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Effects of transgenic and conventional *gypsophila* on beneficial arthropod diversity

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ABSTRACT

Gypsophila paniculata (L) (Baby's Breath) is a common commercial variety, with predominantly white or light pink flowers. Through genetic insertion of *pap 1* gene, cultivars with altered colour, dark purple and red to light pink have been developed, prompting the need to determine their effect on beneficial arthropod diversity. Five transgenic *Gypsophila* cultivars (TG272, TG292, TG59, TG505, TG143) and conventional cultivar CGMS (control) were established. Each cultivar was a treatment with five replicates. Ten plants were randomly selected for each cultivar and lady bird beetles, predatory mites, syrphids, ants, bees, mummified aphids and spiders recorded at two weeks interval, one month after planting. Transgenic *Gypsophila* TG59 had 2.42±0.6 and 1.79±0.2 bees in first and second seasons, respectively, and the difference was significant (*P*=0.0001). CGMS had the highest mean ladybird beetles (0.32 ± 0.05) in second season and the difference was significant (*P*=0.0001). Transgenic cultivar TG292 recorded the highest mean ants (0.75±0.11; 0.73±0.11) and CGMS had the least mean ants (0.06±0.03; 0.07±0.02) and the difference was significant (*P*=0.0001). TG59 had the highest mean mummified aphids (0.18±0.07) and the difference (*P*=0.0021) was only significant in the second season. Transgenic *Gypsophila* had no negative effect on beneficial arthropods since arthropods were found on it. More bees, ants, mummified aphids and spiders were found on transgenic *Gypsophila,* while conventional *Gypsophila* had more ladybird beetles. Bees are major beneficial arthropods of Gypsophila. Research on effect of transgenic cultivars on other beneficial arthropods and even in other crops is necessary.
Keywords: White flowers; Genetic engineering; *pap 1* gene; Transgenic cultivars

INTRODUCTION

Gypsophila (*Gypsophila* sp.) is an ornamental plant which originated in Eurasia and belongs to the Caryophyllaceae family which also has other popular flowers such as carnation (Mustafa et al., 2010). It is commercially grown worldwide as a cut flower. The most common commercial variety is *G. paniculata* (Baby's breath) which has predominantly white flowers and in rare cases there are varieties with light pink flowers (Williams, 1989). It is used as filler for formal floral arrangements and bouquets, especially with roses and also as a dried flower.

Genetic engineering is a biotechnology tool useful in improving ornamental crops through the addition of desirable traits to existing ornamentally-adapted cultivars for instance flower colour. The first blue rose developed by Australian corporation Florigene was Genetically Modified (GM) for blue colour (ISAAA, 2008). In an effort to increase the range of flower colours in *Gypsophila*, Imaginature Ltd in Israel developed new cultivars with colours ranging from dark purple and red to light pink through introduction of *pap*1 gene, which regulates the production of phenylpropanoids, including anthocyanin pigments. The gene was isolated from *Arabidopsis thaliana* with Binary transformation *vector pCGN1599.* The Binary plasmid doesn't contain oncogenic genes which lead to the pathogenesis of the natural agrobacterium.

The impact of genetic engineering *Gypsophila* for aesthetic value or colour change on arthropod natural enemies and pollinators in Kenya is unknown thus the need to assess the impact of the new varieties. Examination of the environmental consequences involves determination of any major alterations in the insect fauna associated with plants expressing the trait and or marker gene (Dobres, 2008) among other factors. *Gypsophila* is attractive to numerous species of pollinating bees and flies (Darwent and Coupland, 1966) and is considered to be predominately insect-pollinated. Hybridization of *Gypsophila* in nature is facilitated by insect pollination and is only effectively achieved by Hymenoptera and Diptera or pollinating bees and flies, since pollen is not spread by wind. *Gypsophila* f1owers are numerous, but small, which would appear to diminish their relative attractiveness to pollinators (Danziger, 2012).

The most obvious exposure route for non-target herbivores is through direct ingestion of plant material, although this is influenced by the mode of feeding and spatial expression patterns of the transgene product (Gatehouse et al., 2010). In terms of exposure to natural enemies, these routes are more diverse since many predators and parasitoids, particularly in the adult stage, are facultative herbivores. They can thus be exposed to transgene products directly from consuming plant tissues like pollen and nectar, or more usually from consuming insects that have themselves fed on plant tissues where the transgene product has been expressed and accumulated (Gatehouse et al., 2010). It has been found that plant diversity has a positive impact on arthropod richness but not on arthropod abundance. An analysis of arthropod community composition revealed that each flower species attracts a different assemblage of beneficial arthropods (Bennett and Gratton, 2013).

Insects, spiders, predatory mites, and other arthropods are considered beneficial insects when they eat arthropods that humans consider undesirable (Smith and Capinera, (2015). There are two categories of beneficial arthropods like predators and parasitoids. Both can effectively control insect and mite pests. Predators are organisms that kill and feed on preyand are generally larger than their prey. Parasitoids, on the other hand, are typically smaller than their hosts and lay eggs on or within them and when the eggs hatch into larvae; these larvae develop and feed on these host insects, causing their death. The commonly encountered natural enemies are ladybird beetles, lacewings, big eyed bugs, pirate bugs, flower flies, predatory gall wasps, ants, parasitic wasps, parasitic flies and predatory mites. The relative importance varies with insect pest, habitat, and season of the year (Smith and Capinera, 2015)**.**

Most flowering plants (75%) require an animal pollinator (Gullan and Cranston, 2010). There are over 200,000 species of animal pollinators and the vast majority of these are insects (Berenbaum, 2007). Insect pollinators include beetles, flies, ants, moths, butterflies, bumble bees, honey bees, solitary bees, and wasps. Bees are one of the largest groups of pollinators (Berenbaum, 2007) and can be social or solitary animals. Honey bees and bumble bees, common eusocial pollinators, are generalists that visit many plant species to obtain nectar and pollen. Honey bees, the most important crop pollinator, pollinate over 100 different fruits and vegetables.

There is widely held concern over the ecological impacts of GM crops and this has led to the extensive examination of the potential effects of a range of transgene proteins on non-target and beneficial insects (Gatehouse et al., 2011). For any technology to be acceptable to the public at large, the perceived benefits have to outweigh any potential risk, this is equally true for biotech crops (Waltz, 2009). Today's environmentally aware public has demanded a rigorous evaluation of the ecological risks of releasing these transgenic crops into the environment (EC, 2001). The aim of the study was to determine effect of transgenic and non-transgenic *Gypsophila* on beneficial arthropod diversity.

MATERIALS AND METHODS

Gypsophila crop was established in a two acre piece of land with transgenic cultivars under trial set at the centre of the trial area in a Confined Field Trial (CFT) at Beautyline Flower farm in Naivasha as a biosafety requirement. There were five transgenic cultivars namely TG272, TG292, TG59, TG505 and TG143 and one conventional cultivar CGMS. Each treatment was on 3 sub plots of 15 plants each thus total of 45 plants. Each cultivar was treated as a treatment replicates five times. The treatments were laid out in a Randomized Complete Block Design (RCBD) and replicated 5 times. The beneficial arthropods targeted were ladybird beetles, predatory mites, syrphids, ants, bees, mummified aphids and spiders. Ten plants were randomly selected from each plot /treatment where the number of beneficial arthropods was recorded. The number of beneficial arthropods per plant were counted every two weeks and recorded according to plants in a treatment. The data of beneficial arthropods on each of the sampled plants was recorded on data sheets designed for the current work. Data on counts were transformed using $\sqrt{(1 + x)}$ and subjected to Analysis of variance (ANOVA) using SAS 8. Means were separated using Students Newmans Keuls' (SNK) test. Differences at $p<0.05$ level were considered statistically significant.

RESULTS

Beneficial arthropods and pollinators observed on the *Gypsophila* crop included lady bird beetles, spiders, syphids, ants, mummified aphids and bees.

Table 1: Mean number of beneficial arthropods on *Gypsophila* cultivars in first season

Cultivar	Ants	Mummified aphids	Bees	Lady bird beetles	Predatory mites	Spiders	Syrphids
TG272	0.15 ± 0.06b	0a	0.06 ± 0.02b	0.03 ± 0.01a	0a	0.53 ± 0.10a	0.01 ±
CGMS	0.06 ± 0.03b	0a	0.32 ± 0.06b	0.03 ± 0.01a	0a	0.52 ± 0.10a	0.01a
TG292	0.75 ± 0.11a	0.02 ± 0.01a	0.03 ± 0.02b	0.04 ± 0.01a	0a	0.54 ± 0.10a	0b
TG59	0.07 ± 0.03b	0.03 ± 0.02a	2.42 ± 0.59a	0.03 ± 0.01a	0a	0.54 ± 0.10a	0b
TG505	0.19 ± 0.04b	0.03 ± 0.02a	0.09 ± 0.03b	0.04 ± 0.01a	0a	0.54 ± 0.10a	0b
TG143	0.15 ± 0.05b	0.01 ± 0.01a	0.06 ± 0.03b	0.04 ± 0.01a	0a	0.54 ± 0.10a	0b
P-value	0.0001	0.0973	0.0001	0.8889		0.7553	0.0417

Means with the same letter are not significantly different

Table 2: Mean number of beneficial arthropods on *Gypsophila* cultivars in second season (Mean ± SE)

Cultivar	Ants	Mummified aphids	Bees	Lady bird beetles	Predatory mites	Spiders	Syrphids
TG272	0.12 ± 0.04b	0.05 ± 0.02b	0.46 ± 0.07d	0.04 ± 0.01b	0	0.05 ± 0.01a	0
CGMS	0.07 ± 0.02b	0.13 ± 0.04ab	0.41 ± 0.06d	0.32 ± 0.05a	0	0.03 ± 0.01a	0
TG292	0.73 ± 0.11a	0.04 ± 0.01b	0.83 ± 0.10c	0.05 ± 0.02b	0	0.04 ± 0.01a	0
TG59	0.29 ± 0.07b	0.18 ± 0.07a	1.79 ± 0.15a	0.07 ± 0.02b	0	0.01 ± 0.01a	0
TG505	0.28 ± 0.06b	0.03 ± 0.01b	1.25 ± 0.15b	0.04 ± 0.01b	0	0.04 ± 0.01a	0
TG143	0.20 ± 0.04b	0.04 ± 0.01b	0.38 ± 0.06d	0.03 ± 0.01b	0	0.05 ± 0.03a	0
Cv	27.67763	15.09316	31.80653	14.61872		0.984289	
P-value	0.0001	0.0021	0.0001	0.0001		0.4959	0.4161

Means with the same letter are not significantly different

Ladybird beetles

Ladybird beetle is an insect predator (Plate 1 A and B). Most adult ladybird beetles are round to oval, brightly coloured and often spotted.

A B

Plate 1: Ladybird beetle (A) on conventional and (B) on transgenic Gypsophila

The transgenic cultivar TG292, TG505 and TG143 had the highest mean of Ladybird beetles 0.04 ± 0.01 in first season while conventional cultivar CGMS had the highest mean of 0.32 ± 0.05 in second season. There was ladybird beetle population peak in all the cultivars in the 6th data collection period in first season, while in second season the population peak was only in cultivar CGMS, TG59 and TG272 for the same (Figure 1 A and B). There was no significant difference (p=0.8889) in ladybird beetle population between cultivar CGMS and the rest of the cultivars in first season but the difference was significant (p=0.0001) in second season (Table 1 and 2).

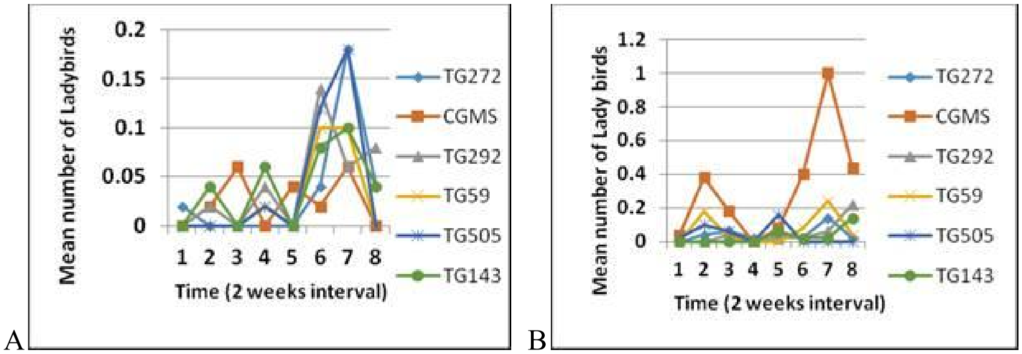

Figure 1: Mean number of Ladybird beetles on Gypsophila cultivars (A) First and (B) Second seasons

Bees

Different types of bees were observed on transgenic and conventional *Gypsophila* (Plate 2 A and B)

Plate 2: Bee on (A) conventional and (B) transgenic Gypsophila

Transgenic cultivar TG59 had the highest pooled mean number of bees 2. 42 ± 0.59 and 1.79 ± 0.15 in first and second seasons respectively (Table1 and 2) followed by TG505 with mean of 1.25 ± 0.15 in the second season. The conventional cultivar CGMS recorded a mean of 0.32 ± 0.06 and 0.41 ± 0.06 (Table 1 and 2). There was significant difference (*P*=0.0001) in number of bees in both seasons between TG59 and all the other cultivars and between TG505 and TG292, TG272, CGMS and TG143 in the two seasons. The bees were more in all the cultivars towards crop maturity (Figure 2 A and B).

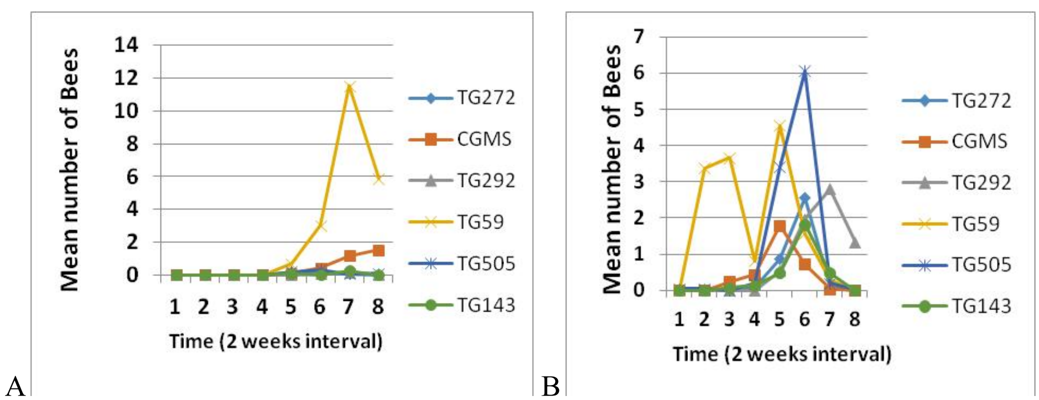

Figure 2: Mean number of bees in Gypsophila crop over seasons: (A) First season (B) Second Season

Ants

Ants were observed moving up and down plants indicating the presence of aphids, mealy bugs, or other sap-sucking insects (Plate 3.3)

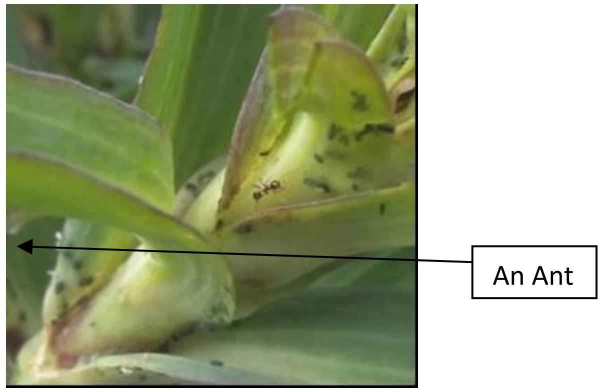

Plate 3: Ants /Aphid interaction

Transgenic cultivar TG292 recorded the highest mean number 0.75 ± 0.11 and 0.73 ± 0.11 of ants in both seasons in the early crop growth stages and declined with maturity (Figure 3 A and B). Cultivar CGMS (Conventional *Gypsophila*) which was the control had the least mean number of ants 0.06 ± 0.03 and 0.07 ± 0.02 (Table 1 and 2). There was significant difference (p=0.0001) between ants population in cultivar TG292 and all other cultivars in both seasons.

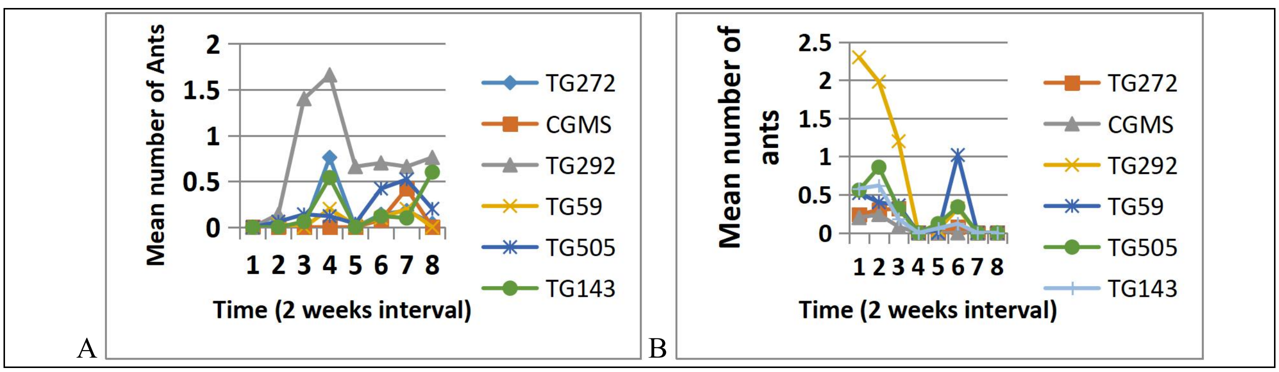

Figure 3: Mean number of ants on transgenic and conventional Gypsophila (A) First season and (B) Second season

Syrphids

Syrphids were absent in both transgenic and conventional *Gypsophila* throughout the crop growth cycle except in cultivar TG272 in the first season (Tables 1 and 2).

Mummified aphids

Transgenic *Gypsophila* TG59 had the highest mean number of mummified aphids in both seasons (0.03 ± 0.02 and 0.18 ± 0.07). There was no significant differences (*P*=0.0973) in mummified aphids between cultivar TG59 and the other cultivars in first season but the difference was significant (*P*=0.0021) between cultivar TG59 in second season (Tables 1 and 2)

Spiders

Transgenic cultivars TG292, TG59, TG505 and TG143 had a mean number of spiders 0.54± 0.10 in first season and cultivar TG272 and TG143 had a mean number of spiders (0.05 ± 0.01; 0.05 ± 0.03) respectively in second season. Conventional cultivar CGMS had a mean of 0.03 ±0.01 and 0.52 ± 0.10 (Tables 1 and 2). There was no significant difference (p=0.7553 and p=0.4959) in the number of spiders between cultivars in both seasons (Tables 1 and 2). The spider peak in all the *Gypsophila* cultivars was observed in 6th week of data collection in first and in second season it was only in TG143 in the 8th week (Figures 4 A and B).

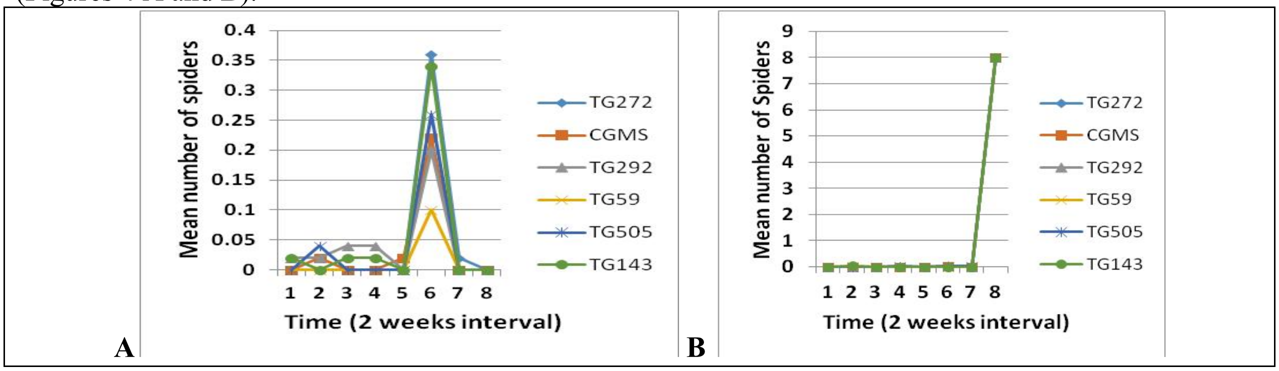

Figure 4: Mean number of spiders on *Gypsophila* cultivars: (A) first season and (B) second season

Predatory mites

There was no predatory mite observed throughout the growth cycle of the *Gypsophila* crop.

Butterflies

Different types of butterflies were observed especially at crop flowering stage in both transgenic and conventional *Gypsophila* (Plate 4)

B D

Plate 4: Butterflies on (A and B) conventional and (C and D) transgenic Gypsophila

DISCUSSION

The transgenic plants especially cultivar TG59 were found to have more of visiting bees due to showy flowers, a typical adaptation to attract pollinators. Since cultivar TG59 came to flowering earlier than the others it was able to attract more bees than the others. It is suggested that the pink-red color of the transgenic cultivars may have contrasted better with background vegetation to the insect eye more than white flowers in conventional *Gypsophila* CGMS. Floral preference in honey bees is controlled by a set of factors including floral abundance, odor, and morphology (Ashman et al., 2005; Chittka et al., 1999), but an important sensory modality influencing floral choice is vision (Chittka and Menzel, 1992). Arthropods visit flowers based on color, scent, and shape of flowers previously experienced, thereby maintaining “constancy” in floral choice (Menzel, 2001). In a study by Jones and Reithel, (2001), bees exhibited remarkably strong preferences for either yellow or red flowers in mixed populations.

It was thus observed that pollinators on transgenic *Gypsophila* used color as a cue for flower visits. Similar findings were reported by Hirota et al. (2012) where Swallowtail butterflies and hawkmoths showed preference for reddish flowers and yellowish flowers. It was also observed that transgenic cultivars emitted a strong scent which could have been an attractant to beneficial arthropods especially bees. Scent composition, rather than scent intensity, plays a more definitive role in determining hawkmoth preference (Hirota et al., 2012). For any nectar or pollen forager, the ability to discriminate, learn, and switch among flowers in the face of an ever-changing environment is critically important. Thus the colour of the transformed cultivars appeared to have impacted greatly on bee population. Lady bird beetles and spiders were also observed and studies analyzing effects of Bt maize on lady beetles, adult and juvenile. The *Theridion impressum* spiders field showed no deleterious effect (Romeis et al., 2006).

Syrphids and predatory mites were not observed in the *Gypsophila* crop so it is suggested that they may not be significant in *Gypsophila* farming. Ants in general were abundant at early growth stages of the crop, declined with time and slightly increased at the end of crop growth. The decline could have been due to increase in crop foliage. It is suggested that the plant tissues were becoming less succulent hence less palatable to sucking insect which are preyed upon by ants. Again towards end of growth cycle there was increase in foliage since the crop had flowered but new tender leaves attracted sap sucking insects, hence slight increase in ants' population.

CONCLUSION

The transgenic cultivars were found to have more of visiting bees due to showy flowers a typical adaptation to attract pollinators. Pink color contrasted better with background vegetation to the insect eye. Syrphid and predatory mites were of no significance in *Gypsophila*. Ants population, just like in other crops is indirectly influenced by succulence of plant tissue; which directly affects abundance of sap sucking arthropods preyed upon by ants. Apart from Ladybird beetles high mean counts in conventional *Gypsophila*, transgenic crop recorded the highest mean number of all beneficial arthropods targeted in the study. It was notable that transgenic G*ypsophila* had no negative effect on beneficial arthropods during the period of the study.

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