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Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects

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Running title: *Arbuscular mycorrhizas and insect pollinators*

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Abstract. 1. Arbuscular mycorrhizal (AM) fungi can increase a number of plant traits to which pollinating insects are known to respond. These include total plant size, flower number, flower size, and amount of pollen produced.

2. It was hypothesised that these effects would lead to a different visitation rate of pollinating insects on mycorrhizal and non-mycorrhizal plants. To test this idea, three species of annual plants (*Centaurea cyanus*, *Tagetes erecta* and *T. patula*) were grown with and without AM fungi and the visits by pollinating insects were recorded over a two month period.

3. In all three species, mycorrhizal plants experienced a greater number of pollinator visits per flower per unit time. Diptera and Hymenoptera were the predominant insects and the latter order showed the strongest response.

4. Here, it is suggested that mycorrhizal fungi increase floral visitation rates by insects, but that the mechanism varies from one plant species to another. In *C. cyanus*, it appears to be due to flower number per plant, in *T. patula* it is individual inflorescence size, and in *T. patula* it is nectar standing crop per inflorescence.

Keywords. Arbuscular mycorrhiza, insect, multitrophic interactions, pollination.

Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with about 70% of all vascular plants (Hodge, 2000). In most environmental conditions, these fungi are beneficial to their host plants, by providing access to limiting soil nutrients, or increasing drought resistance, photosynthetic rate and resistance to insect herbivores and fungal pathogens (Smith & Read, 1997). A number of studies have investigated the interactions between AM fungi and invertebrates, in the quest to understand plant-mediated links between above- and below-ground organisms (Wardle *et al*., 2004).

Virtually all the experiments with insects and AM fungi have been laboratory based, (reviewed by Gange & Brown, 2002 and Gehring & Whitham, 2002). In most cases, these experiments have involved insects confined upon their host plants, whereupon positive or negative effects on insect growth and survival have been recorded. There are very few instances in which host plant selection by phytophagous insects was considered and when it has, mycorrhizal presence seemed to have no effect on insect choice, even though the mycorrhizal plants were larger than non-mycorrhizal individuals (Gange & Nice, 1997; Gange *et al*., 2003). Furthermore, most of the insect species used have been phytophagous, with the potential to harm the plant in some way by their feeding. However, many insects are of great benefit to plants, by facilitating the process of pollination. Pollinating insects have been a major factor in the evolution of angiosperm diversity (Crepet, 1983), and, like mycorrhizal fungi, can be considered as plant mutualists.

To date, only one study has examined whether AM fungi can affect the behaviour of pollinating insects (Wolfe *et al*., 2005). These authors found that mycorrhizal colonization of fireweed (*Chamerion angustifolium* L. Holub) increased pollinator visitation rate to plants, probably caused by the fact that mycorrhizal plants were larger and bore more flowers. This provides an interesting contrast to the lack of herbivore choice of mycorrhizal plants (above)

and suggests that the belowground mutualism may have positive influences on plant selection by above ground mutualists. As Stanton (2003) points out, most studies of mutualisms involve two species or trophic levels only and these must be extended now, so as to understand the importance of multitrophic interactions at the ecological and evolutionary scales (Strauss & Irwin, 2004).

Wolfe *et al*. (2005) comment that more studies are needed to assess whether plant traits other than size affect pollinating insects. Indeed, it is likely that pollinating insects will show different plant choice responses to those of herbivores, because a number of floral parameters known to be altered by AM colonization are important determinants of pollinator behaviour. For example, mycorrhizas are known to increase the size of individual flowers (Gange *et al*., 2005) and the honey bee (*Apis mellifera* L.) preferentially selects larger flowers (Waser, 1983; Martin, 2004). Mycorrhizas increase the number of flowers per plant (Koide, 2000) and pollinator visits may be positively correlated with floral display size (Thompson, 2001). Finally, mycorrhizas may increase the floral rewards for insects, through enhanced pollen production (Poulton *et al*., 2002) or nectar quality. It is therefore hypothesised that mycorrhizal plants would experience greater numbers of pollinator visits and this idea was tested with three species of annual plants, all of which are attractive to pollinating insects (Comba *et al*., 1999).

Materials and methods

Plant propagation

Three species of annual flowering plant, Cornflower *Centaurea cyanus* L., French marigold *Tagetes patula* L. and African marigold *Tagetes erecta* L. were chosen for this

study, due to their known associations with AM fungi and their attractiveness to pollinating insects (Comba *et al*., 1999; Linderman & Davis, 2004). In April 2002, seeds of each species were germinated in sterile sand. Seedlings were transplanted singly into 5 cm diameter pots, containing 150 g of John Innes No. 1 sterilised compost (Gem Gardening, Accrington, U.K.) and grown in a constant environment room (18:6 L:D) at 20°C for five weeks.

Mycorrhizal inoculum was prepared by the method described in Gange *et al*. (2003). Briefly, a field site was sown with a wildflower meadow seed mixture in spring 1996. *C. cyanus* was one of the dominant members of the vegetation in 1996 and persisted in the community until 1998. Spores of mycorrhizal fungi were isolated from the site in summer 1998 and single spore cultures of the two commonest species, *Glomus mosseae* (Nicol. & Gerd.) and *G. intraradices* Schenck & Smith, were established on the roots of *Plantago lanceolata* L. seedlings, grown in inert expanded clay granules (Seramis®, Pedigree Petfoods, Melton Mowbray, U.K.). Bulk inoculum was prepared over a three year period by continually sub-culturing on to new *P. lanceolata* seedlings. At the end of this time, plants were allowed to die and the dry granules, containing roots, spores, and hyphal fragments were used as the inoculum. Observations of soil surrounding *C. cyanus* roots in 1997 showed that *G. mosseae* and *G. intraradices* were consistently found together and so a mixed inoculum was used for this experiment.

In May 2002, after 5 weeks of growth, 20 even-sized seedlings of each plant species were selected and each transplanted into a 13 cm pot, containing 450 g John Innes No. 2 sterilised compost. Plants were inoculated with AM fungi by spreading 1.5 g of mixed (0.75 g of each species) dry inoculum in a layer 5 cm beneath the final surface of the compost, adjacent to the periphery of the root system. There were two experimental treatments, inoculation with mycorrhizas and inoculation with autoclaved granules (control). There were ten replicates of each treatment for each plant species, giving 60 plants in total.

Observations

The 60 plants were placed in a glasshouse and watered twice daily with 50 ml water for a further 8 weeks, by which time all plants were flowering. They were then transferred to an outdoor observation area, measuring 5.5m x 4m, and bordered on all sides by a 1.6m high metal mesh fence to deter mammalian herbivores. Every plant was placed in a plastic tray, half filled with Horticultural Grade Lime-Free Washed Quartzite grit (Sinclair Horticultural, Gainsborough, U.K.) to prevent any mycorrhizal colonization of roots from the surrounding soil, while maintaining drainage. Plants were placed in a randomised block arrangement, with a 30 cm gap between each pot.

Pollinator visits were recorded on 48 separate occasions during June and July 2002. Observations were only made on calm, warm, sunny days that provided optimum foraging conditions for pollinating insects. On each day, recording took place between 13.00 – 14.00, coinciding with the time of peak nectar production and pollinator activity (Comba *et al*., 1999). Within this recording interval, each plant was observed for a one minute period, in which the number and identity (to insect order) of visiting insects was recorded. Insects were not identified to species because of the need to not disturb individuals visiting flowers during each recording period. The order in which plants were observed was randomised on each recording day. This gave a total of 48 minutes recording time for each individual plant, or 16 h per species. The total number of open flowers was recorded on each plant on each sampling occasion. No inflorescences were removed from the plants during the observation period.

Nectar standing crop and secretion rate were measured on three separate occasions at the start, middle and end of the observation period, following the method of Comba *et al*. (1999). On each occasion, three separate flowers on each plant of *C. cyanus* were sampled, while for

the two species of *Tagetes*, three disc florets (which contain the nectar) were sampled at random within each of three capitula per plant and a mean calculated for each capitulum. Nectar was withdrawn into a glass microcapillary and sugar content measured with a hand held refractometer (Corbet, 2003). Flowers that had been emptied for the standing crop measurements were marked with a quick-drying indelible pen and immediately bagged in muslin. These were then re-sampled after 120 min, to estimate nectar secretion rate, as recommended by Comba *et al.* (1999). Flower (*C. cyanus*) or capitulum size (*Tagetes*) was measured on four separate occasions by recording the diameter of three randomly selected mature inflorescences on each plant. At the end of August, plants were harvested and the total number of flowers or capitula produced over the season was recorded. Five intact dry inflorescences were randomly picked from each plant and all seeds in each counted and weighed. Mean total seed number per inflorescence and individual seed weight per plant was calculated for analysis. Each plant was carefully excavated and the roots washed free of soil. A 2 g sample of roots was taken from each plant and stained to reveal mycorrhizal colonization using the acidified ink method (Vierheilig *et al*., 1998). Colonization was measured using the cross-hair eyepiece method of McGonigle *et al*. (1990), with a minimum of 200 intersections per slide. Total dry root and shoot biomass was recorded for each plant, after correction for the loss of 2 g of root.

Statistical analyses

All analyses were conducted using plants as replicates. Insect visits were standardized by calculating the number of visits per flower per plant on each sampling occasion. These data were summarised over the season by taking an average of the 48 observations and then subjected to factorial ANOVA using plant species, AM fungi and Block as the main effects.

The same ANOVA model was used to examine mycorrhizal effects on root and shoot biomass, total inflorescence number, inflorescence diameter, seeds per inflorescence and seed weight. Biomass measurements were log transformed while other plant parameters were square root transformed prior to analysis. Nectar sugar content and secretion rate were subjected to a Repeated Measures Analysis of Variance, employing mycorrhizal treatment and date as main effects. Multiple linear regression was used to examine whether the frequency of insect visits was a function of flower number, flower size or nectar quantity. These analyses were performed using only the replicate control plants, to remove any potential bias of treatment. All analyses were performed with the UNISTAT® statistical package.

Results

Plant traits

No mycorrhizal colonization was detected in any of the control plants, while all plants in the inoculated treatments showed evidence of colonization. For *C. cyanus*, the mean percent root length colonized (% RLC) was 15.4 ± 2.8 %, for *T. patula* it was 14.1 ± 0.9 % and for *T. erecta* it was 11.9 ± 1.5 %. These levels compared well with plants of *C. cyanus* extracted from the wildflower meadow, which had a colonization range of 0 – 26%, with a mean of 13.8 ± 4.9%.

Mycorrhizas had a positive effect on above ground biomass (Table 1), although the effect was only significant in *C. cyanus*, with control plants having a mean of 5.42 ± 1.3 g, compared with 11.2 ± 0.9 g for colonized plants. In *T. patula*, mycorrhizal plants weighed 9.7 ± 1.7 g, 20% larger than controls, while in *T. erecta* mycorrhizal plants (21.3 ± 4.6 g) were only 10% larger. An identical pattern was found for root biomass (data not shown).

Mycorrhizas had a considerable effect on total flower number in *C. cyanus*, increasing this by nearly 70% (Fig. 1, Table 1). In *T. patula*, there was a small but significant increase in the number of capitula on mycorrhizal plants, but no effect was seen in *T. erecta* (Fig. 1).

In contrast to flower number, flower size was unaffected in *C. cyanus*, but increased by mycorrhizas in the two species of *Tagetes* (Fig. 2, Table 1). In *T. patula*, flowers on mycorrhizal plants were, on average, 4 mm greater in diameter than those on control plants (an increase of 7%), while in *T. erecta*, this difference was nearly 10 mm, (an 11% increase).

Mycorrhizas had no effect on nectar sugar content in either *C. cyanus* or *T. patula*, but in *T. erecta*, sugar content was increased by colonization ($F_{1,18} = 10.9$, $P < 0.01$). Control plants had an average of 0.042 ± 0.005 mg sugar per floret, while the value for mycorrhizal plants was 0.065 ± 0.009. Meanwhile, mycorrhizal fungi increased the nectar secretion rate in both species of *Tagetes*. In *T. patula*, control plants had an average rate of 0.027 ± 0.0016 mg sugar floret^{-1} h^{-1}, while mycorrhizal plants produced nectar at the rate of 0.049 ± 0.0021 mg sugar floret^{-1} h^{-1} ($F^{1,18} = 15.2$, $P < 0.001$). Meanwhile, in *T. erecta*, control plants had an average rate of 0.062 ± 0.0011 mg and mycorrhizal plants a rate of 0.093 ± 0.024 mg sugar floret^{-1} h^{-1} ($F_{1,18} = 6.8$, $P < 0.05$).

All three plant species produced a greater number of seeds per flower or capitulum when mycorrhizal (Table 2). A significant interaction between species and mycorrhiza was found (Table 1), because the effect was most clearly seen in *C. cyanus*, where mycorrhizal plants produced nearly twice as many seeds as uncolonized individuals. Mycorrhizas also increased the average seed weight in *C. cyanus* (Table 2) but had no effect on this parameter in either species of *Tagetes*, leading to another significant interaction term in the analysis (Table 1).

Insect visits

Insect pollinators were dominated by Hymenoptera (particularly *A. mellifera* and some individuals of *Bombus* spp.) and Diptera. A few individuals of Lepidoptera and Coleoptera were also noted. In all three plant species, the total number of insect visits flower^{-1} minute^{-1} was significantly increased by mycorrhizal presence (Fig. 3a, Table 1). In *C. cyanus,* the effect was considerable, with flowers on mycorrhizal plants receiving twice as many visits as control plants ($F_{1,16}$ = 17.6, P < 0.001). In *T. patula,* mycorrhizas increased visits by 62% ($F_{1,18}$ = 9.7, P < 0.01), while the most dramatic effect was seen in *T. erecta*, with mycorrhizal plants having over three times the number of visits per flower recorded on control plants. The fact that the strength of the effect differed between plant species was shown by a significant species x mycorrhiza interaction term in the ANOVA (Table 1).

Visits by Hymenoptera were most common and to an extent, effects on this order were responsible for those seen in the total number of visits, with the mycorrhizal effect being strongest on *C. cyanus* and *T. erecta* (Fig. 3b, Table 1). A significant interaction term was again found, because the mycorrhizal effect was not consistent across plant species. Meanwhile, mycorrhizas only caused an increase in visits by Diptera to *C. cyanus* and no effect was seen in either species of *Tagetes* where visits by these insects were far fewer (Fig. 3c).

In *C. cyanus,* plants with more flowers received more visits by all insects per flower (Table 3), while no such effect of plant size could be found in either species of *Tagetes*. In *T. patula,* floral visits were related to flower size in that larger flowers attracted more visits per unit time. There was a similar weak relationship in *T. erecta* (P = 0.066), but in this species a significant positive relation was found between floral visits and nectar sugar content (Table 3).

Discussion

It is clear that the effects of AM fungi on floral parameters vary from one plant species to another. In *C. cyanus*, flower number was increased by mycorrhizas, but flower size unaffected. The flowers of *T. erecta* were relatively large, but far fewer in number and this plant responded to mycorrhizas by increasing flower size, rather than number. Meanwhile, *T. patula*, with intermediate numbers and size of flowers, responded to colonization with an increase in both these traits.

The levels of mycorrhizal colonisation in this study were comparable with field conditions, but lower than some other reports (Linderman & Davis, 2004). What is interesting is that despite these levels, mycorrhizas increased insect pollinator visits in all three species of plants. The effect was particularly noticeable with Hymenoptera, which showed a consistent pattern of increase across all three plant species. Diptera pollinators were relatively rare in this study, but mycorrhizal *C. cyanus* still experienced three times the number of visits by these insects, compared with non-mycorrhizal plants of this species. Insect pollinators may respond to a variety of floral parameters, including colour, size of inflorescence, and floral reward (quality and quantity of nectar). Some of the earliest experiments involving pollinator attraction were reviewed by Waser (1983), where it is shown that target size is more important than exact colour in attracting an insect to flowers on a plant. Insects respond positively to large floral displays (Thompson, 2001) and in *C. cyanus* non-mycorrhizal plants, a significant relationship was found between visits per flower and the total number of flowers on a plant. It is therefore likely that the greater number of flowers on mycorrhizal plants was responsible for the increase in pollinator visits. Wolfe *et al*. (2005) found that mycorrhizal plants of *C. angustifolium* also attracted more pollinator visits, though there are several

interesting differences between their data and those reported here. Firstly, Wolfe *et al*. (2005) did not detect any difference in visits per flower on mycorrhizal and non-mycorrhizal plants. Their conclusion was that AM fungi caused plants to be larger, thus bearing more flowers and so attracting more pollinators. It has been shown in this study that the mechanism is more subtle than this and that mycorrhizas increase the frequency of visits to individual flowers as well. A second difference is that Wolfe *et al*. (2005) only recorded Hymenoptera and these data show that other floral visitors such as Diptera respond in a similar way, thus showing a generality in the effect.

Wolfe *et al*. (2005) did not measure individual flower size, but pollinating insects are known to select larger flowers (Elle & Carney, 2003; Martin, 2004). A significant relation between capitulum size and visitation rate was found, this is likely to be the reason why mycorrhizas increased visitor frequency to *T. patula*. In this plant species, no relation between flower number and floral visits was found. Although AM fungi did cause a small, but significant increase in flower number, it is less likely that this factor resulted in the increased visitation rate. Capitulum size in *T. erecta* was also increased by AM colonization, though the relation between this trait and visitation rate was less clear. Mycorrhizas are known to increase individual flower size in other plant species (Koide, 2000; Gange *et al*., 2005) and this may have important consequences for the behaviour of pollinating insects.

Nectar reward is also important in the floral selection process (Comba *et al*., 1999) and in this study, mycorrhizas were found to increase the sugar content and secretion rate in *T. erecta*. To present knowledge, this is the first report of mycorrhizas affecting nectar quantity and quality, and it may also have contributed to the increased visitor number on mycorrhizal plants. *T. erecta* was the only plant in which a significant relation was found between visitation rate and nectar standing crop, showing that the mycorrhizal effects on pollinating insects may be very subtle indeed. The original hypothesis was therefore upheld, although the

mechanism by which AM fungi affect pollinating insects seems to vary from one plant to another.

The mycorrhizal-induced increase in quantity and quality of resource for pollinators may be important for these beneficial insects. AM fungi are known to increase the size of pollen grains and the total amount of pollen per flower (Lau *et al*., 1995; Poulton *et al*., 2002) and it has been shown that flowers on mycorrhizal plants are more attractive to insects that require pollen. Thus the process of gathering pollen by a bee or hoverfly may be more efficient on a mycorrhizal plant and it would be rewarding to measure pollen loads of insects visiting mycorrhizal and non-mycorrhizal plants. The fact that mycorrhizas appear to alter nectar sugar content is also important, and may be due to an enhancement of carbon fixation in mycorrhizal plants (Smith & Read, 1997). Stabentheiner (2001) has shown that honey bees returning to a hive communicate the location of high quality nectar sources through their dancing patterns. If mycorrhizal plants provide higher quality sources, then this may result in more efficient foraging and more visits per plant.

Mycorrhizal effects on pollinating insects may also have important consequences for the reproduction of plants. In all three of the study plants, seed production was increased on mycorrhizal plants. This phenomenon has been reported before (Koide, 2000) and has been explained by the mycorrhiza providing an enhanced supply of limiting nutrients, particularly phosphate. These results suggest that increased visits by pollinating insects may also be a reason for enhanced seed set. It would be interesting to perform controlled experiments involving pollen addition to mycorrhizal and non-mycorrhizal plants, similar to that performed by Nuortila *et al*. (2004) but with and without insect pollinators, to really tease apart the direct and indirect effects of the fungi and insects.

These results may also have significant implications for plant community structure. It is accepted that the plants studied here do not co-occur in nature and it would be instructive to

repeat this study with co-occurring plants in natural communities. Clearly, certain plants in a population that are mycorrhizal could be visited more by pollinators and have an enhanced seed set, compared with non-mycorrhizal conspecifics. Given that these mycorrhizal plants are likely to be larger and may have reduced herbivore attack (Gehring & Whitham, 2002, though see also Gange *et al*., 1999) and that their offspring may be more vigorous (Koide & Lu, 1995) they could provide a disproportionate contribution of genetic material to the next generation (Shumway & Koide, 1995; Koide & Dickie, 2002). It is known that mycorrhizas can change the structure of plant communities through differential effects on growth and competition (Hartnett & Wilson, 2002) but these results suggest that they may have other, more subtle effects on plant population genetic structure also. That these effects are mediated by higher trophic level organisms emphasises the intricacy of the multitrophic interactions that exist in communities.

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Table 1. Results of statistical analyses of plant and insect parameters d.f. for plant species effect = 3, 54, for AM fungi = 1, 54 and interaction term = 2, 54. Bold values indicate significant differences ($P < 0.05$).

	Plant species		AM fungi		Interaction (species x fungi)	
Parameter	*F*	*P*	*F*	*P*	*F*	*P*
Root biomass	91.3	**< 0.001**	0.79	0.376	2.76	**0.072**
Shoot biomass	73.5	**< 0.001**	10.63	**0.002**	2.35	0.104
Total inflorescence number	50.51	**< 0.001**	18.86	**< 0.001**	15.11	**< 0.001**
Inflorescence diameter	331.9	**< 0.001**	8.39	**0.0054**	1.44	0.245
Seeds per inflorescence	1198.5	**< 0.001**	12.54	**< 0.001**	5.99	**0.004**
Seed weight	5.57	**0.006**	14.4	**< 0.001**	7.42	**0.001**
Total insects visits per inflorescence	7.64	**0.001**	39.57	**< 0.001**	7.39	**0.001**
Hymenoptera visits per inflorescence	9.55	**< 0.001**	31.06	**< 0.001**	9.92	**< 0.001**
Diptera visits per inflorescence	6.84	**0.002**	7.71	**0.008**	2.08	0.134

Table 2. Seed production of the three plant species. Values tabulated are means ± one standard error. For statistical results, see Table 1.

	Seed number per inflorescence		Seed weight, mg	
Plant	Control	Mycorrhizal	Control	Mycorrhizal
C. cyanus	14.8 ± 3.3	22.2 ± 0.9	1.88 ± 0.51	5.48 ± 0.72
T. patula	76.8 ± 1.8	83.3 ± 1.9	2.39 ± 0.17	2.53 ± 0.14
T. erecta	347.9 ± 12.6	401.5 ± 13.5	3.55 ± 0.63	3.95 ± 0.41

Table 3. Summary of results from multiple regression analysis, examining effects of plant traits on total insect visits per flower. All degrees of freedom: 1, 8. Bold values indicate significance ($P < 0.05$).

	F	r^2	*P*
C. cyanus			
Flower number	12.24	0.605	**0.008**
Flower size	0.68	0.078	0.434
Sugar content	0.45	0.086	0.462
T. patula			
Capitulum number	0.934	0.104	0.366
Capitulum size	8.08	0.502	**0.022**
Sugar content	1.53	0.266	0.251
T. erecta			
Capitulum number	0.866	0.097	0.379
Capitulum size	4.51	0.363	0.066
Sugar content	7.54	0.444	**0.038**

Figure legends

Fig. 1. Mean total number of inflorescences produced by *Centaurea cyanus, Tagetes patula* and *T. erecta*, grown with mycorrhizas (shaded bars) or without mycorrhizas (open bars). Vertical lines represent ± one standard error. Asterisks above bars indicate significant pairwise differences between means, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 2. Mean inflorescence size of *C. cyanus, T. patula* and *T. erecta* with mycorrhizas (shaded bars) or without mycorrhizas (open bars). Vertical lines represent ± one standard error. Asterisks above bars indicate significant pairwise differences between means, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3 (a) Mean total insect pollinator visits per flower (or per capitulum) per minute, (b) visits by Hymenoptera and (c) visits by Diptera, over a two month observation period, with mycorrhizas (shaded bars) or without mycorrhizas (open bars). Vertical lines represent ± one standard error. Asterisks above bars indicate significant pairwise differences between means, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 1

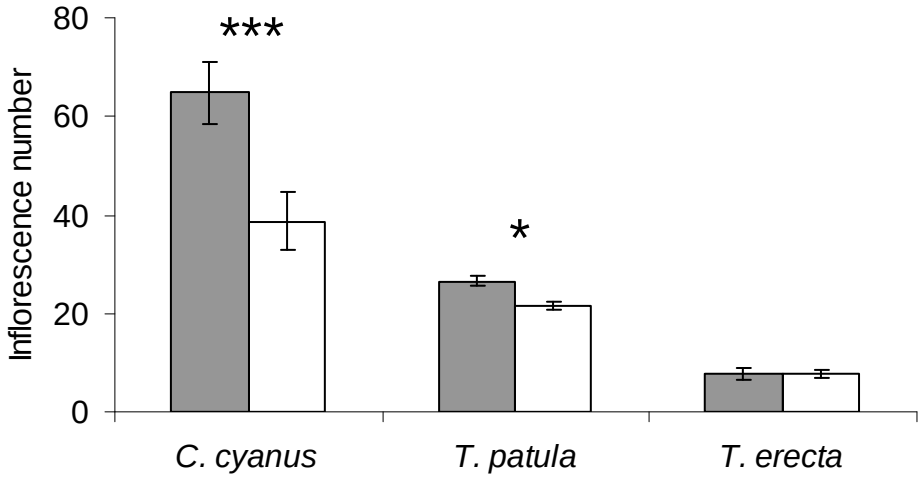


Figure 2

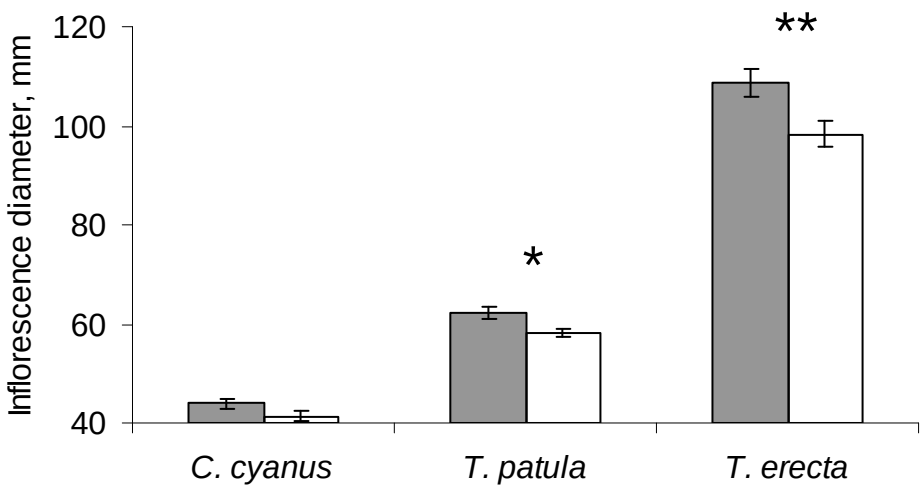
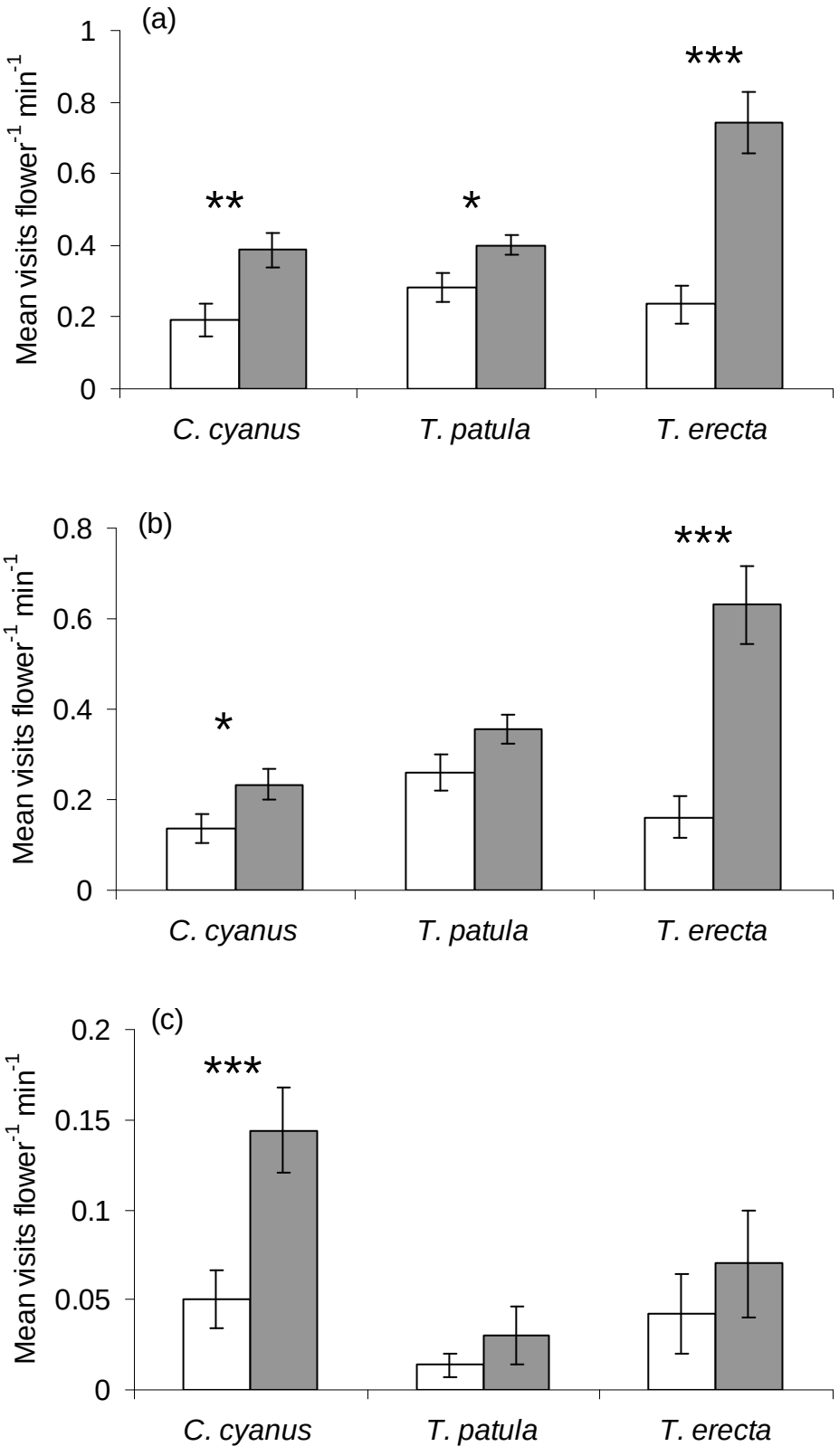

Inflorescence diameter, mm
120
100
80
60
40
*
**
C. cyanus
T. patula
T. erecta

Figure 3


(a)
Mean visits flower-1 min-1
1
0.8
0.6
0.4
0.2
0
**
*
***
C. cyanus
T. patula
T. erecta
(b)
Mean visits flower-1 min-1
0.8
0.6
0.4
0.2
0
*
***
C. cyanus
T. patula
T. erecta
(c)
Mean visits flower-1 min-1
0.2
0.15
0.1
0.05
0
***
C. cyanus
T. patula
T. erecta