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An insect-plant interaction in the Sinai desert ecosystem

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ABSTRACT

In the arid mountains of St Katherine Protectorate, a heteropteran plant bug, *Copium teucrii* (Host), attacks inflorescences of the Felty Germander plant, *Teucrium polium* L. causing galls on its flowers and morphological, anatomical and hormonal changes in the plant. Within galled flowers, *Copium teucrii* (Host) develops through 5 nymphal instars, and the corolla increases in size, becoming swollen and spherical. The reproductive organs of galled flowers reduce in size, and the flower fails to set any seeds. On galled plants, most galls are concentrated in the central parts of the plant, and most are unilocular. Microscopic examination of the leaf near a galled inflorescence showed a reduction in the midrib region, with a smaller stem, especially the conductive tissues. Endogenous plant growth regulators (both auxin and cytokinin) greatly increased, and gibberellin increased, in galled inflorescences relative to ungalled ones, whereas abscisic acid content decreased.

KEYWORDS: *Copium, Teucrium*, interaction, biology, gall, hormone, St Katherine, Sinai, Egypt

INTRODUCTION

Galls and other symptoms of disease that develop on host plants under the influence of insects have stimulated many entomologists and botanists to conduct investigations into the causes of gall formation (Hori 1976). All plant organs are subject to galling including roots, stems, leaves, flowers and fruits, as well the various parts of these organs are preferred by certain gall insects. The effect of gall-insect attack on different plant organs varies depending not only on the type of organ attacked but also on the developmental state of the organ at the time of attack occurs (Birch 1974). Some insects attack the reproductive system of the plant and usually occur early in the development of the plant, normally in the bud stage. The result of such attacks includes swollen flower buds and/or flower heads. Some investigators ascribe the induction of galls to plant-growth substances (auxin etc.) in the insect saliva (Schaller 1968). These secretions are usually accompanied by chemical changes in the plant tissues. Such changes may cause serious imbalances in plant chemistry and result in alterations of plant physiology that may be more important than the galls themselves (Birch 1974).

In the wadis of St Katherine (South Sinai, Egypt), mainly in Wadi Gebel, the Felty Germander plant, *Teucrium polium* L. (Family: Labiatae) is very common. It is a dwarf shrub with downy leaves and stems with small white flowers gathered into a compact inflorescence which appears in May-July (Zalat & Gilbert 1998). A heteropteran plant bug, *Copium teucrii* (Host) (Family: Tingidae) induces galls on the flowers of this plant. Tingid bugs are the only gall inducers in the Heteroptera. Members of this family usually attack the flowers and inflorescence of various plants. For example, *Copium teucrii* (Host) induces giant flowers on *Teucrium montanum* and the development of the flower is inhibited by this tingid bug, provoking cell hyperplasia and hypertrophy (Shorthouse & Rohfritsch 1992). Mond &

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Carayon (1958) mentioned that the species of *Copium* are mainly herbivores on a specific host plant of *Teucrium*, galling *T. montanum, T. capitatum, T. polium* L. and *T. marum*. The same authors gave some notes on the gall formation induced by *Copium teucrii* on *T. montanum*.

No studies have been done on the interaction between *Copium teucrii* (Host) and its host plant *Teucrium polium* L. The present work is intended to focus on the insect-plant relationship, dealing with the insect life cycle, the changes in the plant morphology and anatomy together with the endogenous growth regulators such as auxins, gibberelline, abscisic acid and cytokinin.

MATERIALS AND METHODS

In Wadi Gebel (Sinai, Egypt), field data were collected during gall formation mainly in July and August 1997. Twenty plants were surveyed, taking ten inflorescences from each. Various parameters have been measured such as number of galls per inflorescence, gall position on the plant and the gall type. The position of the gall on the plant has been classified into three categories: top (on the top third of the plant), bottom (on the bottom third of the plant) and middle (central third). The number of chambers in each gall was recorded (1-5), this referred in the text as gall type. An extra twenty plant replicates (10 ungalled and 10 galled), were randomly collected, a set variables measured on flowers including the length and width of the sepal, petal, filament, anther, ovary, style and stigma (all in mm).

Anatomical sections of the leaf and stem were done at the region near the inflorescence in both galled and ungalled inflorescences. Normal and galled plant material were fixed and preserved in FAA and 70% ethanol. Using a hand microtome, sections of about 10-20μm were obtained and double stained with safranin (1% safranin solution in 50% ethanol) and light green (1% light green solution in absolute ethanol and clove oil) (Corgan & Widmayer 1971).

The effects of galling on the endogenous auxins, gibberellins and abscisic acid were investigated within the plant. These different growth regulators were extracted in pure forms and quantified by HPLC. The tissues used in this analysis were both galled and ungalled inflorescences. The extraction of indole acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA), benzyladinine (BA) and kinetine followed the method adopted by Guinn *et al.* (1986). The measurement of the hormones followed the method described by Smith & Davies (1985). The proper concentrations of endogenous plant growth regulators (IAA, GA3, ABA, BA and kinetine) were obtained by comparing their respective peak areas in the plant extracts with their corresponding areas obtained with the authentic samples. The peak areas and plant growth regulator concentrations were measured automatically by the HPLC software.

RESULTS AND DISCUSSION

The life cycle of *Copium teucrii* (Host) is usually completed within the galled flower of *Teucrium polium* L., where it undergoes five nymphal instars (Table 1 and Figure 1). The egg is about 0.5mm in length, and conical. The first nymphal instar is very small (about 0.5mm long), yellow in colour, with indistinct spines (clypeal, jugal and occipital) and wing buds. It develops to the slightly larger second instar, with no serious morphological changes. The third nymphal instar grows in size, with a slight change in colour from yellow to yellowish orange, the jugal spines begin to develop slightly, and the occipital spines become distinct and extend below the lower margin of the eyes. The fourth nymphal instar showed changes in the same characters especially the tibial segment that elongates and lengthens, and the clypeal spines become distinct. The last fifth instar increases again in size to about 2mm in length; it is orange-brown in colour and has large eyes. The jugal, occipital and clypeal spines, the third and fourth antennal segments, the wing buds and the tibial segments are larger and more distinct.

Table 1: The main morphological differences between the nymphal instars of the insect *Copium teucrii.*

Characters	Nymphal Instars				
	First	Second	Third	Fourth	Fifth
Body length	0.5mm	0.75mm	1.0mm	1.2mm	1.9mm
Body colour	yellow	yellow	yellow to yellowish- orange	orange brown	orange brown
Size of eyes	very small	small	Moderate	large	large
Jugal spines	indistinct	indistinct	slightly developed	slightly developed	well developed
Occipital spines	indistinct	indistinct	distinct & extended below lower margin of eyes	distinct & extended between eyes	distinct & extended beyond upper margins of eyes
Clypeal spines	indistinct	indistinct	Indistinct	distinct	distinct
3rd antennal segment	shorter than 2nd	as long as 2nd	longer than 2nd	longer than 1.5X of 2nd	longer than 2.5X of 2nd
4th antennal segment	short & rounded	medium & fusiform	medium & fusiform	elongate & fusiform	elongate & spindle-shaped
Wing buds	indistinct	indistinct	extended to mesothorax	extended to upper margin of 1st abdominal segment	extended to upper margin of 5th abdominal segment
Tibial length	very short	short	moderate	long	very long

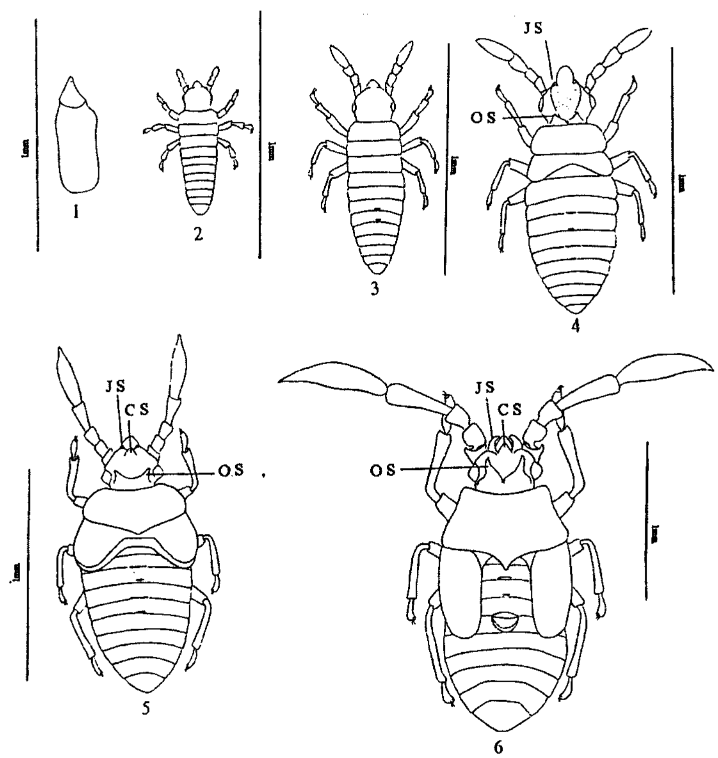

Figure 1: The egg and the five nymphal instars of *Copium teucrii* (Host) (1. egg; 2. first nymphal instar; 3. second nymphal instar; 4. third nymphal instar; 5. fourth nymphal instar; 6. fifth nymphal instar; JS= jugal spine; CS= clypeal spine; OS= occipital spine).

The data on the morphology of the plant as a result of infestation by the insect showed that there was no correlation between the number of heads per branch and the number of galls ($r= 0.31$, $p =0.06$) and there was high significant difference between the number of galls and the gall position ($p<< 0.01$), since most galls are concentrated on the middle parts of the plant. Moreover, there was a significant difference between the gall type and the gall position ($p<< 0.01$), most galls have a single chamber and are concentrated on the middle part of the plant, but 2- and 3-chambered galls appear to be randomly distributed on the plant. There was a correlation between gall type and gall number ($p<< 0.01$).

The data obtained on the reproductive organs of galled and ungalled flowers is shown in Table 2. The results showed that the normal flower is more elongate and pyriform in shape, being about three times longer (7mm) than wide (2.4mm), while the ungalled flower is much bigger and more spherical in shape (9.4 x 5.5mm). The calyx showed slight differences, the galled being slightly shorter and more spherical (4mm depth x 4mm width) than the ungalled flower (4.5mm depth x 3mm width). The increase in corolla depth and width of the galled flower is due its expansion as a consequence of gall formation.

For the androecium and gynoecium of the flowers, it is clear from Table 2 that there was a great reduction and malformation in the size and shape of the galled reproductive system in comparison to the ungalled state. All reproductive organs of galled flowers showed reduction in size, and were enclosed within the enlarged and swollen corolla. The seed set of these flowers was zero (i.e. no seed set). The reduction in the reproductive organs of galled flowers was the direct reason for their failure to make seeds. This result was the same as Hori (1976), who recorded that infested flower buds and/or flower heads do not develop into normal flowers and do not produce seeds.

Table 2: Morphological and reproductive characteristics of *Teucrium polium* L. (galled and ungalled). All morphological measurements are in mm; n = 20, CL= corolla length, CW= corolla width, XL= calyx length, XW= calyx width, FL= filament length, FW= filament width, AL= anther length, AW= anther width, OL= ovary length, OW= ovary width, TL= style length, TW= style width, GL= stigma length, GW= stigma width.

		Ungalled			Galled		
		Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Corolla	CL	6.5	7.5	7.0 ± 0.42	8.0	11.0	9.4 ± 1.5
	CW	2.0	3.0	2.45 ± 0.43	5.0	6.0	5.6 ± 0.45
Calyx	XL	4.5	4.5	4.5 ± 0.0	4.0	4.0	4.0 ± 0.0
	XW	3.0	3.0	3.0 ± 0.0	4.0	4.0	4.0 ± 0.0
Androecium	FL	4.0	5.0	4.6 ± 0.51	1.0	1.5	1.45 ± 0.15
	FW	0.2	0.2	0.2 ± 0.0	0.1	0.1	0.1 ± 0.0
	AL	1.2	1.5	1.41 ± 0.14	0.5	0.5	0.5 ± 0.0
	AW	0.5	0.5	0.5 ± 0.0	0.3	0.4	0.32 ± 0.04
Gynoecium	OL	1.5	2.0	1.8 ± 0.25	1.0	1.0	1.0 ± 0.0
	OW	1.5	2.0	1.8 ± 0.25	1.0	1.0	1.0 ± 0.0
	SL	6.0	7.0	6.5 ± 0.52	2.0	2.0	2.0 ± 0.0
	SW	0.1	0.1	0.1± 0.0	0.1	0.1	0.1 ± 0.0
	GL	0.2	0.3	0.27 ± 0.04	0.2	0.2	0.2 ± 0.0
	GW	0.2	0.3	0.28 ± 0.04	0.05	0.05	0.05 ± 0.0

The microscopic examination of the leaf showed that the leaf cells (Figure 2A) of the ungalled inflorescence are well developed and the conducting tissues (xylem and phloem) are broad and situated near or at the center of the midrib. The same tissues (Figure 2B) near the galled inflorescence showed a reduction in the number of cells at the midrib region, especially the conductive tissues and the cells underneath. It is obvious from both figures that the leaf development of the infested inflorescence is abnormal. The reduction and changes of plant tissues are possibly a defence mechanism by the plant to reduce the passage of nutrients to the newly growing gall.

(A) (B)

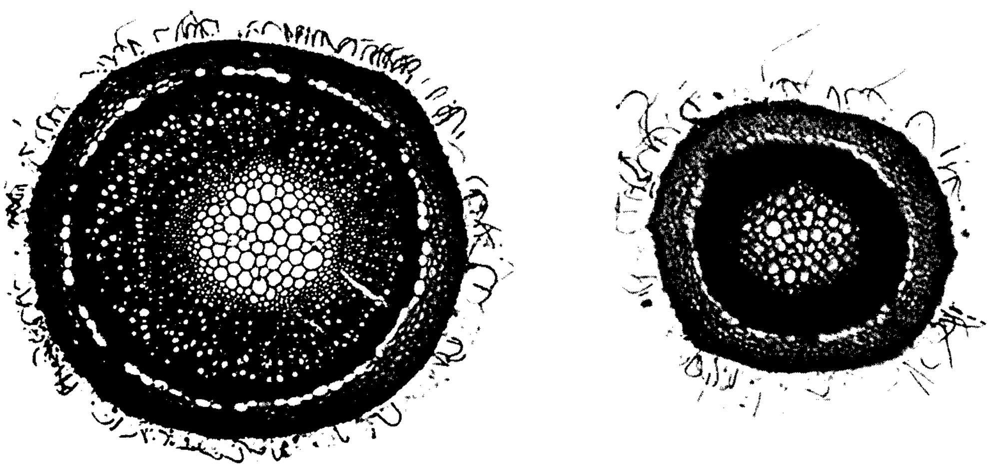

Figure 2: Microscopic cross section of the *Teucrium polium* L. leaf (X= 100). (A) the tissues of the leaf of the ungalled inflorescence; (B) the tissues of the leaf of the galled inflorescence.

Furthermore, the sectioning through the stem of the infested inflorescence (Figure 3B), showed that the xylem region is obviously affected: it looks reduced and more compact than normal (Figure 3A), and the region at the ends of the primary xylem and beginning of the pith is more lignified. It is possible that this compactness of the tissues is due to stress-induced changes in the stem caused by resource demands.

(A) (B)

Figure 3: Microscopic cross section of the *Teucrium polium* L. stem (X= 100). (A) the tissues of the stem of the ungalled inflorescence; (B) the tissues of the stem of the galled inflorescence.

The concentration of endogenous plant growth regulators of both galled and ungalled inflorescences of *Teucrium polium* L. (Table 3 and Figure 4) showed big differences. There was a large increase in auxin content in the galled inflorescence, and an obvious increase in cytokinins. These accord with the idea of Salisbury & Ross (1992) that cytokinins and auxins are important in controlling formation and development of tumors (galls). The data in Table 3 show that the auxin level was lower than the cytokinin content in galled tissue, and this may be a factor in increasing the probability for gall formation. Skoog & Leonard (1968) and Skoog & Armstrong (1970) found that if a high cytokinins -to- auxin ratio is maintained, meristematic cells produce a callus. The involvement of cytokinins in insect galls was first suggested by McCalla *et al.* (1962) who observed that galls of *Pontania proxima* were always initiated adjacent to vascular tissue, a known source of cytokinin. Other workers have also implicated cytokinins involvement in insect gall development (for example Engelbrecht 1971; Abou-Mandour 1980). They all reported higher levels of cytokinins in gall tissues than in uninfected tissues when measured by using a bioassay. Abou-Mandour (1980) has suggested that the elevated levels of cytokinins might be responsible for redirecting nutrients required for gall development. The data in Table 3 also show that the level of abscisic acid was lower in ungalled tissues. The increased level of gibberellins in ungalled inflorescences is obvious in Table 3, and this may explain their greater ability to produce well-developed flowers than galled inflorescens, since gibberellins stimulate the mobilization of nutrients towards the flower (Salisbury & Ross 1992).

Table 3: Effect of galling by *Copium teucrii* on endogenous plant growth regulator concentrations in the inflorescence (fresh weight) of the *Teucrium polium* L. plant.

	GA3 (mg/100g)	IAA (µg/100g)	ABA (µg/100g)	Benzylanine (µg/100g)	Kinetine (µg/100g)
Galled Inflorescence	44.012	1313.668	115.449	117	3890.38
Ungalled Inflorescence	93.296	259.59	27.823	42.606	1861.787

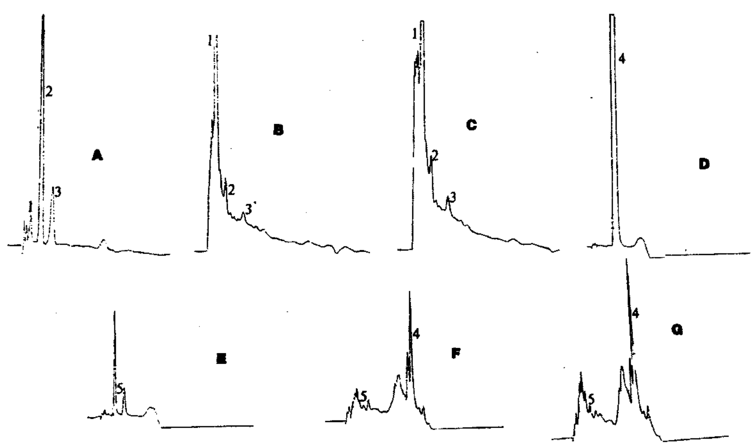

Figure 4: A High Performance Liquid Chromatography (HPLC) profile of the endogenous hormones in both the galled (C, G) and ungalled (B, F) inflorescence of *Teucrium polium* L. plant. (1= GA3; 2= IAA; 3= ABA; 4= Benzyladenine; 5= Kinetine). **A)** Standards for GA3, IAA and ABA; **B)** GA3, IAA and ABA in ungalled inflorescence; **C)** GA3, IAA and ABA in galled inflorescence; **D)** Standard for benzyladenine; **E)** Standard for Kinetine; **F)** Kinetine and benzyladenine in ungalled inflorescence; **G)** Kinetine and benzyladenine in galled inflorescence.

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الملخص العربى

علاقة الحشرات بالنبات فى النظام البيئى لشبه جزيرة سيناء

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تعتبر الدراسة واحدة من سلسلة الدراسات عن علاقة الحشرات بالنبات فى النظام البيئى المتميز لمحمية سانت كاترين بسيناء. فى هذا البحث وجد أن هناك حشرة من أنواع بق النبات (*كوبيم تيكرى*) تهاجم أزهار نبات الجعدة (*تيكريام بوليام*) مسببة أورام زهرية وتغييرات مورفولوجية وتشريحية وهرمونية فى النبات المصاب. تمر الحشرة (*كوبيم تيكرى*) بخمس أطوار يرقية داخل الأزهار المصابة وتتباين تلك الأطوار فى الشكل الظاهرى والحجم. تسبب الإصابة انتفاخ وزيادة حجم الكورولا فى الزهرة، ضمور فى الأعضاء التناسلية للنبات وهذا يؤدى إلى فشل النبات فى إنتاج بذور مما يؤثر على تواجد واستمرار النبات. أيضا أوضحت الدراسة أن أغلب تلك الأورام تتكون من حجرة واحدة، تتركز على أزهار الجزء الأوسط للنبات. عند الفحص المجهرى لقطاعات من الأوراق والسيقان القريبة من الأزهار المصابة وجد حدوث إختزال كبير فى العرق الوسطى للأوراق وضمور خلايا الساق وخصوصاً الخلايا الموصلة. أيضا أثبتت التحاليل الفسيولوجية للنباتات المصابة زيادة إفراز منظمات النمو (الأوكسين والسيتوكانين) بينما قل محتوى حمض الأبسيسيك فى النباتات المصابة مقارنة بالسليمة.