DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: Tropical Biology Association Uganda 2012

Travel Dates: 27/06/2012 – 05/08/2012

Location: Kibale Forest National Park, Uganda

Group Members: Huw Richards

Aims: To learn about tropical ecosystems and carry out a project

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Outcome (not less than 300 words):-

This course vastly exceeded my own expectations. The course is set in an incredible field station surrounded by local communities on one side and a remarkable forest on the other. This expanded my understanding of the wildlife conflicts affecting this and other areas and the solutions being used. The tropical forest is an area of supremely high primate biodiversity with 9 diurnal and 3 nocturnal species being recorded.

The course itself was very well run, with several field exercises and many lectures from people involved in conservation in Uganda and other fields. These were incredibly useful as it gave me many different skills. Some I had some previous experience in, such as using mist netting to capture and ring birds, whilst others were completely new to me, such as how to record animal behaviours and profiling a forest stand structure. The lectures were very informative with them being given by many guest lecturers that were experts in their field with case studies they had been part of.

The final part of the course was a research project carried out in small groups (3 students maximum). This was the most valuable part of the course for me personally because I do not have that much experience of undertaking fieldwork. My group and I found out first hand the difficulty of doing fieldwork in a field setting and in such a short time frame (projects had to be planned, carried out and presented in 10 days). Finally, we got a workable project and managed to get some good data but it was really the planning and trouble shooting part of this project that I think will benefit me the most in my future studies.

Another aspect of this course that I found deeply rewarding was mixing with the African students. Not only to learn from them academically as some had some excellent experience and knowledge that I could learn from but it was also the cultural differences we found between us that was very interesting. I would like to think that they learnt from me as much as I learned from them.

This course is one of the most valuable things that I have done and would highly recommend it to any of my friends. I would again like to thank the Davis Trust Fund for making it possible for me to go.

Prevalence of ant repellents in floral scent in Kibale Forest National Park, Uganda

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Abstract

Many plant species attract ant guards in order to reduce herbivory. However, this bears a potential disadvantage for the plant as ants could restrict pollinator access or damage pollen and floral structures and thus reduce reproductive success. Therefore it is crucial that plants develop mechanisms to keep ants away from their reproductive parts. One of these mechanisms is the use of volatile organic compounds to repel ants. This study looks at the prevalence of ant repellents in floral scent in a number of flowering plants. Findings indicate that plant species do not differ in their effect on ants' reactions but they all have a significant repellent capacity. This suggests that ant repellence may not only be present in ant-plant interactions but may be a widely used defence in many plant taxa.

INTRODUCTION

An individual's fitness is dependent upon balancing a number of key trade offs, including those between defence and reproduction (Ness, 2006; see Heil *et al.*, 2002). In plants, increased defence could deter potential pollinators, whilst reduced defence for increased reproduction can lead to greater herbivory. Both scenarios could potentially result in a lower seed set, thus lowering an individual's fitness. Hence it could be expected that selection would act to provide a mechanism to balance this trade off.

An effective way of plant defence against herbivory occurs through the attraction of ant guards by providing a range of benefits as part of a mutualistic relationship. Ants may be attracted by direct food rewards such as extra-floral nectar (EFN) and protein-rich secretions (Beltian or Müllerian bodies) in certain plants. Extra-floral nectar provides a predictable, energy-rich food source to ants, whilst Beltian bodies supply essential proteins. Other rewards include domatia, plant structures which provide safe, ready made nesting sites. In return for these rewards, ants continuously patrol plants, chase off herbivores both large and small (Ghazoul, 2001), kill off potential plant competitors and remove parasitic fungi (see Michelangeli, 2003; see Nicklen & Wagner, 2006). However, there is a conflict between these mutualists as the ants can deter potential pollinators visiting the flowers. Furthermore, ants do not make good pollinators (Ghazoul, 2001) as they lack specialised pollen carrying structures, the ability to carry pollen over large distances and cross-pollinate plants (see Willmer *et al*., 2009). In addition, they are capable of damaging floral structures both physically, or through secreting anti-microbial compounds which affect pollen viability (see Nicklen & Wagner, 2006; see Willmer *et al*., 2009).

Plants have thus evolved a number of mechanisms to keep ants away from their reproductive parts. These can be categorised into bribes, physical and chemical defences (see Willmer *et al*., 2009). Bribes include the above mentioned EFNs and protein-rich secretions, which not only attract ants to plants, but are also positioned away from floral structures (e.g. tip of leaves or at base of stem) in order to attract ants away from flowers. Plants may also physically restrict ant access to flowers by producing a hairy or sticky stem which act as a barrier to ant movement. Finally, many plants produce volatile organic compounds (VOCs) to repel ants from flowers. A well known example of this chemical defence mechanism occurs in certain ant-acacia mutualisms in Africa, where *Acacia* sp. emits these ant deterring compounds at a certain time of the day (dehiscence window), repelling their ant guards and allowing pollinators to access its flowers (Ghazoul, 2001).

It has been hypothesised that the production of ant-deterring VOCs is widespread amongst angiosperms as the conflict between ant and plant is not restricted to ant-plant mutualisms. Indeed, most plants interact with many different genera of ants. As a result VOC action needs to be broad spectrum to repel all potential ant visitors, although level of response induced by one floral VOC may vary considerably between different ant species (see Willmer *et al*., 2009).

Our study aims to determine the prevalence of ant deterring VOCs in five flowering plant species in Kibale Forest National Park, Uganda. We hypothesise that all plants will have ant deterring floral VOCs of some sort, although the reaction they induce in different ant species may be varied.

MATERIALS AND METHODS

Study Area

Our study was carried out at Kibale Forest National Park, Makarere University Biological Field Station (MUBFS), Uganda from 20^{th} to 25^{th} July 2012 between 09:00-13:00 and 15:00-18:30.

Experimental Setup

Responses of ants to VOCs were studied *in situ* by puffing 5 ml air on individual ants using 20 ml disposable plastic syringes. These either contained clean air (control), or were filled with wild flowers and were left sealed off for 10 minutes before use in order to allow VOCs to volatilise. The flowers for each study session were picked at the same time before this session and kept in clear plastic bags until use. Flowers in the syringes were changed after two trials (one trial being two puffs) so as to maintain constant VOC levels. Puffs were performed from a standard maximum distance of 2 cm at approximately right angles to the ant's anterior-posterior axis. Reactions of ants were scored on a scale of 0 to 3, where 0 means no response; 1 – moving antennae; 2 – arching abdomen beneath the body; 3 – running away (after Willmer *et al*. 2009).

Syringes were rinsed with cold water before being washed with ethanol and rinsed again with hot water every time before a new species of flower was placed into the syringe. This was done to avoid mixing VOCs from different plants. The control syringes were also washed in this manner so as to control for any effect of the washing.

To control for observer bias, syringes were wrapped into paper, therefore the observer never knew the content of syringes and had no expectations regarding ant reaction. Both ant and plant species used in this study were selected on an ad-hoc basis, but it was our aim to study as many ants residing on trees or herbaceous plants as possible.

Study Species

Four species of ants and six species of flowering plants were included in our final analysis. The four ant species studied were from the genera *Myrmicaira*, *Polyrhachis*, *Tetramorium*, and *Camponotus*. Plant species used were *Crassocephalum montuosum*, two *Bothriocline* sp., *Lantana camara*, *Barleria* sp. and *Desmodium setigerum.*

Data Analysis

Reaction of the ants was analysed using Minitab Statistical Software 14. The data was incorporated into an ANOVA GLM analysis to determine whether the number of individuals performing a certain response varied between ant species and stimuli. Reaction 2 and ant species 2 (unidentified) were excluded from the data analysis due to low sample size compared to other reactions and ant species, making any conclusions drawn from these data potentially invalid. Paired t-tests were carried out to see if there was a difference between the proportions of individuals performing the same behavioural action for the control against an average of the treatment proportions.

RESULTS

There was no significant difference in the number of individuals performing the same reaction for different stimuli (ANOVA GLM: 0 – $F_{1,20}$= 0.02, p=0.897; 1 – $F_{1,20}$= 0.00, p=0.972; 3 – $F_{1,20}$= 2.61, p=0.122), indicating that different stimuli do not play an important role in determining the reaction of different ant species. The difference in number of individuals of different species performing a certain reaction showed no significance either (ANOVA GLM: 0 – $F_{1,20}$= 0.11, p= 0.748; 1 – $F_{1,20}$= 0.05, p=0.832; 3 – $F_{1,20}$= 0.427), implying that ants do not react in a manner specific to their own species.

There were no significant interactions between the treatment and ant species for the three behavioural reactions (ANOVA GLM: 0 – $F_{1,20}$= 0.45, p=0.509; 1 – $F_{1,20}$= 0.27, p=0.607; 3 – $F_{1,20}$= 1.34, p=0.261). This indicates that the ant species did not differ in their behavioural reactions to the different treatment stimuli.

Paired t-test

Frequency of *no response* was significantly higher in each species when treated with air (control) than when treated with any floral scent (Paired t-test: $T_{4,4}$=5.56, p=0.011) (Figure 1).

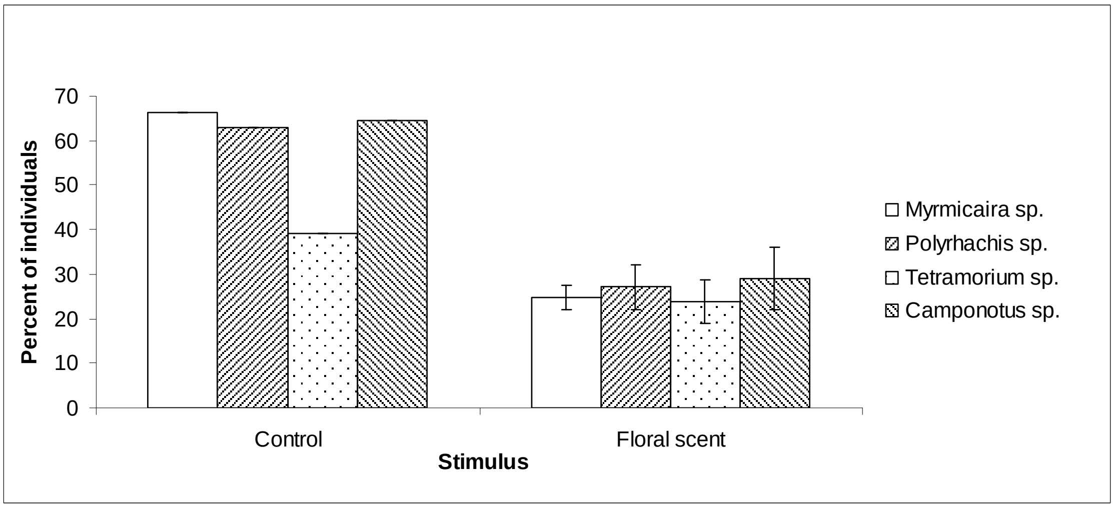

Figure 1 Comparison between control and floral scent for the percent of individuals giving *no reaction*.

The chart shows how the percent of individuals with *no reaction* is significantly higher for the control than the floral scent, for all ant species (p=0.011). The error bars on the floral scent bars represent the standard error, which was not possible to calculate for the control bars as these were not averages.

Conversely, there were a significantly higher proportion of individuals who ran away when puffed with floral scent compared to air (Paired t-test: $T_{4,4}$=-4.69, p=0.018) (Figure 2).

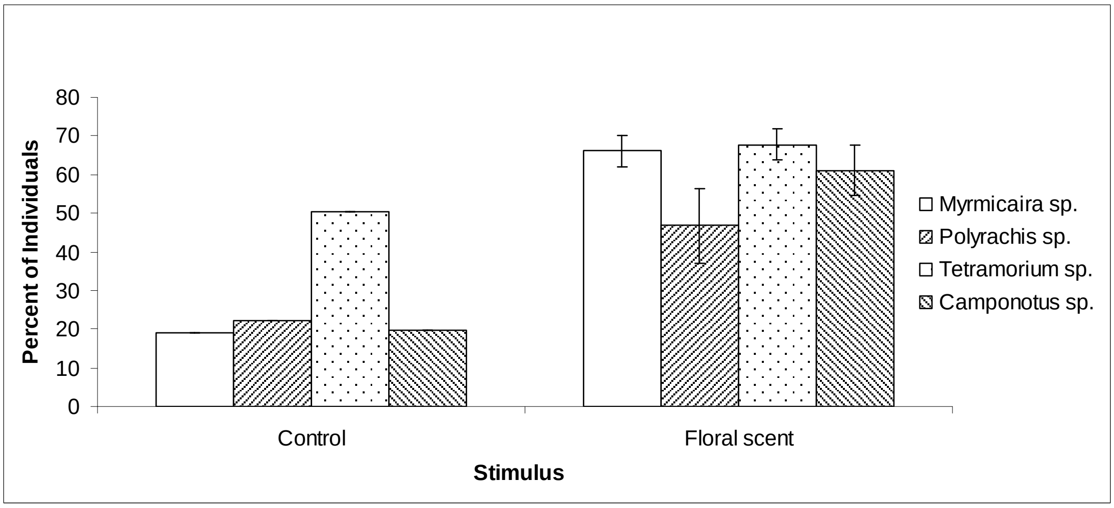

Figure 2 Comparison between control and floral scent for the percent of individuals *running away*. The chart shows how the percent of individuals *running away* is significantly higher for the floral scent than for the control, for all ant species (p=0.018). The error bars on the floral scent bars represent the standard error, which was not possible to calculate for the control bars as these were not averages.

The above results imply that the ant repellent effect of floral scent is significantly greater than that of air. There was no significant difference in the proportion of individuals waving antennae when puffed with air or floral scent (Paired t-test: $T_{4,4}=0.11$, $p=0.922$).

DISCUSSION

Variation in the number of individuals expressing a specific response is not explained by either the stimuli or the ant species. There was no significant interaction between the stimuli and the ant species for a given behavioural response; indicating that the ant species respond in a similar way to all stimuli. This is consistent with the hypotheses that ant-repelling compounds should work against a number of different potentially damaging ant species (Willmer *et al.*, 2009) and that they should be present in most plants that attract pollinators through the use of floral resources (Ghazoul, 2001). No specific interaction between any one stimulus and an ant species have been found, possibly because no resident ants or plants with ant guards were included in the study, which have been shown to have strong specificity (Willmer *et al.*, 2009).

The difference in the mean percentage of individuals expressing a certain response between the control (air) and all other floral stimuli combined was significant for *no* *response* and *running away*. These results provide an indication of the presence of non-target specific ant repellent compounds in these plant species, as the percentage of individuals that ran away was significantly higher for floral scent than control (Figure 2), with the control having a higher percentage of *no response* (Figure 1). This is again in agreement with the already mentioned hypothesis regarding commonality of ant repellent compounds (Ghazoul, 2001).

A previous study by Willmer *et al.* (2009) described a specific case of ant repellence in some ant-acacia mutualisms where the tested species of Acacias differed in their repellent effect on the different resident ant species. This difference related to the dominance and aggression hierarchy of the ant species, thus plants will possibly defend themselves primarily against the most aggressive ant residents as these could deter the most pollinators. In this study they discovered that the ant repellence comes from the pollen of the flowers as repellence varied with the species of Acacia but was correlated with release of pollen. Our findings complement these results by demonstrating that floral ant repellence has not only evolved in specific ant-plant mutualisms, but it may be a widely used defence mechanism in a number of plant taxa.

There are a number of issues however that may have influenced our results. First, the ethanol we used to rinse the syringes between experimental sessions was strongly scented, and despite washing them multiple times with hot water we believe the scent may have influenced the response of ants. Second, flower heads used in the study were picked at different times of the day. This may influence nectar and pollen availability in flowers, which in turn can modify the response of ants. Third, two ant species (*Tetramorium* sp. and *Camponotus* sp.) were tested in the immediate proximity to their nest, which we believe may have exaggerated the response level of these ants.

Further studies could be conducted to confirm our conclusions and to expand our knowledge in this area. We suggest testing ant species for the entire day with fresh flower heads picked at consistent frequent intervals to investigate the level of ant repellence over the day. Since intensity of ant repellence has been linked to pollen production by earlier studies (Willmer *et al.*, 2009), it would also be instructive to establish the daily release of pollen of different plant species and relate this to the level of ant repellence. Further laboratory work could be conducted to test this effect of pollen by placing pollen grains/anthers in one half of a petri-dish and looking at the proportion of time the ant spent in this half compared to the control half (empty) (after Willmer *et al.*, 2009).

Based on the results from this study we can accept our hypothesis that ant repellence will be present in a number of different plant species. However, it cannot be concluded that the variety of ants' responses depends upon the plant species they are exposed to.

ACKNOWLEDGEMENTS

We would like to thank Clive Nuttman for his patience and guidance in this project; Ryszard Laskowski for his statistical knowledge and advice; Brian Fisher for his help in identifying our ant genera. We would also like to thank the MUBFS staff for their hospitality and help. We would also like to thank the British Ecological Society, University College London and the Davis Trust Fund from the University of Edinburgh for helping to fund this project.

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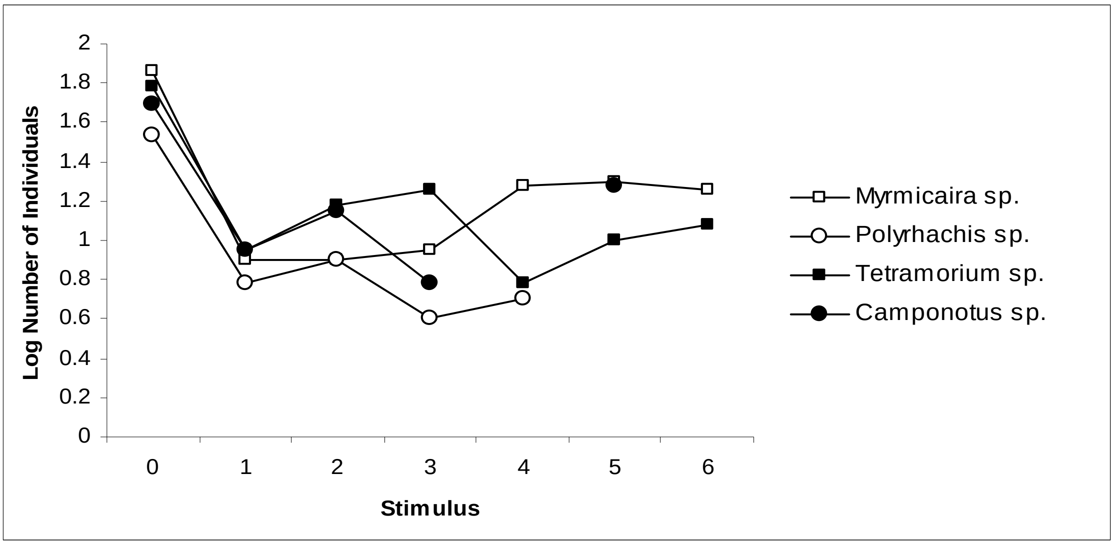
APPENDICES

Appendix A: Sample number for each of the five ant genera for each stimulus

Ant Species	Treatment						
	Air (control)	*Crassocephalum montuosum*	***Bothriocline* sp.**	***Lantana camara***	***Barleria* sp.**	***Desmodium setigerum***	***Bothriocline sp. 2***
***Myrmicaira* sp**	110	29	29	79	73	69	67
Unidentified	8	18	11	-	-	-	-
***Polyrhachis* sp.**	54	20	20	23	24	-	-
***Tetramorium* sp**	153	49	52	40	58	53	54
***Camponotus* sp**	44	41	39	-	41	-	-

Appendix B: Interactions of the ant genera and the different stimuli, for the different reactions



Number of individuals giving *no reaction* to different stimuli.

The graph shows the interaction of the four ant species to the different stimuli when they gave *no reaction*. Stimulus: 0 – control; 1 – *Crassocephalum montuosum*; **2 – *Bothriocline* sp. 1; 3 – *Lantana camara*; 4 – *Barleria* sp.; 5 – *Desmodium setigerum*; 6 – *Bothriocline* sp. 2.**

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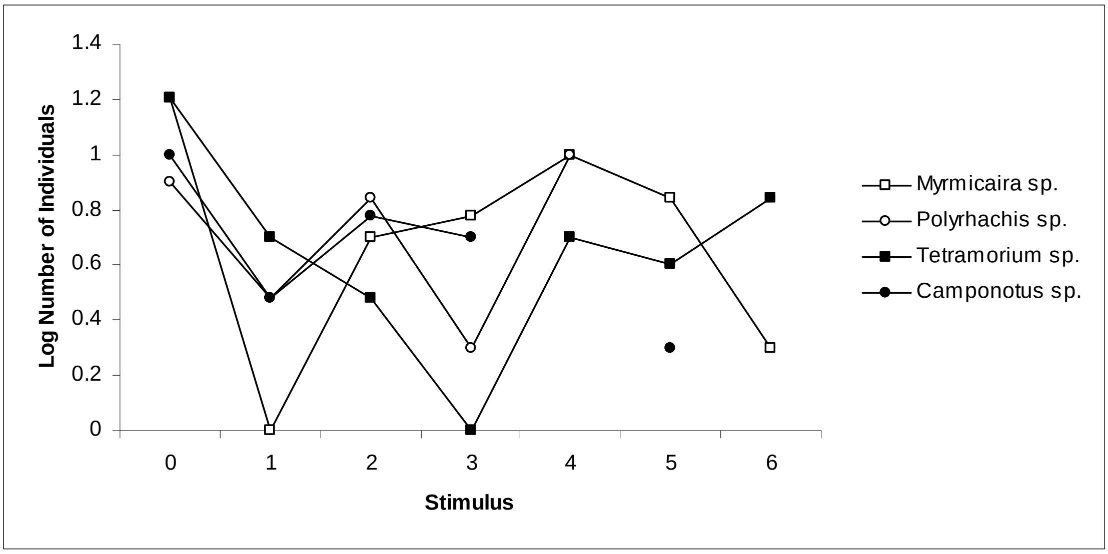
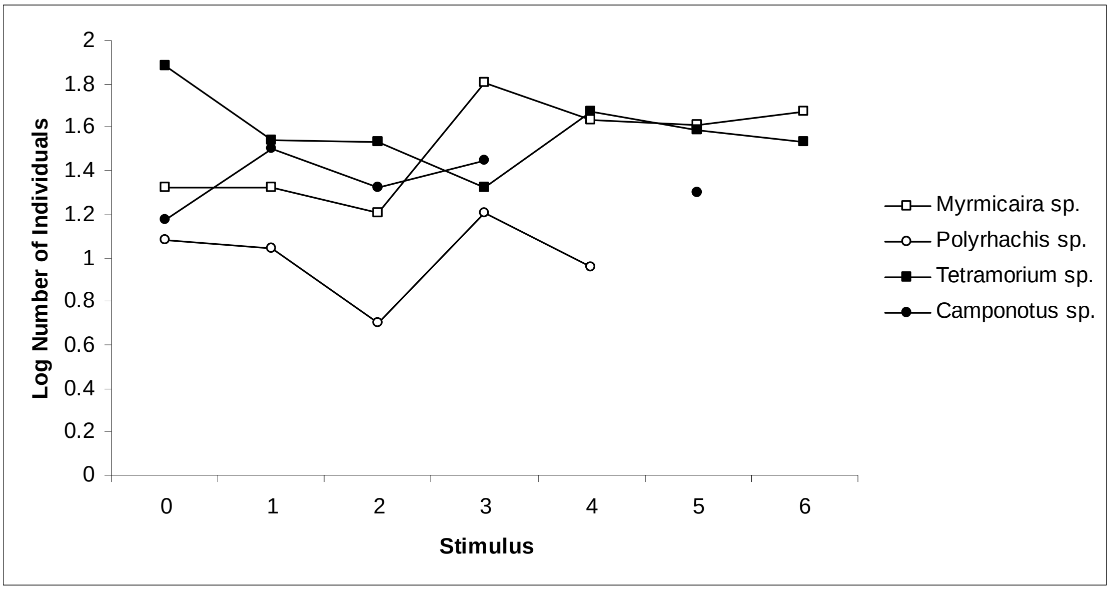


Number of individuals *waving antennae* to different stimuli.

The graph shows the interaction of the four ant species to the different stimuli when they *waved antennae*. Stimulus: 0 – control; 1 – *Crassocephalum montuosum*; **2 – *Bothriocline* sp. 1; 3 – *Lantana camara*; 4 – *Barleria* sp.; 5 – *Desmodium setigerum*; 6 – *Bothriocline* sp. 2.**



Number of individuals *running away* to different stimuli.

The graph shows the interaction of the four ant species to the different stimuli when they *ran away*. Stimulus: 0 – control; 1 – *Crassocephalum montuosum*; **2 – *Bothriocline* sp. 1; 3 – *Lantana camara*; 4 – *Barleria* sp.; 5 – *Desmodium setigerum*; 6 – *Bothriocline* sp. 2.**