REGULAR ARTICLE

Different arbuscular mycorrhizal interactions in male and female plants of wild *Carica papaya* L.

Rocío Vega-Frutis · Roger Guevara

Abstract The different resource demands on male and female plants of dioecious species can lead to secondary sexual dimorphisms. Male and female plants might also interact differently with antagonists and mutualists. We used a repeated measures natural experiment in five subpopulations to investigate secondary sexual dimorphism in *Carica papaya* including interactions with arbuscular mycorrhizal fungi. Male and female plants did not differ in size or growth rate, but male plants flowered earlier than female plants. We observed different patterns of root colonization by arbuscular mycorrhizal fungi in male and female plants of *C. papaya*. These differences between the sexes preceded the flowering of most female plants. Female plants were sensitive to changes in soil fertility and adjusted the extent of their root colonization by arbuscular mycorrhizal fungi accordingly. Overall, the different resource demands on male and female plants seem to modulate the interactions of *C. papaya* with arbuscular mycorrhizal fungi.

Introduction

In seed plants, studies have consistently shown a greater overall resource allocation to the female function compared with that to the male function (Goldman and Wilson 1986). There are different resource demands on each sex (Obeso 2002; Case and Ashman 2005) and in dioecious species, in which male and female sexual functions are entirely independent, there are different resource demands on different individuals. Dioecy has evolved in 38–43% of flowering plant families and in around 6% of angiosperm species (Renner and Ricklefs 1995). However, in tropical regions, dioecy is much more common, occurring in over 21% of flowering flora (Bawa 1980). In dioecious species, sex-specific resource allocation patterns have been consistently reported (Lloyd and Webb 1977), and different investments in reproductive functions by male and female plants are associated with male-biased sex ratios in unfavourable environments (Bierziychudek and Eckhart 1988), faster growth rates in males (Obeso et al. 1998), extended longevity in males (de

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R. Vega-Frutis (✉) · R. Guevara
Departamento de Biología Evolutiva, Instituto de Ecología,
A.C. Km 2.5 Carretera Antigua a Coatepec No.351,
Congregación El Haya,
C.P 91070 Xalapa, Veracruz, México
e-mail: rociovegaf@yahoo.com.mx

Present address:
R. Guevara
Department of Biological Sciences, Stanford University,
Stanford, CA 94305, USA

Jong and Van der Meijden 2004), increased flowering frequency in males (Thomas and LaFrankie 1993), and increased resource allocation to anti-herbivore defences in females (Cornelissen and Stiling 2005).

The different resource demands on the male and female plants of dioecious species become evident in ecological correlates such as those listed above. Nevertheless, there has been a conspicuous lack of investigation of sex-related effects on mineral cycling. If male and female plants of dioecious species have different resource demands, it is likely that they differ in their uptake of soil nutrients. The work of Rhoades et al. (1994) with *Simarouba amara* is perhaps the only study in which the sex-specific effects on mineral cycling have been investigated. They suggested that sex-dependent controls on phosphorus may include below-canopy enrichment of the available phosphorus by fruit and litter cycling or increases in phosphorus transformation from bound to labile forms. The latter control on phosphorus may be linked to the activity of arbuscular mycorrhizal (AM) fungi, which access inorganic and organic phosphorus sources that are unavailable to non-mycorrhizal plants (Cardoso and Kuyper 2006). Although some efforts have been made to understand the effects of AM fungi on resource allocation to male and female functions in monoecious and hermaphroditic species (Lu and Koide 1994; Pendleton 2000; Philip et al. 2001; Poulton et al. 2001a, b, 2002), similar studies on dioecious species have just started to appear in the literature (Varga and Kytöviita 2008).

Around 80% of plant species and 92% of plant families (Wang and Qiu 2006) form mycorrhizal interactions. In general, mycorrhizal fungi enhance the uptake of soil minerals and possibly water by plants (Koide 1991; Lee and George 2005). Mycorrhizal fungi also increase plant resistance to drought (Kaya et al. 2003; García et al. 2008) and pathogen attack (Cardoso and Kuyper 2006; Carlsen et al. 2008). Conversely, mycorrhizal interactions are expensive for plants because the fungi can consume up to 4–30% of the plant's photosynthates (Jakobsen and Rosendahl 1990; Finlay and Söderström 1992). However, mycorrhizal interactions in most plants seem to be sensitive to changes in soil fertility (facultative mycotrophy). In general, root colonization by mycorrhizal fungi correlates negatively with the concentration of readily available phosphorus in the soil (Treseder 2004).

Because there are different resource demands on male and female plants, and mycorrhizal fungi enhance

the uptake of soil minerals in most plant species, we investigated sex-dependent patterns of root colonization by AM fungi in dioecious wild *Carica papaya*. A variety of cultivars of *Carica papaya* are grown in most tropical regions and wild populations are common in all those areas. Nevertheless, genuine wild populations are restricted to tropical America (Badillo 1971). *Carica papaya* is classified in the Caricaceae family, and native populations are strictly dioecious (Badillo 1971), whereas hermaphrodite flowers are common in cultivars (Storey 1941). Sexual expression in *C. papaya* is genetically determined by a pair of homomorphic sex chromosomes (Yu et al. 2007). Although some cultivars are know to undergo sex changes when the stems are mechanically damaged (Irons 1908) or with changes in environmental factors: temperature, carbon dioxide, acetylene (Lange 1961), there is no evidence of sex change in native wild populations of *C. papaya*. *Carica papaya* is pollinated by Lepidoptera, but wind-assisted pollination has also been reported (Hernández and Acosta 1992). *Carica papaya* blooms and fruits all year, and cultivars have been reported as mycotrophic (Trindade et al. 2006).

In this study, we aimed to answer three questions. Is there secondary sexual dimorphism in the sizes and pre-reproductive growth rates of *C. papaya* plants? Are there sex-specific patterns of root colonization by AM fungi in *C. papaya*? Does soil fertility affect AM interactions in male and female plants? Because the resource demands on female plants are generally greater than those on male plants, we predicted greater root colonization by AM fungi in female plants, potentially enhancing the uptake of mineral nutrients compared with male plants (see Delph 1990). However, because AM fungi interactions are expensive for the plant, we also hypothesized that female plants are more sensitive to changes in soil fertility than are male plants and that they achieve the best possible balance in resource allocation to growth, defence, reproduction, and the maintenance of mutualists (Lloyd and Webb 1977; Obeso 2002).

Materials and methods

Study site

This study was performed in southern Mexico in the region know as Los Tuxtlas (18°35′07.51″–18°35′

11.12″ N and 95°04′05.09″–95°04′00.41″ W) in an area of 5.8 km² at an elevation of 60–165 m above sea level in the vicinity of the road between La Palma and the Universidad Nacional Autónoma de México's field station. The natural vegetation in the region is tropical low-mountain rainforest. The weather is warm and humid for most of the year, with an average annual rainfall of around 4,700 mm. Most rainfall occurs in the summer (June–September) and autumn–winter (October–February), and the latter is associated with predominantly northerly winds. There is also a marked dry season from March to May. The mean annual temperature is around 26°C, with the highest (36°C) and lowest (12°C) monthly temperatures usually occurring in May and January, respectively (González-Soriano et al. 1997). The soils are andisols, rich in organic matter and mineral nutrients, but phosphates are usually bound to aluminium and iron cations, and phosphorus is a limiting nutriment for plant growth (Sommer-Cervantes et al. 2003).

Sex ratio, time to flowering, sexual dimorphism, and sex change

In February 2006 (cohort-1), we located and marked five subpopulations of C. papaya composed predominantly of 3- to 4-week-old seedlings. All seedlings were tagged, and their sizes were estimated as the height to the apical bud and the basal diameter of the stem. Then, in May 2006, we recorded the height, basal diameter, and diameter at breast height of each of the initially tagged plants. We also tagged newly established plants (cohort-2) and recorded their heights and basal diameters. In September 2006 and February 2007, we recorded the heights and basal and breast-height diameters of all the tagged. In addition to the tagged plants, in September 2006 we recorded the heights and basal and breast-height stem diameters of 100 reproductive plants: 50 males and 50 females. These plants were located along the main road within a distance of 2 km from the main study site. These 100 plants were then mechanically damaged following the standard practice used by farmers to change the sexual expression of male plants in papaya cultivars. We pierced the stem of all 100 plants twice with a machete about 30 cm above the ground, forming a cross-like wound. Then, over a period of 6 months, we checked all the pierced plants for any evidence of sex change.

Arbuscular mycorrhizal fungi

We collected the fine roots from all the tagged plants in February, May, and September 2006 and February 2007. The roots were processed according to the method of Koske and Gemma (1989) and stained with trypan blue. To quantify the percentage of root length colonized by vesicles or arbuscules, we prepared 15–30 root segments (15 mm long) per plant. Each root segment was examined in three equally spaced sections under a light microscope, using the hairpin method (McGonigle et al. 1990). We also estimated the average length of the hyphae of the AM fungi in the root cortex of C. papaya per millimetre of root, and we refer to this hereafter as the "average length of the hyphae". To estimate the average length of the hyphae, we followed Buffon's needle theorem (Schroeder 1974). We used a 1 mm² grid in the ocular of an E600 Nikon microscope with an opening at ×10 magnification of 100 μm and applied the following formula: $LH(\mu m) = \frac{(100\pi I)}{(4F)}$, where LH is the average length of the hyphae, I is the total number of intersections between hyphae and the grid, and F is the total number of observation fields. This method is a modification of that proposed by Giovannetti and Mosse (1980). We also estimated root colonization by AM fungi in the 100 pierced adult plants. The roots of these plants were collected before we inflicted the mechanical damage on their stems. In all cases we traced individual roots to any given focal stem by carefully excavating single roots until reaching the profuse branching of fine roots.

Soil chemistry and root colonization by arbuscular mycorrhizal fungi

In February, May, and September 2006, we collected soil samples from all five subpopulations of C. papaya in which plant growth and root colonization by AM fungi were followed over time. The soil samples were taken from the top 10 cm, and we collected three–six samples from each subpopulation. Because the soil samples cannot be linked to a single plant, the samples from each subpopulation were homogenized before chemical analysis. We quantified soil pH in water (1:2). Readily available phosphorus was estimated with the method of Bray and Kurtz (1945). Carbon and nitrogen concentrations were estimated in an automatic system (LECO, TruSpec),

and soil organic matter was estimated using the Walkley–Black method (Nelson and Sommers 1982). In addition we calculated the C:N and the P:N ratios.

Statistical analysis

To test whether the observed sex ratio departed significantly from the 1:1 expectation, we used a proportion test with expected $P=0.5$. We used mixed effect models (repeated measures, i.e., different seasons) to investigate sexual dimorphism throughout the development of *C. papaya* plants. Sex was defined as a fixed factor, whereas the seasonal effect was incorporated into the random component of the model. We also used mixed effects models to test temporal changes in the average length of the hyphae and in the percentages of roots colonized by vesicles and arbuscules. To meet the model assumptions, the Neperian logarithmic transformation was applied to the average length of the hyphae. We used one-way analysis of variance (ANOVA) to test the size differences between adult male and female plants (pierced plants) and also to test the differences in root colonization by AM fungi. Because root colonization estimates are not independent, ANOVA models were preceded by a multivariate analysis of variance (MANOVA). For the 40 plants that flowered in cohort-1, we used a *G*-test to test the independence of the observed frequencies between the time to flowering (May 2006, September 2006, and February 2007) and sex (male and female plants). We also used a generalized linear model to test whether the average lengths of the hyphae differed significantly between male and female plants once their reproductive activity had been initiated. We used as covariates the estimates of plant size at establishment, and the initial fitted model included up to double interactions. The model was fitted with a gamma error structure, with identity as the link function. The model was simplified until the minimum significant model was achieved. We used mixed effects models to investigate the relationships between soil fertility and root colonization by AM fungi. The response variable was the Neperian logarithm of the average length of the hyphae at the subpopulation level. To summarize soil fertility attributes and to avoid non-independent tests, we used principal components analysis on the variance–covariance matrix. The scores of the first

principal component were used as the random explicative variable. Because there were no male plants in one of the five subpopulations, we performed separate analyses for male and female plants. Model simplification was based on the maximum likelihood ratio test (Pinheiro and Bates 2000). All statistical analyses were performed in the statistical environment R (R Development Core Team 2007).

Results

Sex ratio, time to flowering, sexual dimorphism, and sex change

Overall, we marked 139 seedlings of *C. papaya*, 72 in the autumn–winter rainy season (February 2006), 64 in the dry season (May 2006), and only three in the rainy season (September 2006). Forty plants of the 72 plants tagged in February 2006 reached reproductive maturity: 23 produced female flowers, 17 produced male panicles, and 32 died before any reproductive activity. In contrast, only three of the 64 plants tagged in the dry season flowered: two plants produced female flowers, one produced male flowers, and 61 plants died before becoming sexually differentiated. None of the three plants established in the rainy season survived to the next season. Among the 43 tagged plants that flowered, the operational sex ratio for *C. papaya* did not depart significantly from 1:1 ($\chi^2=0.84$, *d.f.*=1, $P=0.36$) and there was no spatial segregation of the sexes among the subpopulations.

Considering the 40 plants in cohort-1 that flowered, we found a statistically significant departure from the expected independent frequencies of male and female plants in the three periods when the plants were first recorded as flowering ($G=12.0$, *d.f.*=2, $P=0.004$). Male plants tended to flower earlier than female plants. Six of the 17 male plants were first seen to flower in May 2006, whereas only two male plants first flowered in February 2007. In contrast, none of the 23 female plants was seen to flower in May 2006, whereas six were recorded as first flowering in February 2007.

We found no evidence of morphological secondary sexual dimorphism in *C. papaya* (Fig. 1). Male and female plants did not differ in their growth rates (height: *L*. ratio=0.66, *d.f.*=3, 4, $P=0.417$, Fig. 1a; and basal area: *L*. ratio<0.00, *d.f.*=3, 4, $P=0.997$, Fig. 1b), nor was there a significant difference in the

Fig. 1 Growth rate and size of female (*black bars*) and male (*open bars*) plants of *Carica papaya*. **a**, **b** Growth rate was estimated from seedling to reproductive maturity on 23 female plants and 17 male plants. **c**, **d** Size estimates were made on an independent set of plants (50 male and 50 female) engaged in reproductive activity for various months. Values are expressed as means ± SE
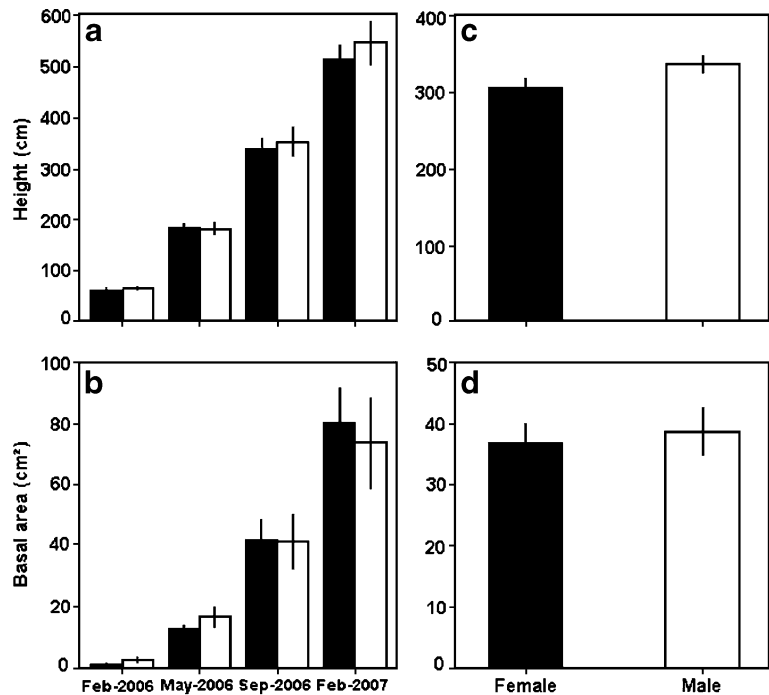
sizes of the male and female pierced plants, which had been reproductively active for several months (height: $F=3.17$, $d.f.=1$, 98, $P=0.078$, Fig. 1c; basal area: $F=0.15$, $d.f.=1$, 98, $P=0.701$, Fig. 1d). Moreover, mechanical damage to the stem caused no sex change in *C. papaya*. Six months after piercing, none of the mechanically damaged plants showed the slightest sign of altered sexuality.

Arbuscular mycorrhizal fungi

The average length of the hyphae increased as the plants aged (Fig. 2a), and the rate of increase differed significantly between male and female plants (*L*—ratio=4.97, $d.f.=3$, 4, $P=0.026$). The average length of the hyphae increased faster in female plants than in male plants. In contrast, the percentage of root length colonized by vesicles (*L*—ratio=2.29, $d.f.=3$, 4, $P=0.130$) and arbuscules (*L*—ratio=1.64, $d.f.=3$, 4, $P=0.200$) did not show a significant temporal trend, and there was no difference between the male and female plants (Table 1). By modelling the length of the hyphae at the time when all the plants were reproductively active (February 2007), we found that female plants (281.9±33 μm) had a significantly ($t=2.6$, $d.f.=29$, $P=0.01$) greater average length of the hyphae than that of the male plants (198.1±41.4 μm).

Furthermore, the average length of the hyphae in male plants (Fig. 2b) just becoming reproductively active correlated negatively with their size at establishment ($\chi^2=6.34$, $d.f=1$, $P=0.012$), whereas no significant correlation was observed in the female plants.

The MANOVA model showed significant effects of sex (Pillai=0.082, $F_{approx}=2.81$ $d.f.=1$, 3, $P=0.044$) on the estimates of root colonization by AM fungi, but there were no significant effects of the interaction between sex and size (Pillai=0.032, $F_{approx}=1.03$, $d.f.=1$, 3, $P=0.384$) or of covariate size on its own (Pillai=0.041, $F_{approx}=1.33$, $d.f.=1$, 3, $P=0.268$). Nor did the average length of the hyphae ($F=1.24$, $d.f=1$, 98, $P=0.27$) or the percentage of root length colonized by vesicles ($F=0.66$, $d.f=1$, 98, $P=0.42$) differ significantly between adult male and female plants. In contrast, arbuscules (Fig. 2c) were over four times more frequent, on average, in the roots of female plants (untagged) than in the roots of male plants, and this difference was statistically significant ($F=7.61$, $d.f=1$, 96, $P=0.007$).

Soil chemistry and root colonization by arbuscular mycorrhizal fungi

Temporal changes in the soil fertility parameters in each of the five subpopulations are presented in Table 2. We
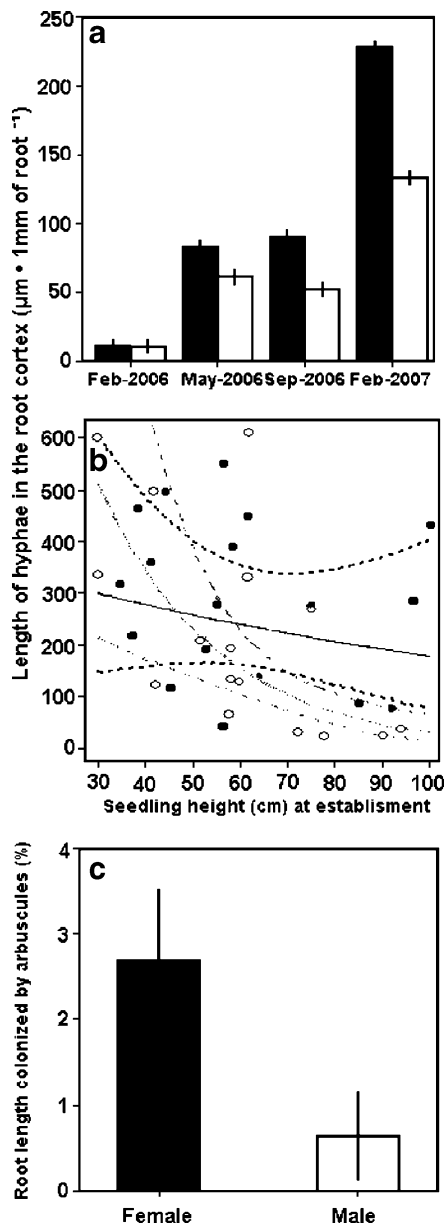
Fig. 2 Root colonization by arbuscular mycorrhizal fungi in female (*black bars* and *circles*) and male (*open bars* and *circles*) plants of *Carica papaya*. **a** Root colonization was estimated from seedling (February 2006) to reproductive maturity. Most male plants first flowered between May 2006 and September 2006 whereas female plants first flowered between September 2006 and February 2007, values are expressed as means ± SE. **b** Size of seedlings and the average length of hyphae of arbuscular mycorrhizal fungi in the root cortex of 1 year old plants of *C. papaya* (February 2007), *dotted lines* are 95% confidence intervals. **c** Percentage of root length with incidence of arbuscules in 50 female and 50 male plants engaged in reproductive activity for various months, values are means ± SE

applied separate principal components analysis to female plants (as described) and male plants because no male plants were recorded in one of the five subpopulations. In relation to female plants, the first principal component fitted to the soil fertility parameters accounted for 98.2% of the overall variability. The N:P ratio showed the highest correlation index (0.996) with the first principal component, whereas all the other variables had positive correlation indices less than 0.001 (Fig. 3a). For male plants, the N:P ratio was the variable with the highest correlation index (0.997) in the first principal component, and the analysis accounted for 98.5% of the overall variability. Because the N:P ratio correlated so strongly with the scores of the first principal component, we used the actual values for the N:P ratio in the subsequent analysis and ignored the other soil mineral nutrients. In the female plants (Fig. 3b), the average root length per observation field correlated positively with the observed N:P ratios, but this correlation differed among seasons (*L*—ratio= 14.12, *d.f.*=8, 12, *P*=0.0069). There was a weak correlation between the N:P ratio and the average length of the hyphae in the two rainy seasons, whereas the correlation was strong and positive in the dry season. In other words, the average length of the hyphae in the female plants increased as the phosphorus limitation increased in the system during the dry season. In

Table 1 Means ± SE root colonization by arbuscular mycorrhizal fungi in female and male plants of *Carica papaya* in 2006 and 2007

	Female	Male
Average length of the hyphae (μm)		
February	12.1±1.6	12.2±1.9
May	125.3±16.7	118.9±27.1
September	134.7±23.1	107.2±25.7
February[a]	281.9±33	198.1±41.4
% of vesicles		
February	0.4±0.3	0.05±0.05
May	2.0±0.5	0.9±0.2
September	1.0±0.4	1.5±0.9
February[a]	1.4±0.5	0.7±0.5
% of arbuscules		
February	0	0
May	0	0
September	4.3±1.5	3.1±1.5
February[a]	2.6±1.3	0.8±0.6

[a] Root colonization by arbuscular mycorrhizal fungi in female and male plants of *Carica papaya* in 2007

Table 2 Soil fertility parameters analysis in the five subpopulations of *Carica papaya* in three seasons of 2006

| | February | | | | | May | | | | | September | | | | |
| | Subpopulations | | | | | Subpopulations | | | | | Subpopulations | | | | |
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
pH	6.0	6.4	6.1	6.4	5.9	5.8	6.2	6	6.3	5.8	6.5	6.6	5.9	6.4	6.2
P Bray mg/Kg	7.1	14.7	4.5	4.2	20.8	7.3	3.7	11.3	2.0	11.9	12.7	6.9	3.3	5.6	9.8
Total C %	4.3	8.1	5.7	6.4	8.9	4.4	6.2	10.7	4.9	6.2	12.7	9.4	4.8	5.2	11.6
Total N %	0.4	0.7	0.5	0.6	0.8	0.5	0.6	1.0	0.4	0.6	1.0	0.8	0.4	0.5	0.9
M.O %	7.5	13.9	9.8	11.0	15.4	7.6	10.7	18.4	8.4	10.7	21.9	16.3	8.3	8.9	20.1
C:N %	10.8	11.6	11.4	10.7	11.1	8.8	10.3	10.7	12.3	10.3	12.7	11.8	12.0	10.4	12.9
N:P mg/Kg	56.3	47.6	111.1	142.9	38.5	68.5	162.2	88.5	200.0	50.4	78.7	115.9	121.2	89.3	91.8

contrast, the average length of the hyphae in the male plants varied significantly among seasons (L—ratio= 19.60, *d.f.*=4, 7, $P<0.001$) but was not significantly affected by the N:P ratio (L—ratio<0.001, *d.f.*=6, 7, $P=$ 0.98) or by the interaction between the season and the N:P ratio (L—ratio=4.73, *d.f.*=7, 10, $P=0.192$).

Discussion

We found that the growth rates of *C. papaya* shoots did not differ significantly between male and female plants. Similarly, the sizes of the adult plants did not differ significantly between the two sexes. In contrast, we found that male plants tended to flower earlier than female plants. The absence of secondary morphological sexual dimorphism in *C. papaya* contrasts with various other examples of fast-growing dioecious perennial species that are sexually dimorphic (Dawson and Geber 1999), whereas the earlier flowering time in the male plants relative to that of the female plants is consistent with a vast number of studies (see Lloyd and Webb 1977; Obeso 2002).

Spatial segregation of the sexes is known in at least 30 dioecious species in over 20 families (Bierziychudek and Eckhart 1988; Eppley 2005). However, we found no evidence of this phenomenon in *C. papaya*. The sympatric distribution of the sexes of *C. papaya* is in agreement with the fact that sexual expression in *C. papaya* is genetically determined by a pair of homomorphic chromosomes (Yu et al. 2007), whereas in most species that display spatial segregation of the sexes, sexual expression is environmentally determined (Bierziychudek and Eckhart 1988). Our data also suggest that sexual expression is immutable in native

wild populations of *C. papaya* at Los Tuxtlas, because even after their stems had been mechanically damaged, none of the plants modified its sexual expression. Overall, the sympatric distribution of the sexes and the genetically determined sex expression of *C. papaya* are consonant with the 1:1 operational sex ratio observed in the five subpopulations at the study site.

We have no estimates of below-ground mass or the below-ground growth rates for focal plants of *C. papaya*. However, Niklas (2005) found strong regression coefficients ($R^2>0.96$) by modelling root:shoot ratios and root and shoot growth rates based on 1,406 data entries for 257 species, including both perennial and annual species. Therefore, it is conservative to assume that the sizes and growth rates of the root systems of the male and female *C. papaya* plants do not differ given that the sizes and growth rates of the shoots do not differ significantly between male and female plants. If the root mass or the growth rates of the roots differed significantly between the male and female plants, then the observed differences in root colonization by AM fungi between plants of the opposite sex could solely reflect changes in the frequencies of stochastic root colonization events by these fungi. Large root systems have more opportunities to encounter AM fungi than do small root systems. Thus, based on the work of Niklas (2005), it is conservative to predict that the sizes and growth rates of the root systems of *C. papaya* will not differ significantly between male and female plants. If this prediction holds true, the hypothesis that differences in root colonization by AM fungi between male and female *C. papaya* plants are the result of stochastic root colonization events by AM fungi has no support. We also found that the size (shoot height) of the male plants at establishment correlated negatively with the average

Fig. 3 a Principal component analysis for soil fertility parameters (including pH, phosphorous; P, carbon, nitrogen, organic matter; OM, nitrogen:phosphorous; N:P and phosphorus:carbon ratios) in five subpopulations of *Carica papaya* (*numbers*) in three different seasons, the winter-rainy season in February (*F*), the dry season in May (*M*) and the summer-rainy season in September (*S*). b Relationship between the N:P ratio in the soil of five subpopulations of *C. papaya* and the average length of hyphae of arbuscular mycorrhizal fungi in the root cortex of female plants of *C. papaya*
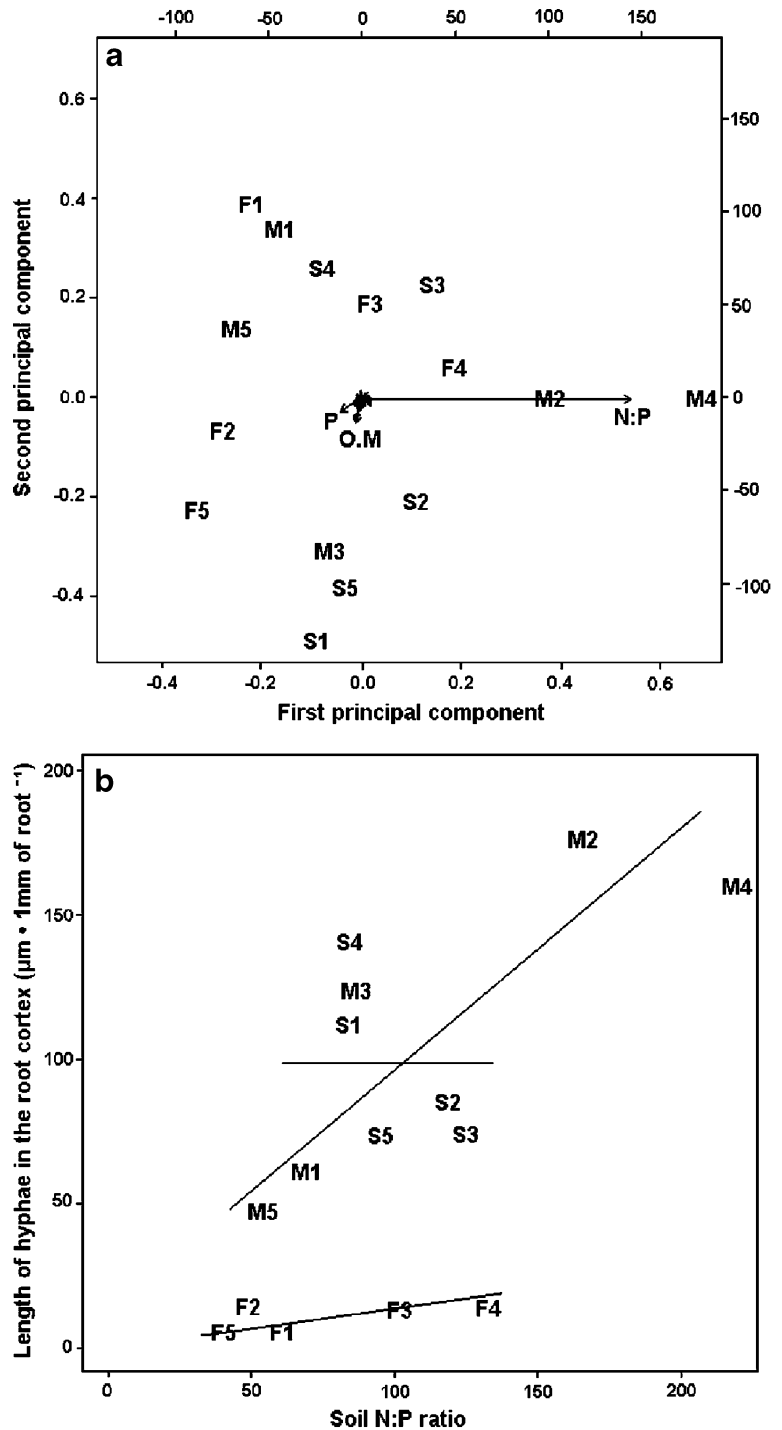
length of the hyphae of AM fungi in the root cortex when the plants were 1 year old. In contrast, in the female plants, the size at establishment did not correlate with the average length of the hyphae. These results suggest that root colonization by AM fungi in *C. papaya* is not primarily governed by stochastic root colonization events. On the contrary, large male seedlings might have a greater capacity to take up mineral

nutrients than do small seedlings, and it seems that the former have less need to interact with AM fungi than do small seedlings. However, there was no correlation between size and root colonization by AM fungi at the seedling stage. Root colonization at the seedling stage was rather low in both male and female plants, and although the variability was not particularly high any potential correlation may have been obscured.

The average length of the hyphae in the root cortex of *C. papaya* increased as the plants aged, and differences in root colonization by AM fungi between male and female plants preceded flowering in most female plants. Furthermore, the observed differences between male and female plants in the average length of the hyphae in the root cortex became even more marked once most of the male and female plants had initiated their reproductive activity.

Competing explanations for the observed differences in root colonization by AM fungi in male and female plants are: (i) different resource demands on male and female plants; and (ii) environmental sex determination. The latter hypothesis seems unlikely because sex expression in *C. papaya* is genetically determined by a pair of homomorphic sex chromosomes. Nevertheless, there have been reports of sex change in *C. papaya* (Lange 1961), but they have been restricted to cultivars, in which the strictly dioecious reproductive system of wild populations has been mutated to favour the occurrence of hermaphrodite individuals (Campostrini and Glenn 2007). We also found no evidence of sex change in *C. papaya* at Los Tuxtlas, and the operational sex ratio did not differ from the 1:1 expectation, whereas in most organisms with environmental sex determination mechanisms, the sex ratios are biased (Werren and Beukeboom 1998). All these observations argue against the hypothesis of environmental sex determination in *C. papaya* mediated by stochastic patterns of root colonization by AM fungi.

On the contrary, the hypothesis that the different resource demands on male and female plants drive the AM interactions in *C. papaya* is supported by most of our findings. The differences in root colonization were statistically significant even before most female plants flowered (May 2006). These differences between males and females increased once both male and female plants were fully engaged in reproduction. Extensive root colonization by AM fungi in female plants favours the acquisition of soil mineral nutrients by these plants (Koide 1991), but female plants would require higher

photosynthetic activity compared with that of males to meet the cost of AM interactions and maintain their growth rates in parity with the growth rates of male plants. Increased photosynthetic activity by plants in response to AM interactions has been reported in various species (Allen et al. 1981; Black et al. 2000; Zuccarini and Okurowska 2008). Various studies have also documented that female plants allocate more resources to leaf tissues in the early stages of development, allowing them to acquire more photosynthates for later allocation to reproduction (Wallace and Rundel 1979; Delph 1990; Delph et al. 1993). Female plants are usually able to do this because although their investment in reproduction is higher overall, their investment early in the season is often lower than that of males (Delph 1990). We also observed a positive correlation between the N:P ratios in the soils of the five subpopulations and the average length of the hyphae in the root cortex of the female plants. These effects of soil fertility on AM interactions in *C. papaya* are consistent with a vast number of experimental (Treseder 2004) and field studies (Guevara and López 2007). Finally, we observed differences in the average length of the hyphae between young male and female plants (tagged) but not in adult plants (untagged). Conversely, we detected significant differences in the frequencies of arbuscules between male and female adult plants but not in young plants. This evidence also suggests that adult female plants have a more vigorous nutrient exchange with AM fungi than do male plants and supports the overall hypothesis that the different resource demands on male and female plants drive the AM interaction in *C. papaya*. The low frequency of arbuscules observed in *C. papaya* is consonant with the Paris-type AM colonization previously reported for this species (Smith and Smith 1997).

Overall, our findings are in complete agreement with the study of Gehring and Whitham (1992), which reported the patterns of root colonization by AM fungi in male and female plants of a dioecious species. Gehring and Whitham (1992) identified a competitive relationship between a parasite (mistletoe) and AM fungi within the male and female plants of *Juniperus monosperma*. They observed that the parasite reduced the resource allocation of *J. monosperma* to AM fungi in both sexes but that this reduction was slightly greater in the female plants than in the male plants. They suggested that the greater effect of the competition between the parasite and AM fungi in the female plants

is a consequence of the higher resource demands on female plants because of their reproductive activity compared with those on male plants. In contrast Varga and Kytöviita (2008) found no differences in root colonization in male and female plants of *Antennaria dioica*, not even when the plants were grown in a water stress regime that simulated the environmental conditions in which male plants grow in wild populations. However, Varga and Kytöviita (2008) found that female plants derived larger benefits (biomass and phosphorous content in shoots) from AM interactions than did male plants; these benefits are in agreement with our hypothesis and observations on *C. papaya*.

In conclusion, *C. papaya* showed no secondary sexual dimorphism in the size and growth rate of the shoot, but the time to flowering was shorter in male plants than in female plants. We also observed different patterns of root colonization in the male and female plants of *C. papaya*, and these differences seem to be related to the different resource demands on male and female plants. Females had a greater investment in AM fungi compared with that of male plants, presumably to enhance their uptake of soil nutrients. Female plants were also sensitive to changes in soil fertility, and their investment in AM interactions decreased as the concentration of readily available phosphorus increased in the soil.

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