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Effect of Induced Plant Expression on Ants and Extrafloral Nectaries Number in Cotton and Castor

Pengaruh Ekspresi Tumbuhan Terinduksi terhadap Populasi Semut dan Nektar Ekstrafloral pada Tanaman Kapas dan Jarak

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ABSTRACT

Plant response to the attack of herbivores is a usual phenomenon, but the number of extrafloral nectaries (EFNs) in response to herbivore attack is least studied and recorded. The current study was undertaken to document the response of cotton (*Gossypium hirsutum* Linnaeus) and castor *(Ricinus communis* Linnaeus) to herbivore and artificial induction. This field research was carried out experimentally on cotton and castor in two study models. The first study was the presence of ants on plants induced by herbivore (with herbivore, without herbivore) and the number of EFNs produced by each plant. The second study was the presence of ants on artificially induced plants (damaged leaves, undamaged leaves) and the number of EFNs produced by each plant. The results revealed that EFNs numbers in cotton and castor were increased by herbivore and artificial inductions, which also induced the number of ant recruitment events in cotton and castor. Artificial induction techniques can be utilized in pest management programs to attract and conserve plant guards, *viz*., biocontrol agents, including ants in the field. EFN is a cheap resource in quickly and effectively maintaining consistent population levels of biocontrol agents within the crops, even during pest-free times.

Key words**:** Ants, artificial induction, extrafloral nectaries, herbivore induction

ABSTRAK

Respon tanaman terhadap serangan herbivora adalah fenomena biasa, tetapi studi tentang jumlah nektar ekstrafloral (EFN) yang dihasilkan tanaman sebagai respons terhadap serangan herbivora masih sedikit dipelajari. Penelitian ini dilakukan untuk mendokumentasikan respon kapas (*Gossypium hirsutum* Linnaeus) dan jarak (*Ricinus communis* Linnaeus) terhadap kehadiran herbivora dan kerusakan bagian tanaman. Penelitian lapangan ini dilakukan secara eksperimental pada tanaman kapas dan jarak dalam dua model penelitian. Model pertama adalah kehadiran semut pada tanaman yang diinduksi herbivora (dengan herbivora, tanpa herbivora) dan jumlah EFN yang dihasilkan. Model kedua adalah kehadiran semut pada tanaman yang diinduksi secara buatan (daun rusak, daun tidak rusak) dan jumlah EFN yang dihasilkan oleh masing-masing tanaman. Hasil penelitian menunjukkan bahwa jumlah EFN pada kapas dan jarak meningkat dengan kehadiran herbivora dan kerusakan bagian tanaman, yang mendorong kehadiran semut pada kapas dan jarak. Teknik induksi buatan dapat digunakan dalam pengendalian hama untuk menarik dan melestarikan musuh alami, termasuk semut. EFN adalah sumber daya yang murah dengan cepat dan efektif mempertahankan tingkat populasi musuh alami yang konsisten di dalam tanaman, bahkan ketika tidak ada hama.

Kata kunci**:** Induksi buatan, induksi herbivora, nektar ekstrafloral, semut

INTRODUCTION

Adaptations in plants were evolved by the biotic and abiotic factors (Marquis, 1992) and are divided into direct and indirect defenses. Direct defenses effect on insect pests by structural (thorns, spines/urticating, hairs and trichomes, sclerotizations) and chemical (waxes, canal secretions latex/resins, and a great diversity of secondary metabolites) factors (Wackers and Wunderlin, 1999). Indirect defenses are the protection attained in plants through the attraction of natural enemies (Dicke et al., 1990) against pests by providing food like extrafloral nectaries or EFNs (Koptur, 1989; Whitman, 1996). EFNs are situated in plants in important parts, which is important for their potential vigor (Heil, 2015).

EFNs are the best example to demonstrate how plants express dynamically to insect pests through the attraction of natural enemies to defend themselves. When herbivores attack the plants, they increase the production of EFNs by 2.5-fold in *Vicia sativa* Linnaeus (Koptur, 1989), 3-fold in *Ricinus communis* Linnaeus, and 12-fold in *Gossypium herbaceum* Linnaeus (Wackers et al. unpublication). Also, the chemical composition of EFNs was altered due to herbivore attack by 5.6 times increase of amino-acid concentration than unharmed *Impatient sultani* (Smith et al., 1990).

Ants are found to respond to both quantitative and amino acid changes in EFNs (Del- Claro and Oliveira, 1993; Lanza et al., 1993). Plants increase EFN secretions only in damaged areas by herbivores, thereby guiding natural enemies to the specific parts as an immediate effect to safeguard themselves effectively (Wäckers et al., unpublication). Yamawo et al. (2019) observed that *M. japonicus* plants controlled the ants foraging on their leaves using several types of EFNs in reaction to leaf damage by *Spodoptera litura*, which resulted in a successful biotic defense against herbivores by ants.

Cotton is attacked by 24 species of insects, of which nine are key pests (Sundramurthy and Chitra, 1992); In addition, *Pectinophora gossypiella* has become a menace in cotton (Naik et al., 2018; Kranthi, 2012). Castor is attacked by 107 species of insects; eight are significant pests (Puneet et al., 2020). This study was undertaken to document the response of cotton (*Gossypium hirsutum* Linnaeus) and castor *(Ricinus communis* Linnaeus) to herbivore and artificial induction. Thus, this study has been initiated to exploit EFNs secretion to manage pest population in both the crops by attraction and conservation of natural enemies, including ants, with no additional cost.

METHODOLOGY

The experiments were conducted at the experimental plots of Department of Entomology, Annamalai University, Annamalai Nagar, Tamil Nadu, during 2019.

Methods

This field research was carried out experimentally on cotton and castor plants aged 60 days after planting, in two study. The first study was the presence of ants on plants induced by herbivore (with herbivore, without herbivore) and the number of EFNs produced by each plant. The herbivore used was *Spodoptera litura*. The second study was the presence of ants on artificially induced plants (damaged leaves, undamaged leaves) and the number of EFNs produced by each plant. A damaged leaf was made using scissors. Each treatment was done on 12 samples of two plants.

Castor and Cotton Planting

The seeds of cotton (hybrid) were sown with the spacing of 90 × 60 cm and castor (hybrid) with the spacing of 120 × 90 cm in the plot size of 15 × 15 m^2. There were no pesticides sprayed for both the crops throughout the study. Agronomic practices were done as per crop production guide (2012).

Larvae Preparation

Spodoptera litura was reared following the procedure of PDBC (1998) in the laboratory. The larvae were fed with castor in the plastic box (22 x 13.5 x 8 cm) and reared till pupation. The pupae were collected, cleaned, and surface sterilized with 0.05% sodium hypochlorite solution, placed in vermiculite inside a plastic bucket (22 x 20 cm) covered with khada cloth. The nerium twig was placed inside the cage as an oviposition substrate. The eggs thus laid were used for further mass rearing.

Herbivore Induction

A field experiment was conducted to know the effect of herbivore (*S. litura*) on the presence of ants and the number of EFN produced by cotton and castor (hybrid). Twelve plants of age 60 DAP were randomly selected in each treatment (with herbivore, without herbivore). Plants were checked for any visible damage.

On the with herbivore treatment, two 3rd instar larvae of *S. litura* were released, and the plant then was covered with nylon meshed cages (60 x 30 cm), while the without herbivore treatment was left without larva. After 48 hours, larvae and cages were removed from the plant. Immediately, the number of ants present on each plant of both treatments were recorded five times lasting five minutes during the following 5 hours. The number of ants recruitment events was also observed for each plant. Ant recruitment event means the occurrence of three or more ants of the same species on a single plant simultaneously.

Artificial Induction

The twelve plants of age 60 DAP were selected randomly in each treatment (damaged leaves, undamaged leaves) in cotton and castor (hybrid). Mechanical leaf damage (50%) was made on leaves 1-5 of the treatment plant (leaf one being the most apical opened leaf) by cutting each leaflet in half horizontally using scissors. Immediately, the number and species of ants present on each plant of both treatments were recorded for five times lasting five minutes in each plant during the following 5 hours. The number of ant recruitment events was also observed for each plant.

Counting the Extrafloral Nectaries (EFNs)

After one week of treatments, EFNs numbers were counted. EFNs numbers were counted at the abaxial surface of leaf on a major vein, mid vein, bract in cotton, and at the abaxial surface of leaves, leaf base, petiole, peduncle, and stem in castor.

Ant Identification

Ants collected were preserved in 75 percent ethyl alcohol. Identification of presserved ants to species level was done at Insect Ecology and Behavioural Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University following the taxonomic keys of Bolton (1994); Tiwari (1999) and Hashimoto (2003), using Stemi DV4 Stereo (Zeiss) microscope.

Data Analysis

The data were subjected to the calculation of standard deviation using Microsoft Excel.

RESULTS

Herbivore Induction

Based on herbivore induction, the highest number of ants was found in castor, which were attacked by herbivore (with herbivore). In both cotton and castor, the number of ant presence for the with herbivore treatment was highest at the 5th hour, and the number of ant presence for the without herbivore treatment was highest at the 3rd in cotton and the 1st hour in castor (Table 1).

The EFNs number was produced more in plants that were attacked by herbivore (with herbivore). The highest EFNs number was produced by castor, which was attacked by herbivore (*S. litura*), 7 times higher than cotton (Table 2).

Table 1. Effect of herbivore induction on ant presence in cotton and castor

Treatments	Hour	Ant presence per plant ± SD (inviduals)	
		Cotton	Castor
With herbivore	1st	1.80 ± 0.03	1.85 ± 0.07
	2nd	2.20 ± 0.10	2.00 ± 0.09
	3rd	2.40 ± 0.09	3.57 ± 0.14
	4th	2.00 ± 0.07	3.22 ± 0.16
	5th	**2.60 ± 0.10**	**4.00 ± 0.07**
Without herbivore	1st	**1.34 ± 0.05**	1.27 ± 0.04
	2nd	1.20 ± 0.04	1.78 ± 0.01
	3rd	0.40 ± 0.01	**2.34 ± 0.09**
	4th	0.50 ± 0.23	2.00 ± 0.10
	5th	1.00 ± 0.05	1.54 ± 0.02

Table 2. Effect of herbivore induction on extrafloral nectar (EFNs) number in cotton and castor

Treatments	EFNs number per plant ± SD	
	Cotton	Castor
With herbivore	23.00 ± 0.90	171.05 ± 9.75
Without herbivore	19.58 ± 0.76	118.14 ± 4.75

The herbivore induction has attracted the presence of five species of ants. Three species were found on cotton and castor: *Camponotus rufoglaucus, C. sericeus*, and *Pheidole* sp. Two species were only found on cotton: *Crematogaster* sp. and *Solenopsis geminata*. Two other species were only found on castor: *C. irritans* and *Monomorium criniceps* (Table 3).

Artificial Induction

Based on artificial induction, the highest number of ants was found in castor, which were damaged leaves. The number of ant presence for the damaged leaves treatment was highest at the 5th hour in cotton and at the 2nd hour in castor. In both cotton and castor, the number of ant presence for the undamaged leaves treatment was highest at the 3rd hour (Table 4).

The highest EFNs number was produced by castor on the damaged leaves treatment, 7 times higher than cotton. This EFNs was also produced more in the damaged leaves treatment (Table 5).

Table 3. Effect of herbivore induction on species number of ants in cotton and castor

Species	Ant species in cotton		Ant species in castor	
	With herbivore	Without herbivore	With herbivore	Without herbivore
Camponotus irritans	-	-	*v*	*v*
Camponotus rufoglaucus	v	v	v	v
Camponotus sericeus	v	v	v	v
Crematogaster sp.	v	v	-	-
Meranoplus bicolour	-	-	-	-
Monomorium criniceps	-	-	*v*	*v*
Monomorium scabriceps	-	-	-	-
Monomorium sp.	-	-	-	-
Pheidole sp	v	v	v	v
Solenopsis geminata	*v*	*v*	-	-
Total	5	5	5	5

Table 4. Effect of artificial induction on ant presence in cotton and castor

Treatments	Hour	Ant presence per plant ± SD (inviduals)	
		Cotton	Castor
Damaged leaves	1st	2.50 ± 0.10	2.20 ± 0.08
	2nd	1.00 ± 0.04	**5.54 ± 0.26**
	3rd	2.60 ± 0.10	4.33 ± 0.17
	4th	2.20 ± 0.08	2.59 ± 0.14
	5th	**2.85 ± 0.11**	3.32 ± 0.05
Undamaged leaves	1st	0.80 ± 0.03	0.86 ± 0.03
	2nd	0.60 ± 0.02	1.40 ± 0.06
	3rd	**1.00 ± 0.04**	**3.00 ± 0.12**
	4th	0.60 ± 0.02	2.32 ± 0.12
	5th	0.60 ± 0.02	1.39 ± 0.02

Table 5. Effect of artificial induction on extrafloral nectar (EFNs) number in cotton and castor

Treatments	EFNs number per plant ± SD	
	Cotton	Castor
Damaged leaves	25.75 ± 1.05	178.95 ± 7.30
Undamaged leaves	21.43 ± 0.87	121.55 ± 6.35

Artificial induction has attracted the presence of eight species of ants, eight species were found in cotton and seven species were found in castor. Seven species were found in cotton and castor: *Camponotus rufoglaucus, C. sericeus, Crematogaster* sp*, Meranoplus bicolour, Monomorium scabriceps, Monomorium* sp, and *Pheidole* sp. One species was only found in cotton, namely: *S. geminata* (Table 6).

Table 6. Effect of artificial induction on species number of ants in cotton and castor

Species	Ant species in cotton		Ant species in castor	
	Damaged leaves	Undamaged leaves	Damaged leaves	Undamaged leaves
Camponotus irritans	-	-	-	-
Camponotus rufoglaucus	v	v	v	v
Camponotus sericeus	v	v	v	v
Crematogaster sp.	v	v	v	v
Meranoplus bicolour	*v*	*v*	*v*	*v*
Monomorium criniceps	-	-	-	-
Monomorium scabriceps	*v*	*v*	*v*	*v*
Monomorium sp.	*v*	*v*	*v*	*v*
Pheidole sp	v	v	v	v
Solenopsis geminata	*v*	*v*	-	-
Total	8	8	7	7

DISCUSSION

Based on herbivore induction, the highest number of ants was found in castor, which was attacked by herbivore (with herbivore) (Table 1). Based on artificial induction, the highest number of ants was also found in castor, which was damaged leaves (Table 4). This may be due to more extrafloral nectaries' secretions.

In both cotton and castor, the number of ant presence for the with herbivore treatment was highest at the 5th hour, and the number of ant presence for the without herbivore treatment was highest at the 3rd in cotton and the 1st hour in castor (Table 1). The number of ant presence for the damaged leaves treatment was highest at the 5th hour in cotton and at the 2nd hour in castor. In both cotton and castor, the number of ant presence for the undamaged leaves treatment was highest at the 3rd hour (Table 4). This may be due to abiotic factors.

The EFNs number was produced more in castor that were attacked by herbivore (with herbivore) (Table 2) and in the damaged leaves treatment (Table 5). Herbivore attacks and damage to plant stimulated plants to produce more EFNs. Mondor et al. (2013) recorded that many plant species secrete more EFNs when damaged (Heil et al., 2000; Heil et al., 2001; Wackers and Wunderlin, 1999; Koptur, 1989). Multiple broad bean cultivars produce additional EFNs in response to leaf damage, but none of these plants increase nectar secretion rates. *Senna chapmanii* plants produced more EFN in response to leaf damage, and that the same leaf damage elicits increased ant activity on the plants (Radhika et al., 2010).

Ness (2003) found that ant attendance at caterpillar-affected leaves rose two- to three-fold within 24 hours of herbivory compared to attendance at the surrounding, undamaged leaves. Prior to the commencement of herbivory, these injured leaves attracted the fewest ants, suggesting that the specialized caterpillar avoids or excluded from leaves with more guardians. The presence of ants at damaged leaves was unaffected by removing leaf tissue using scissors. Compared to nearby unattacked plants, ant attendance per leaf on attacked plants rose 6- to 10-fold following the introduction of the caterpillar. The plant's biotic defense works on two levels: following an assault, the number of bodyguards (ant workers) on the plant rises, and this augmented workforce is oriented towards the attacked leaves inside the plant. Plants that attracted more ants had fewer caterpillars, implying that these plants had fewer caterpillars. The above observations indirectly support the present findings.

Similar to present study results were reported by Agrawal and Rutter (1998) and Heil et al. (2001), who pointed out that the overall number of EFNs on a bean plant is also a phenotypically plastic trait in response to leaf damage, not to disrupt herbivore feeding directly, but presumably to attract mutualist partners such as ants, to reduce herbivory.

Yamawo and Suzuki (2017) experi-mentted on plants cultivated in a greenhouse or the field under control or leaf damage settings. They measured EFNs secretion and the number of ant workers on plants after causing fake leaf injury. After leaf injury, they counted the quantity of EFNs on each of the seven leaves. Following leaf injury, EFNs production was triggered within one day, attracting many ant workers, and the EFNs secretion declined to its original levels after seven days. After leaf injury, the number of EFNs was highest on the first leaf and lowest on the sixth leaf, but the overall number of EFNs did not differ across treatments. After leaf injury, *M. japonicus* quickly promotes EFNs production, followed by relaxing. Furthermore, it is possible to reduce the cost of induction by lowering the number of EFNs on later generated leaves after EFNs induction on newly produced leaves.

In the present study, the damaged leaves treatment produced more EFNs number after one week, which is in line with the Pulice and Packer (2008) observations who performed a greenhouse experiment, in that continuous damage to seedlings was simulated in two treatments intended to mimic different types of herbivores: (i) 50% of the area of each leaf was removed using a paper hole punch (e.g., insect herbivore simulation), and (ii) 50% of the area of each leaf was removed using scissors (e.g., browsing mammal simulation). Seedlings in the control group were not damaged. Post-treatment, damaged plants produced significantly more EFNs per leaf on pre-existing leaves and those that emerged following the onset of damage than plants in the control group. Regardless of treatment, leaves emerging earlier supported EFNs than leaves emerging later in the experiment. Herbivory, mechanical injury, and treatment with methyl jasmonate (MeJA) all stimulated EFNs release in the myrmecophilic plant *Macaranga tanarius* (Heil et al., 2001). Recent research in other systems has shown that various species within the same genus (*Populus*) might have varied EFNs activity patterns (e.g., constitutive or inducible) (Escalante-Perez et al., 2012).

The herbivore induction has attracted the presence of five species of ants (Table 3), and the damaged leaves have attracted the presence of eight species of ants (Table 6) due to their preference for the specific plant host.

CONCLUSION

Extrafloral nectaries (EFNs) number in cotton and castor were increased by herbivore and artificial inductions which also induced the number of ant recruitment events in both cotton and castor. Artificial induction technique can be utilized in pest management program to attract and conserve plant guards *viz*., biocontrol agents including ants in the field. Thereby reduces the pesticide dumping and minimizes pest resistance problem in commercial crops. EFN is a cheap resource in maintaining consistent population levels of biocontrol agents within the agricultural crops, even during pest-free times quickly and effectively.

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