico and evolved during long 106 periods of isolation (Valiente-Banuet et al., 2004). 107

In this study we describe the primary seed dispersal of *B. longipes* by frugivorous birds in a TDF of the Balsas basin of Guerrero. Belonging to the Simaruba sub-group of the diverse *Bursera* genus, *B. longipes* forms part of a larger clade that emerged in the Miocene just over 8.5 million years ago (De-Nova et al., 2012). This tree is a dominant species (Almazán-Núñez et al., 2012) in this biotic region, considered to be the center of diversification for this genus in Mesoamerica (Rzedowski, Medina & Calderón de Rzedowski, 2005). While *B. longipes* 

114 abundance increases in the most well-preserved sites, it also settles in disturbed areas
115 (Rzedowski, Medina & Calderón de Rzedowski, 2005).

The questions of this study were: (1) Which bird species remove *B. longipes* seeds along the TDF successional mosaic? (2) Do seeds that pass through the tract of birds have higher germination rates than those that fall down from trees? (3) Are there differences in the quality of seeds dispersed by birds? (4) Does *B. longipes* need nurse plants to establish in different sites? (5) Do dispersal birds preferentially use nurse plants for perching in the successional mosaic?

121

#### 122 Materials & Methods

#### 123 Study sites

We conducted the study at three sites corresponding to different successional phases of 124 TDF that have been largely unmanaged for varying periods of time since their last major 125 disturbance (i.e., clear-cutting or burning). The three sites are described, as follows: (1) the early 126 successional site (last disturbed ca. 20 y ago) consisted of vegetation regrowth but was once used 127 for cattle ranching and, to a lesser extent, seasonal agriculture. (2) The intermediate successional 128 site (last disturbed *ca*. 35 y ago) corresponded to a transitional phase between a mature forest and 129 130 fragmented areas. The matrix surrounding the site consisted of pasture and seasonal corn and bean fields. This site was previously used for seasonal corn production and cattle ranching. 131 Nonetheless, to a large extent, structural and floristic elements have developed so that they begin 132 133 to mirror the original dry forest vegetation. (3) The mature successional stage is characterized by a closed canopy and the presence of a tree cover that is typical of mature dry forest (i.e., 134 135 dominance of the *Bursera* spp.). This site has not experienced a large scale disturbance for more 136 than 50 years.

#### 137

#### 138 Bursera longipes

Bursera longipes is endemic to TDF in the states of Mexico, Morelos, Puebla, Guerrero and Oaxaca in the Balsas basin (Fig. 1; Rzedowski, Medina & Calderón de Rzedowski, 2005). It is a deciduous species with trivalvate fruits that turn red at maturity. The seeds have a slightly orange pseudoaril (Guízar & Sanchez, 1991). The fruits are  $1.3 \pm 0.02$  cm in length (the data represent mean  $\pm$  SE) and  $0.87 \pm 0.04$  cm in width, with a fresh weight of  $0.62 \pm 0.01$  g (N = 100fruits). The flowering season begins with the onset of the rainy season (May or June) and fruiting occurs in early June or from May–July. Most fruits ripen between November and May.

146

#### 147 Seed dispersal effectiveness

The effectiveness of seed dispersal in each successional stage was estimated as proposed 148 by Schupp (1993), by considering the quantity and quality components. The quantity was 149 estimated based on the abundance of frugivorous birds, their frequency of visits to B. longipes 150 tree and average number of fruits removed per visit. The quality was estimated based on the 151 percentage of germination after seeds passed through the digestive system of birds, probability of 152 153 seed deposition at nurse plants (adult plants that positively influence the recruitment of young seedlings) and the contribution of bird species to the establishment sites (Schupp, Jordano & 154 Gómez, 2010). Seed dispersal effectiveness of each frugivore is calculated as the product of the 155 156 components of quantity and quality, using the following subcomponents (Schupp, 1993): Effectiveness = relative abundance x frequency of visits x average number of removed fruits x 157 seed germination x seed deposition probability x contribution of birds to the site of 158 159 establishment.

160

#### 161 **Component of quantity**

#### 162 *Relative abundance*

The relative abundance of birds was estimated using nine mist nets (12 m long x 2.5 m 163 wide) placed between *B. longipes* trees in each of the successional stages. The use of nets to 164 165 capture birds was employed to corroborate and supplement information on species that feed on fruit and to capture data on birds' use of the habitat, relative abundance and feeding activities 166 (Loiselle & Blake, 1999; Figueroa-Esquivel et al., 2009). Nets were placed during the months of 167 168 greatest availability of mature B. longipes fruits (May and December 2010, from January-May, December 2011 and March–May 2012). During each period a total of 15 days of sampling per 169 site was carried out. Mist nets remained open from 0700 to 1800 hours, resulting in a total of 170 171 1485 net-hours per site and 4455 net-hours for all sites.

For each species, the relative abundance was calculated based on the number of individuals captured per net hour, and the data were analyzed in a  $\chi^2$  contingency table to determine whether differences in number of individuals existed between sites. The null hypothesis in this case would be the existence of an equal number of individuals at each site.

176

#### 177 Frequency of visits and average amount of removed fruit

The frequency of visits was determined by focal observations using binoculars (8 x 40 mm). These observations were randomly focused on seven *B. longipes* individuals with ripe fruits at each of the successional stages. The observations were made during January–May 2011 and March–May 2012 in both the morning (0700–1130 h) and afternoon (1600–1830 h) when bird activity is higher. A total of 70 hours of observation were recorded for each site (10 hours / tree), with a total of 210 hours for all three sites.

184 Each tree was observed at a distance of  $\sim 20$  to 30 m for an observational period of 30 min, during which the visitor species, number of individuals per visit, total time per visit (from 185 arrival to departure), number of visits and number of fruits eaten were recorded. The frequency 186 of visits was analyzed in a  $\chi^2$  contingency table to determine differences between successional 187 stages. The null hypothesis would indicate the existence of an equal number of visits between 188 189 successional stages. The number of removed fruits was compared between sites with an unbalanced one-way ANOVA. For this analysis, data were transformed ( $\log x + 1$ ) to meet the 190 assumptions of normality and homogeneity of variance. 191

192

#### 193 Component of Quality

#### 194 Seed germination

195 To determine the effect of the digestive system of birds on the proportion of germinated seeds, we used seeds obtained from the excrement of birds captured by the mist nets. These birds 196 were placed in individual cages (40 x 40 cm) lined with mosquito netting and fed ad libitum with 197 ripe B. longipes fruits for a day after capture. Retention time of seeds was estimated from the 198 199 moment of fruit consumption until defecation. The premise is that longer retention times likely 200 contribute to seeds being spread farther from the mother plant (Westcott & Graham, 2000). After evacuation, the feces were collected, and the birds were released. The techniques used to collect 201 vomit and feces were non-invasive and it was no necessary to have a special authorization. 202

The viability of the seeds was tested with a flotation test, where floating seeds were considered nonviable due to the lack of embryonic development (Thompson, Grime & Mason, 1997). Viable seeds were washed with 10% sodium hypochlorite, planted in cotton on petri dishes at ambient temperature and moistened daily with distilled water. This procedure was

performed to the following three treatments, representing seeds obtained from varying sources:
a) control group 1: seeds with pseudoaril obtained directly from the trees, b) control group 2:
seeds without pseudoaril obtained from trees and c) seeds that passed through the digestive
system of collected birds.

211 For the final category, the germination experiment was performed only with bird species 212 from which the largest number of seeds was obtained: Myiarchus nuttingi (N = 67), Myiodynastes luteiventris (N = 58), Myiarchus cinerascens (N = 33), Melanerpes chrysogenys (N213 = 29) and *Myiarchus tyrannulus* (N = 27). Meanwhile, a total of 50 seeds per site were used for 214 215 each of the controls (fruits obtained from trees). Germination experiments were performed directly in the field, placing boxes with seeds of *B. longipes* under the canopy of nurse plants 216 Mimosa polyantha and Senna wislizenni, which are commonly used by disperser birds for 217 perching. The boxes were protected with mesh mosquito netting and boric acid was poured 218 around the perimeter to avoid predation by ants. Over the course of 20 days, the boxes were 219 220 checked daily to count the number of germinated seeds. Germination was indicated by the emergence of a radicle. 221

The time that seeds remained in the digestive system of birds and the proportion of germination were compared between treatment and controls groups with a one-way ANOVA, using as a null hypothesis the same proportion of germination for all treatments. The data on germination percentages were transformed to arcsine square root, as this is an appropriate technique for analyzing percentages and ratios (Sokal & Rohlf, 1987). Multiple comparisons were analyzed with a Tukey HSD. Analyses were performed in the SPSS 17.0 software (SPSS, 2008).

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#### 230 Seed deposition in secure sites (nurse plants)

231 The deposition of seeds under the cover of trees or shrubs (potential nurse plants) was estimated by focal observations to record the number of visits to these perching sites by birds 232 after fruit consumption. To facilitate the monitoring of birds after they finished eating and 233 departed to fly in another direction or roost on another plant, one person was dedicated to post-234 consumer observations. The number of visits to each of the following categories of perch plants 235 236 was recorded: 1) Permanent, indicating that the individual remained in the same plant where they ate fruit, including displacement to the ends of branches. 2) Mimosa and Acacia trees and shrubs, 237 which have been identified as potential nurse plants in semi-arid environments (Godínez-Álvarez 238 239 & Valiente-Banuet, 1998). 3) Other tree or shrub plants, including nopals or cacti. Focal observations ended when eve contact with the observed individuals was lost. 240

A contingency table of  $\chi^2$  was used to compare the number of bird visits with each category of perch plant. The null hypothesis would indicate of an equal number of bird visits among all perch categories in the three successional stages. Standardized residuals were used to evaluate the preferential use by birds of certain perching sites (Valiente-Banuet et al., 1991; Godínez-Alvarez, Valiente-Banuet & Rojas-Martínez, 2002). These residuals are distributed around a mean of 0 with a standard deviation of 1, so any resulting value  $\geq 2$  (approximately 5% of the normal distribution) was considered to be a significant deviation.

The probability that the seeds were deposited in safe places (*Acacia* and *Mimosa* trees and shrubs) was determined by the proportion of frugivore visits to these perch plants in relationship to the total number of recorded visits.

251

#### 252 Contribution of birds to seedling establishment in different successional stages

Two plots with a radius of 30 m (2 828 m<sup>2</sup> per plot) were randomly chosen in each of the successional sites. In each plot seedlings and young individuals of *B. longipes* (height <50 cm)

255 were counted underneath trees or shrubs used by birds to roost after ingesting *B. longipes* fruits. The number of young *B. longipes* plantlings observed underneath nurse plants was compared to 256 the number of individuals expected to be recruited at random, derived from examining a 257 proportional area and counting *B. longipes* underneath all plants with a DBH > 10 cm (Valiente-258 Banuet et al., 1991). The fulfillment of the null hypothesis would indicate a number of seedlings 259 260 proportional to the coverage of woody plants for the two plots. Standardized residuals were obtained to analyze the significance. Plant cover was determined in a previous study 261 corresponding to the study sites (Almazán-Nuñez et al., 2012). 262

Finally, each bird species was assigned a value of 0-1 according to their contribution towards the establishment of *B. longipes*. This value was estimated from observations of individuals of each bird species after feeding on *B. longipes* drupes, their flight destination, and number of visits to other plants. The maximum value was assigned to birds with the highest frequencies of flights to nurse plants under which the largest number of seedlings or young *B. longipes* individuals had been observed with respect what would be expected by chance, according to the standardized residuals for each plot.

270

271 Results

272 Component of quantity

#### 273 *Relative abundance*

A total of 20 bird species were registered eating *B. longipes* fruits (Table 1). Differences in the abundance of bird species across the three successional stages were significant ( $X^2 =$ 48.26, df = 30, p < 0.05). *Melanerpes chrysogenys, Myiarchus cinerascens, Myiodynastes luteiventris* and *Icterus pustulatus* were abundant and present at all sites (Table 1). In the early successional stage the species with the highest relative abundance was *Spinus psaltria*, while
flycatchers *Myiarchus tyrannulus* and *Tyrannus vociferans* were the most abundant in the mature
stage.

281

#### 282 Frequency of visits and number of removed fruits

The frequency of visits to remove fruit varied between sites ( $X^2 = 54.78$ , df = 38, p < 0.05). *Myiarchus tyrannulus* and *Tyrannus verticalis* were the most frequent visitors to the early successional site (Table 1), *T. vociferans* and *T. verticalis* to the intermediate site and *M. cinerascens* to the mature site. *Spinus psaltria* removed the greatest number of fruit at the early ( $5.00 \pm 1.58$ ) and intermediate ( $4.40 \pm 0.51$ ; Table 1) sites and *E. canicularis* at the mature site 288 ( $11.00 \pm 4.00$ ).

Overall, 17.9% of the counted fruit was removed (n = 825) at the early site, 42.2% at the intermediate site and 39.9% at the mature site, although no significant differences were found among sites ( $F_{2,275} = 1.57$ , p = 0.210). The flycatcher *T. verticalis* remained for the longest time in the trees of the early site ( $6.78 \pm 1.30$  min), while in the intermediate site *T. vociferans* remained the longest ( $6.33 \pm 1.13$  min) and in the mature site *E. canicularis* did so ( $8.00 \pm 4.00$ min; Table 1).

295

#### 296 Seed germination

The shortest average seed retention time from fruit intake until evacuation was recorded for *M. nuttingi* and the highest for *M. tyrannulus* (Table 2). The latter had the widest range in seed evacuation time (minimum = 10 min and maximum = 230 min). The shortest range

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300 corresponded to *M. luteiventris* (minimum = 12 min and maximum = 155 min; Table 2). 301 However, these differences in retention time were not significant ( $F_{4,122} = 0.98$ , p = 0.420).

None of the seeds with pseudoaril (control group 1) germinated (Fig. 2). Seeds without 302 pseudoaril (control group 2) had a germination rate of 10%. The seeds that passed through the 303 digestive tract of *Myiarchus cinerascens* had the highest germination percentage (27%, n = 33), 304 followed by Myiarchus tyrannulus (26%, n = 27), Melanerpes chrysogenys (24%, n = 29), 305 Myiarchus nuttingi (15%, n = 67) and Myiodynates luteiventris (12%, n = 58) (Fig. 2). The 306 resulting differences among treatments were significant ( $F_{5.114} = 9.11$ , p < 0.001). The Tukey 307 308 HSD test indicated that the three bird species with the highest percentage of germination (M). *cinerascens*, *M. tyrannulus* and *M. chrysogenys*) significantly differed in the proportion of seeds 309 germinated in the seeds without pseudoaril (control group 2, Fig. 3). However, significant 310 311 differences were not found in the germination between seeds without pseudoaril (control group 2) seeds eaten by *M. nuttingi* and *M. luteiventris*. 312

313

#### 314 Seed deposition in secure sites (nurse plants)

After consuming the fruits, the birds perched in three categories of plants (Fig. 4). The preference was for *Acacia* and *Mimosa* plants at all three sites ( $X^2 = 22.98$ , df = 12, p < 0.05;  $X^2$ = 55.33, df = 20, p < 0.05;  $X^2 = 54.98$ , df = 20, p < 0.05 for the early, intermediate and mature sites respectively) (Fig. 4a-c). At the intermediate and mature sites, the flycatchers *M. nuttingi* and *M. tuberculifer* remained for the longest period of time in *Acacia* and *Mimosa* plants following feeding episodes, while *Tyrannus verticalis* and *Vireo gilvus* spent more time in trees where they obtainded the seeds. Thus, flycatchers of the genus *Myiarchus* were the most likely

species to deposit *B. longipes* seeds beneath *Mimosa* and *Acacia* trees and shrubs throughout the
 three successional sites (Table 4).

324

#### 325 Contribution of birds to the site of seedling establishment

The lowest density of *B. longipes* seedlings and non-reproductive individuals was found at the early site (0.002 ind / m2). The average height of plants was  $54.07 \pm 7.90$  cm. At the intermediate and mature sites, the densities of seedlings and non-reproductive individuals were 0.007 ind / m2 and 0.008 ind / m2, with and average height of  $50.93 \pm 3.90$  cm and  $53.19 \pm 3.80$ cm, respectively, and did not differ significantly in density ( $F_{2,5} = 0.89$ , p = 0.50) or in average height ( $F_{2,104} = 0.12$ , p = 0.89).

At the early site, the number of seedlings and young *B. longipes* individuals was significantly higher underneath *Tecoma stans*, *Plocosperma buxifolium* and *Mimosa polyantha* plants (Table 3). At the intermediate site, the largest number of seedlings was found under *Mimosa polyantha* and *Calliandra eryophylla*, and for the mature site under *Eysenhardtia polystachya*, *Senna wislizeni*, *Sebastiana pavoniana* and *Acacia cochliacantha* (Table 3). *Acacia subangulata* was the only legume that presented a lower number of observed seedlings than expected by chance (Table 3).

The largest contribution to the establishment of *B. longipes* seedlings (that was calculated based on the number of flights to nurse plants with the largest number of observed seedlings with respect what would be expected by chance), was attributed to *M. cinerascens* at the early site and to *M. nuttingi* at the intermediate and mature sites (Table 4).

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#### 345 *Effectiveness in seed dispersal*

The effectiveness of seed dispersal was estimated for five bird species whose number of 346 visits allowed for a reliable estimation, which varied depending on the site (Table 4). For other 347 species, dispersion was not determined due to lack of defecated seeds or other subcomponents 348 that would allow for assessment. At all sites the best dispersers belonged to the genus Myiarchus. 349 At the early site, only *M. cinerascens* contributed to seed dispersion (Table 4), at the 350 351 intermediate site, *M. nuttingi* was the largest contributor to seed dispersion while in the mature site, five species participated in seed dispersion, and *M. cinerascens* had the highest effectiveness 352 353 (Table 4).

354

#### 355 Discussion

The bird species involve in fruit consumption, removal and dispersion of *B. longipes* seeds were 356 studied in terms of abundance and frequency of their visits to perching sites in the three study 357 sites with different successional phases. B. longipes fruits were consumed by birds in all 358 successional stages, but the identity of the species participating in seed dispersion, their 359 importance and the plants used for perching after feeding varied among sites. Both abundance 360 and the frequency of bird visits were lower in early successional site. This result concurs with the 361 362 reports of Cordeiro & Howe (2003) and Figueroa-Esquivel et al., (2009), whom note that at disturbed sites the number frugivorous bird species and their relative abundance tends to 363 decrease, due to the lower supply of resources. 364

Although several bird species removed many fruits and constantly visited *B. longipes* trees at all three sites, some of them did not contribute to the effective dispersal of its seeds. For example, *S. psaltria* and *E. canicularis* had the highest rate of fruit removal at all three sites. *S. psaltria* bits the seeds' pseudoaril, discarding the seeds underneath the parent plant that reduces

the chances of germination and establishment, due to competition with other conspecifics (Jordano & Schupp, 2000; Bas, Pons & Gómez, 2005). *E. canicularis* destroyed or damaged the seeds upon handling them, thus their contribution to dispersion was negative. In addition, the mortality rate of plant seeds in species of the Malvaceae in the Neotropics has been shown to be high due to similar predation by birds related to this species (i.e., Psittacidae; Francisco et al., (2008)).

Previously it was suggested that species of the *Tyrannidae* family, particularly from the 375 Myiarchus genus, despite being largely insectivorous, could be the main legitimate dispersers of 376 377 Bursera fruits (Ramos-Ordonez & Arizmendi, 2011). In our study, ca. 70% of the seeds at the three sites were removed by Tyrannidae. *Myiarchus spp.* individuals, whose behavior is less 378 379 gregarious compared with other birds that also consume *B. longipes* fruits (e.g., genus *Tyrannus*, 380 Eupsittula, Spinus), removed about 30% of fruits at all of the sites. Two of these species are latitudinal migratory (M. cinerascens and M. nuttingi), and others two make local altitudinal 381 382 migrations (*M. tuberculifer* and *M. tyrannulus*).

In the case of the first two flycatchers, the ripening time of *B. longipes* fruits coincides 383 with the winter presence of these birds. In winter, energy demands increase (Telleria, Ramírez & 384 385 Pérez-Tris, 2005) and the fruits of *Bursera* spp. represent a source of lipids, which are present in the pseudoaril overlaying the seeds (Ramos-Ordoñez, Arizmendi & Márquez-Guzmán, 2012). 386 The distribution pattern of *M. tuberculifer* and *M. tyrannulus* apparently is determined by the 387 388 supply of *Bursera* fruits (Almazán-Núñez et al., 2015), as the two bird species were only present at the study sites during fruiting season. These flycatchers were also observed during the fruiting 389 390 of B. morelensis in the Valley of Tehuacan in Puebla (Arizmendi & Ramos-Ordoñez, 2011), as 391 well as the White-eyed Vireo (Vireo griseus), whose presence and abundance was correlated

with the number of *B. simaruba* fruits in secondary growth forests in the Yucatan Peninsula
(Greenberg, Foster & Márquez-Valdelamar, 1995).

The distribution of *Bursera* spp. and particularly *B. longipes* apparently coincides with 394 that of the Myiarchus genus throughout the Neotropics, and in this study these flycatchers have 395 proven to be its most effective seed dispersers (sensu Schupp, 1995). The distribution of both 396 397 groups is characteristic of semi-arid environments, and both the birds and the plants diversified during the Miocene (Ohlson, Fjeldsa & Ericson, 2008; De-Nova et al., 2012). Both groups are 398 also adaptable to anthropogenic disturbances throughout their range (Howell & Webb, 1995; 399 400 Rzedowski, Medina & Calderón de Rzedowski, 2005), and this also has been a determinant factor of their recent success throughout Mesoamerican tropical forests. 401

402 Overall, the minimum retention time of seeds by frugivorous seed dispersers was greater 403 than the time that they remain on *B. longipes* trees. This indicates they do not defecate 404 immediately after feeding, and therefore seeds are transported and deposited to other sites far 405 from the mother plant, such as under nurse plants (Schupp, 1995; Godínez-Alvarez & Valiente-406 Banuet, 1998; Padilla & Pugnaire, 2006).

Moreover, the germination rate of seeds that have passed through the digestive tract of M. 407 408 cinerascens, M. tyrannulus and M. chrysogenys was significantly higher than seeds without 409 pseudoarile (control group 2), although not in the case of *M. nuttingi* and *M. luteiventris*. While 410 Bursera seeds responded to similar physiological treatments during endozoochory (Stevenson et 411 al., 2002), germination differences between species can potentially be explained by time spent in the digestive system (Dominguez-Dominguez, Morales-Mávil & Alba-Landa, 2006), as M. 412 413 nuttingi and M. luteiventris had a lower retention time (although the differences were not 414 significant). B. longipes seeds are hard and possibly require a longer time in order to soften the

endocarp. The fact that none of the seeds sown with pseudoaril germinated, highlights the importance of frugivorous birds in removing this tissue, as it may contain substances that inhibit the germination of seeds as found in *B. fagaroides* (Ortiz Pulido & Rico-Gray, 2006). Moreover, these bird species consume insects throughout much of the year, and their intestines are usually small and require additional enzymatic attack, contrary to strict frugivores whose digestive system is more long and simple (Jordano, 1986).

The probability that B. longipes seeds are deposited by flycatchers of the genus 421 Myiarchus, in suitable sites for germination and the subsequent development of seedlings, 422 423 confirms that these flycatchers are the most efficient seed dispersers across different stages of successional dry forest. These dispersers often select perches belonging to plants of the Acacia 424 425 and *Mimosa genera*, which have been previously identified as nurse plants throughout the arid environments of Mexico (Valiente-Banuet et al., 1991; Godínez-Alvarez & Valiente-Banuet, 426 1998 Castillo & Valiente-Banuet, 2011). This demonstrates the importance of legumes in aiding 427 seedling establishment as they foster nutrient recycling, accumulation of organic matter and a 428 shadow effect, thereby creating more favorable temperatures that promote the development of 429 native plants (Franco & Nobel, 1989; Bonanomi et al., 2007). 430

Other members of the Tyrannidae family, such as *T.verticalis* and *T. vociferans*, were also considered as dispersers in this study, since they remove fruits in large quantities, swallow whole seeds and typically perch on the top branches of *B. longipes* trees for long periods of time. However, the quality of dispersal by these species was low, as conspecific trees recruitment was not observed at our study sites (see also Godínez-Alvarez, Valiente-Banuet & Rojas-Martínez, 2002).

The observed number of recruited seedlings was lower in the early successional site with respect to the intermediate and mature sites. *Mimosa polyantha* was one of the most preferred nurse species by dispersers for perching, resulting in a higher recruitment of seeds and consequently, the number of seedlings was higher underneath this plant than expected by chance at all stages of succession. Similar results were obtained for other species of the same nurse genus (Castillo & Valiente-Banuet, 2011).

At the mature and intermediate sites, density of recruited seedlings increased due to seed 443 rain from dispersers, but it is also likely influenced by the seed bank in the soil, which may be of 444 445 lower density in earlier successional stages, as found for other DTF sites (Uhl, 1987; Hammond, 1995). The presence of a seed bank fosters a higher rate of germination at the advanced 446 successional stages that along with a high number of disperser visits improve the 447 448 microenvironmental conditions favoring seedling establishment (Valiente-Banuet et al., 1991 Godínez- Alvarez & Valiente-Banuet, 1998; Orozco-Almanza et al., 2003; Padilla & Pugnaire, 449 2006). 450

Although the number of frugivorous birds was lower in the earlier successional stages 451 (Almazán-Núñez et al., 2015), the presence of dispersers that can tolerate disturbed sites 452 453 ultimately contributes to the regeneration of these forests (Griscom et al., 2007). At a lesser density, the number of seedlings recruited underneath mother plants in the early site was greater. 454 However, it is also likely that the process of germination and establishment at this stage is slower 455 456 than for other stages of succession, mainly due to the more inhospitable conditions facing seeds once they are dispersed (Padilla & Pugnaire, 2006; Obeso et al., 2011). In this scenario, the 457 increased presence of legumes at early successional stages, the preference of various dispersers 458 459 to perch on these plants and the adaptability of some zoochorous plants to these new conditions

460 generates greater chances of recovering these habitats. Regional or genetic studies to analyze the 461 spatial variability of seed dispersal are necessary to further understand both the preferences and 462 the movements of the frugivorous birds (Carlo, Aukema & Morales, 2007; Gonzalez-Varo et al., 463 2014), as well as differences they experience plants in different environments.

464

#### 465 Conclusions

The study of sites with different levels of disturbance and local environmental variations can aid in the understanding of biotic seed dispersal at a regional level (Carlo, Aukema & Morales, 2007). It is also possible to analyze mutualistic interactions and local adaptations, where these may be considered to be active processes that have shaped co-evolutions in the geographic mosaic (Thompson, 2005; Kiers et al., 2010; Guimaraes et al., 2011).

471 In this study, the sites varied locally in their soil and climatic conditions and demonstrated differences in structure and species composition (Almazán-Núñez et al., 2012). 472 Although the interaction of the genus *Myiarchus* spp. with the *B. longipes* fruits varied slightly 473 between successional sites, these flycatcher species were the most efficient dispersers of its 474 seeds, as has been previously reported for other species of Bursera (Ramos-Ordoñez & 475 476 Arizmendi, 2011). This suggests that *Bursera* has co-evolved with Tyrannidae family, in particular with Myiarchus flycatchers, positioning both Bursera and Myiarchus as successful 477 members of the arid environments in Mesoamerica. 478

479

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673	Table 1. Relative abundance (individuals/hr-net), frequency of visits (visits/hr-observation),
674	fruits and time spent per visit of birds that consumed B. longipes fruits in three successional
675	stages of tropical dry forest in the Balsas basin of Guerrero. The values shown are as the mean $\pm$
676	standard error.
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#### 680 Table 1.

681 The species with a dash (-) were not collected (relative abundance) or observed visiting trees (frequency of visits).

Family	Species _	Early succession			Intermediate succession				Mature succession				
		Rel.Ab.	Freq. Visit	Fruits/ visit	Time/ visit	Rel. Ab.	Freq. Visit	Fruits/ visit	Time/ visit	Rel.Ab.	Freq. Visit	Fruits/ visit	Time/ visit
Psittacidae	Eupsittula canicularis	-	-	-	-	-	-	-	-	-	0.029	$11.00\pm4.00$	$8.00\pm4.00$
Picidae	Melanerpes chrysogenys	0.0061	-	-	-	0.0074	0.057	$2.25\pm0.25$	$2.75\pm0.48$	0.0054	0.071	$2.66\pm0.56$	$2.83\pm0.53$
Tyrannidae	Myiarchus tuberculifer	-	-	-	-	-	0.043	$2.00\pm0.58$	$2.40\pm0.83$	-	0.086	$1.83\pm0.31$	$4.17\pm0.65$
	M. cinerascens	0.0067	0.086	$2.50\pm0.43$	$3.33\pm0.99$	0.0061	0.257	$2.11\pm0.42$	$2.56\pm0.37$	0.0135	0.243	$2.52\pm0.37$	$4.07\pm0.34$
	M. nuttingi	0.0013	-	-	-	0.0189	0.143	$2.20\pm0.53$	$4.00\pm0.68$	0.0135	0.1	$2.77\pm0.62$	$4.17\pm0.76$
	M. tyrannulus	0.0027	0.129	$3.77\pm0.52$	$5.00\pm0.76$	0.0047	0.143	$2.40\pm0.37$	$4.90 \pm 1.22$	0.0128	0.114	$1.75\pm0.49$	$2.00\pm0.46$
	Myiodynastes luteiventris	0.0054	0.029	$3.50\pm1.50$	$2.75\pm0.25$	0.0108	-	-	-	0.0054	0.071	$4.33\pm0.56$	$5.50\pm0.99$
	Tyrannus melancholichus	-	-	-	-	-	0.014	5	10	-	-	-	-
	T. vociferans	0.0013	-	$1.50\pm0.50$	$1.75\pm0.25$	0.002	0.3	$3.00\pm0.43$	$6.33 \pm 1.13$	0.0054	0.214	$2.56\pm0.52$	$4.41\pm0.60$
	T. verticalis	0.0027	0.129	$4.11\pm0.98$	$6.78 \pm 1.30$	0.002	0.3	$3.52\pm0.59$	$5.76 \pm 1.02$	0.0061	0.129	$5.45\pm0.76$	$6.31 \pm 1.13$
Corvidae	Calocitta formosa	0.0007	-	-	-	-	0.029	$3.00\pm1.00$	$2.50\pm0.50$	0.0013	-	-	-
Vireonidae	Vireo gilvus	-	-	-	-	0.0007	-	-	-	0.0013	0.029	$1.00\pm0.00$	$1.50\pm0.50$
Cardinalidae	Passerina caerulea	0.004	0.043	$1.00\pm0.00$	$1.83 \pm 1.09$	0.0013	0.029	$1.50\pm0.50$	$5.00\pm2.00$	-	-	-	-
	P. versicolor	0.004	0.029	$1.00\pm0.00$	$3.00\pm2.00$	0.0088	0.114	$2.38\pm0.46$	$4.31 \pm 1.02$	0.0067	-	-	-
	P. lechlacherii	0.0074	-	-	-	0.0141	0.029	$2.00\pm0.00$	$3.50\pm0.50$	0.0034	0.029	$1.50\pm0.50$	$5.00 \pm 1.00$
	Pheucticus melanocephalus	-	-	-	-	0.0007	-	-	-	0.0007	0.043	$3.33 \pm 1.86$	$6.83 \pm 4.28$
Emberizidae	Haemorhous mexicanus	0.0027	-	-	-	0.004	0.043	$3.00\pm0.00$	$3.67\pm0.88$	0.002	-	-	-
Icteridae	Icterus wagleri	-	0.029	$2.00\pm0.00$	$2.00\pm0.00$	-	0.029	$2.50\pm1.50$	$5.00\pm2.00$	-	0.029	$5.00 \pm 1.00$	$3.25\pm1.75$
	I. pustulatus	0.0088	0.086	$3.83\pm0.87$	$3.33\pm0.80$	0.0189	0.257	$2.17\pm0.26$	$3.27\pm0.49$	0.0135	0.086	$4.83 \pm 1.33$	$8.50 \pm 1.72$
Fringillidae	Spinus psaltria	0.0074	0.057	$5.00\pm1.58$	$4.25\pm1.16$	0.0007	0.071	$4.40\pm0.51$	$5.00\pm0.89$	0.0061	0.029	$2.00\pm0.58$	$2.83\pm0.17$

682 Table 2. Average seed retention time from point of seed consumption to defecation by

683 individuals. S.E. = Standard error.

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Statistics	Myicin	Myinut	Myityr	Myilut	Melchr
Average time (min)	104	60	129	69	80
Standar Error	11.3	5.8	23.0	8.2	11.0
Minimum	22	18	10	12	7
Maximum	225	179	230	155	155

685 Myilut (Myiodynastes luteiventris), Myinut (Myiarchus nuttingi), Melchr (Melanerpes chrysogenys),

686	Myityr (Myiarchus tyrannulus).
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Table 3. Standardized Residuals (StaRes) for the number of young *B. longipes* seedlings (<50

cm) according to observed (Obs) and expected (Exp) coverage under nurse plants. Residual

values > 2 are significant at a 95% confidence level for a normal distribution.

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		Early			Ir	Intermediate			Mature		
		Succession				Succession			Succession		
Species	Family	Obs	Exp	StaRes	Obs	Exp	StaRes	Obs	Exp	StaRes	
Tecoma stans	Bignoniaceae	1	0.1	3	1	0.3	1.3	0	0	-0.2	
Cordia sp	Boraginaceae	1	1.9	-0.7	0	1.2	-1.1	1	0.8	0.3	
Opuntia sp	Cactaceae	0	0.2	-0.4	1	0.2	1.6	0	0	0	
Senna wislizeni	Caesalpinaceae	2	0.7	1.5	3	1.1	1.8	8	2.9	3	
Senna skinneri	Caesalpinaceae	0	0	0	2	1.2	0.8	0	0.3	-0.5	
Ipomoea pauciflora	Convolvulaceae	0	0.5	-0.7	0	2.7	-1.6	1	1.2	-0.1	
Euphorbia schlechtendalii	Euphorbiaceae	0	0.1	-0.3	0	2.4	-1.6	1	0.7	0.4	
Sebastiana pavoniana	Euphorbiaceae	0	0	0	0	0	0	1	0.2	2.2	
Eysenhardtia polystachya	Fabaceae	0	0.5	-0.7	0	3.5	-1.9	2	0.2	4.4	
Gliricidia sepium	Fabaceae	3	3.5	-0.3	0	3.3	-1.8	0	0.8	-0.9	
Havardia acatlensis	Fabaceae	2	1.5	0.4	3	4.3	-0.6	1	2.7	-1	
Pterocarpus acapulcensis	Fabaceae	0	0.3	-0.6	2	4.1	-1	3	8.5	-1.9	
Plocosperma buxifolium	Loganiaceae	1	0.1	3.1	0	2.7	-1.6	1	0.6	0.5	
Acacia cochliacantha	Mimosaceae	1	1.4	-0.3	2	0.7	1.5	9	3.1	3.3	
Acacia subangulata	Mimosaceae	0	0.9	-1	3	6.1	-1.3	5	13.6	-2.3	
Calliandra eryophylla	Mimosaceae	0	0	0	4	0.7	4	0	0	0	
Desmanthus balsensis	Mimosaceae	0	0	0	0	0.7	-0.9	3	3.5	-0.2	
Lysiloma tergemina	Mimosaceae	0	0.5	-0.7	5	2.5	1.5	2	2.2	-0.2	
Mimosa polyantha	Mimosaceae	2	0.4	2.3	10	1.9	5.9	9	4.6	2.1	
Ziziphus amole	Rhamnaceae	0	0	0	0	0	0	1	0.8	0.2	
Randia sp	Rubiaceae	0	0	0	1	0.3	1.2	0	0	0	
Cissus sp.	Vitaceae	1	1.3	-0.2	6	3	1.8	0	0.5	-0.7	

707 Significant values of standardized residuals (StaRes) for each successional stage are highlighted in bold.

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Table 4. Probability of seed deposition, contribution to the establishment of seedlings and
effectiveness of *B. longipes* seed dispersal by frugivorous birds in a successional gradient of

715 tropical dry forest in southwestern Mexico.

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Species	Probability of <i>B. longipes</i> seed deposition in secure sites			Value establis seedl	of contribution shment of <i>B. lor</i> ings in secure s	to the <i>agipes</i> ites	Seed dispersal effectiveness			
	Early	Intermediate	Mature	Early	Intermediate	Mature	Early	Intermediate	Mature	
C. formosa	-	0.02	-	-	-	-	-	-	-	
I. pustulatus	0.15	0.07	0.05	0.16	0.6	-	-	-	-	
I. wagleri	0.05	0.01	0.02	-	0.2	-	-	-	-	
M. chrysogenys	-	0.02	0.06	-	0.2	0.25	0	1.1 x 10 <sup>-4</sup>	4.2 x 10 <sup>-4</sup>	
M. cinerascens	0.29	0.18	0.17	1	0.2	0.5	0.011	0.003	0.023	
M. luteiventris	0.05	-	0.09	-	-	0.25	0	0	5.4 x 10 <sup>-4</sup>	
M. nuttingi	-	0.18	0.09	-	1	1	0	0.016	0.007	
M. tuberculifer	-	0.12	0.08	-	-	0.25	-	-	-	
M. tyrannulus	0.20	0.14	0.08	-	0.4	0.25	0	0.002	0.001	
T. melancholicus	-	0.02	-	-	-	-	-	-	-	
T. verticalis	0.17	0.14	0.11	-	0.4	0.25	-	-	-	
T. vociferans	0.10	0.08	0.25	-	-	0.50	-	-	-	
V. gilvus	-	-	0.01	-	-	-	-	-	_	
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Figure 1. Projection of the study area and distribution of *Bursera longipes* in the biotic province
of the Balsas basin. Photograph of (a) the rainy season with presence of foliage and in (b) the dry
season with the presence of ripe fruit.



### NOT PEER-REVIEWED

## Peer Preprints

Figure 2. Seed germination of *B. longipes* after passing through the digestive system of birds and
compared with control group 1 (seed with pseudoaril) and control group 2 (seeds without
pseudoaril).





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- 753 Figure 3. Proportion of seeds germinated after passing through the digestive system of birds.
- 754 Different letters among treatments indicate significant differences (Tukey, p < 0.05).
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768	Figure 4. Residuals of a contingency table comparing perching sites for birds after consumption
769	of B. longipes fruit in the three stages of succession: a) early, b) intermediate c) mature. Bars
770	represent frequencies, and significant residuals indicate preference (positive residual) or rejection
771	(negative residual). * $p < 0.05$ , ** $p < 0.01$ .
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Figure 4.

