

**The effect of nectar scent on honeybee (*Apis mellifera* L.) behaviour**

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Floral nectar is a complex mixture of primary and secondary compounds. While most of the research has focused on the role of primary compounds for the interaction with pollinators, the role of secondary compounds is much less understood. Volatile organic compounds (VOCs) have only recently been investigated as part of the complex mixture of the chemicals found in nectar. They might play a role as pollinator attractants or as a repellent for herbivores. In addition VOCs in nectar may reduce fermentation of sugars in nectar by microorganisms. However, there might be a trade-off between these different functions. The floral and nectar VOCs of nine bee selected flower species were investigated using GC-MS. The response of honeybees (*Apis mellifera* L.) to four different concentrations (0.01%; 0.1%; 0.5%; 1%) of linalool, phenylethanol, and hexanoic acid were tested in choice tests against an unscented sugar solution (40% sucrose). Rejection of linalool began at 1%. Phenylethanol was rejected at a concentration of 1%. Hexanoic acid was a strong repellent even at low concentrations of 0.01%. Antimicrobial activity was found for all three VOCs over a period of six days. Different concentrations of VOCs (0.01%; 0.10%; 1%) did not have an effect on antimicrobial activity. The data indicates that some VOCs like hexanoic acid in nectar can have a strong repellent effect on honeybees while others like linalool are tolerated even at relatively high concentrations such as 1%. The findings indicate that nectar scent does have an effect on the behaviour of honeybees. The data also suggests that potential trade-offs between the antimicrobial activity of VOCs and repelling pollinators may be compound specific.

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**Essential oil yield and composition of three *Helichrysum* species occurring in the Eastern Cape Province of South Africa**

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Three species of *Helichrysum* namely *H. odoratissimum*, *H. cymosum* and *H. petiolare*, all commonly known as Imphepho (Xhosa, Zulu), are widely used in South Africa for medicinal purposes. The active ingredients assigned to the medicinal properties include both non volatile components like phloroglucanols in water extracts and volatile essential oil components. Main components in the essential oil from the three species harvested in the Kareedow area of the Eastern Cape and extracted using steam distillation included  $\alpha$ -pinene, d-limonene, 1,8-cineole,  $\gamma$ -terpinene,  $\beta$ -caryophyllene and  $\alpha$ -humulene. The concentrations of these components varied considerably between the three species with  $\alpha$ -pinene highest in *H. odoratissimum* with 23.1% and lowest in *H. cymosum* with 8.4%. *H. cymosum* had the highest  $\beta$ -caryophyllene content of 17.8% with *H. petiolare* the lowest content of 1.1%. Oil yield of especially *H. petiolare* showed a large variation between summer and autumn harvesting of 0.05% and 0.15% respectively. A thorough investigation is needed to quantify the variation of oil yield and composition between geographical regions and seasons.

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**Multiple shoot induction from wild lettuce [*Launea taraxacifolia* (Willd) Amin, Ex. C. Jeffrey], an indigenous leafy vegetable**

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*Launea taraxacifolia* (Asteraceae), also known as wild lettuce, is one of the many indigenous leafy vegetables (ILV) in Nigeria. Selection of *L. taraxacifolia* plants with tender leaves of low bitterness would increase its consumption. A limitation to cultivation and domestication of this plant is the lack of variability occasioned by the commonly used vegetative mode of propagation leading to inefficiency of conventional breeding. Molecular breeding will therefore be a more efficient method for genetic improvement of the plant and *in vitro* regeneration protocols are essential. The objective of this study was to compare the effects of a combination of benzyladenine (BA) with naphthalene acetic acid (NAA), and a combination of 2,4-dichlorophenoxy acetic acid (2,4-D) with kinetin (KN) on multiple adventitious shoot regeneration and to develop an efficient micro-propagation protocol for the species. The explants were cultured on MS (Murashige and Skoog, 1962) medium fortified with different concentrations of 2,4-D with KN and BA with NAA. A combination of BA with NAA was more efficient for multiple adventitious shoot regeneration as compared to the combination of 2,4-D with KN. Out of different explants utilized (leaf, stem and nodal), only leaf explants produced multiple adventitious shoots (100%) with an average number of  $13.3 \pm 2.4$  shoots per explants on shoot induction medium (Murashige and Skoog (MS) medium + 2.0 mg/l BA + 0.1 mg/l NAA). Shoot buds elongated rapidly with over 50% of the elongated shoots rooting well on the same medium. This study, to our knowledge is the first report on the multiple adventitious shoot regeneration of *L. taraxacifolia*.

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**Geographic variation in flower color: Spectral composition versus perception of pollinators**

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Geographic variation of flower color can be the result of selective processes mediated by pollinators. Changes in flower color can steer visual attention of pollinators in different ways, thus influencing enhancing plants pollination success. Despite this is a widespread belief in pollination biology, there is no study that analyse at a geographical scale both, the spectral patterns of the light reflected by flowers across the entire wavelength range, and the colour space patterns obtained from pollinators perception of the reflected colours (adaptive component). Here we compare geographical variation structure of reflected flower color versus flower color perceived by pollinators, in 23 populations of the three species of genus *Monttea* (*Plantaginaceae*). There was substantial variation in the coloration of flowers between and within species considering both floral color components. However, for each flower segment measured (petal, floral tube and elaiophore), each color component showed different variation patterns. On one hand, at intraspecific level, coefficients of variation showed that the adaptive component is less variable at geographical scale than the spectral patterns obtained from flowers. On the other hand, nested analyses of variance showed mainly interspecific differences through the perception of pollinators. Moreover, multivariate analyses showed that pollinators could

distinguish among species, whereas there is an important overlap in the spectra of flowers. Results showed differences in floral color patterns considering the spectral analysis versus that one perceived by the pollinators, suggesting different ecological and evolutionary processes underlying the observed variation of each group of data sets: the adaptive component of color would be primarily associated with pollinators assemblage which seems to be species-specific, suggesting an isolation mediated by pollinators among *Monttea* species, while the spectral variation of color, would be associated with other factors, like climatic and/or neutral processes.

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### **Flower morphology and its relationship with the pollination system in the southern South American genus *Jaborosa* (Solanaceae)**

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Flower morphology has been considered an important factor in species isolation because of its dual impact on the attraction and mechanical fit with pollinators. In the present work we quantified the variation in floral shape amongst twelve species of the genus *Jaborosa* Juss. (Solanaceae) to assess whether this variation is spurred by pollinator shifts or is explained by their phylogenetic history. Floral morphology (i.e. corolla limb shape and disposition of stamen and pistil within the corolla) was studied using traditional and geometric morphometrics. Flower shape and pollinator guild were mapped onto a molecular phylogeny of the genus and ancestral states were reconstructed. Two major clades were recovered, one grouping species that clearly exhibit a sphingophilous syndrome and the second one grouping the species pollinated either by saphrophilous flies or moths. Sphingophilous species showed salverform flowers with dissected petals whereas the clade grouping the remaining myophilous and phalaenophilous species showed a greater variation in flower morphology from rotate to urceolate and tubular flowers. Flower architecture determined that pollen was carried in the proboscis of long-tongued hawkmoths and moths and in a nototribic or pterotribic position by carrion flies. These results suggest that pollinator shifts either than phylogenetic ancestry would be responsible of the diversification of flower shapes and patterns of pollen removal/deposition within the genus *Jaborosa*.

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### **Pollination by *Hemimepsis* wasps: A newly described South African guild with an analysis of trait convergence between guild members**

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Pollination syndromes are based on the premise of convergence in floral traits of plants that share a common pollinator. Although syndromes are widely used as a conceptual framework for studies of floral evolution, pollinator-mediated convergence in floral traits has seldom been examined in the context of syndromes. In this study, we describe a guild of plants that are pollinated by *Hemimepsis* wasps, and examine levels of floral trait convergence in this newly recognized pollination system. The *Hemimepsis*-wasp pollination

guild includes 23 plant species from three families (Apocynaceae, Orchidaceae and Asparagaceae) which are pollinated by between one and four species of *Hemimepsis* wasp (Pompilidae: Pepsinae). Of the known guild members, 18 are pollinated exclusively by these wasps. The guild is distributed throughout the grasslands of eastern South Africa. Guild members share several qualitative traits, including dull greenish- or brownish-white flowers, often with purple blotches, mid-summer flowering, sweet spicy scent and exposed nectar. To explore levels of floral trait convergence within the guild we compared the floral traits of guild members to those of congeneric plant species pollinated by other vectors. We found limited evidence of convergence in the nectar properties (volume and concentration) and floral scents of guild members. However, we found evidence of convergence in the floral colours of guild members and loci for the spectral reflectance of guild members were more tightly clustered in the hymenopteran colour hexagon than those of non-wasp-pollinated congeners. Our results firmly establish the existence of a specialized system of pollination by *Hemimepsis* spider-hunting wasps and suggest that some traits do not necessarily evolve during shifts between pollinators.

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### **RAPD and SSR genetic diversity analysis of *Moringa oleifera***

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*Moringa oleifera* is potentially an economically important tree species. It has gained interest globally for its multipurpose uses, in particular as a source of nutrition and oil as well as various medicinal properties. *Moringa* is native to India, Malaysia and the Middle East, but have been introduced to many countries throughout Africa ranging from Niger to South Africa. There is however limited knowledge regarding the genetic variation of both native and introduced populations of *Moringa*, although phenotypic observations suggests the presence of significant genetic diversity. In this study we aim to determine the level of genetic variation found between different populations of *Moringa* from locations including India, South Africa, and Hawaii. Molecular marker such as Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR), will be used to analyse the genetic diversity based on their success in other tropical tree population studies. Their low capability for SSR conservation, make them ideal for the application at the intra-specific level. Various RAPD primers and 17 SSR primer pairs will be used to generate amplification profiles that can be used in a diversity analysis. Initial screening has identified markers that show significant genetic diversity amongst the populations. Furthermore the study will try to identify markers related to quantitative traits such as seed oil content and yield that could potentially be useful in future selection and breeding programs aimed at tree improvement.

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### **Baobab population dynamics - Investigating spatial and temporal patterns in KNP**

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