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Responses of the Weed Parthenium hysterophorus (Asteraceae) to the Stem Gall-inducing Weevil Conotrachelus albocinereus (Coleoptera: Curculionidae)

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A stem-galling weevil *Conotrachelus albocinereus* Fiedler 1940 from Argentina is being field released to regulate populations of the weed *Parthenium hysterophorus* in Queensland, Australia since 1995. In this paper we report the tissue and metabolic responses in *P hysterophorus* in the context of the biology and feeding behaviour of the weevil. *C albocinereus* induces elliptical galls often on the main shoot axes and rarely on the terminal and axillary meristems of the host plant. From oviposition through gall maturation, the structure and metabolism of the host-plant tissues, especially at the gall region, change continuously to accommodate pressure inflicted by the feeding larva. The host plant shows re-direction of its vital metabolites to the gall, and to the metaplasied cells of nutrition in particular. In mature galls, the pith parenchyma elements turn lignified. Larval feeding fractures the vertical continuity of vascular tissues, which affects the host plant's overall metabolism. As the larva tunnels the shoot column, it places the frass at the fissured vascular sites. That activity initiates necrosis and eventual death of the living cells of the vascular tissue complex. Such a development induces water-logging stress in the gall and the evapotranspirational system displays contrasting responses. Permanently closed stomatal apertures and abnormally inflated substomatal chambers indicate that *P hysterophorus* suffers moisture-stress with cecidogenesis. The larval performance triggers moisture inundation in the galled shoot and this appears to be an advantage in using this weevil in the control of *P hysterophorus*.

Key words: *Conotrachelus albocinereus* Fiedler 1940 – *Parthenium hysterophorus* Linnaeus – galling behaviour – host plant responses – water-logging stress

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Um die Population der Unkraut-Art *Parthenium hysterophorus* zu kontrollieren, wird seit 1995 in Queensland, Australien, der argentinische Rüsselkäfer *Conotrachelus albocinereus* Fiedler 1940 freigesetzt, der Gallen am Stengel induziert. In der vorliegenden Studie wurden die Veränderungen des Gewebes und des Metabolismus im Zusammenhang mit der Biologie und dem Fraßverhalten des Käfers untersucht.

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C albocinereus induziert elliptische Gallen an der primären Sproßachse, seltener auch am terminalen und axillaren Meristem der Wirtspflanze. Beginnend mit der Eiablage bis zur fertig ausgebildeten Galle verändert sich ständig die Struktur und der Metabolismus des Wirtspflanzengewebes, insbesondere um die Gallenregion, um sich dem wechselnden Druck durch die fressende Larve anzupassen. Es ist eine Umkehrung des Nährstoff-Flusses zum Gallengewebe hin, vor allem zurück zu den metaplasierten Zellen, zu beobachten. Bei vollständig ausgebildeten Gallen lignifiziert das Markparenchym. Durch den Fraß der Larven werden die Leitungsbahnen zerstört und so der gesamte Metabolismus beeinflußt. Beim Minieren in der Sproßachse werden Fäces an den zerstörten Leitungsbahnen abgelegt. Dies führt zu nekrotischen Reaktionen bis hin zum Absterben der Zellen der Leitungsbahnen. Während dies Trocken-Streß in der Galle verursacht, zeigen sich im Transpirationssystem gegenteilige Veränderungen. Ständig geschlossene Stomata und stark vergrößerte substomatale Kammern deuten auf einen Überschuß an Wasser hin, so daß *P hysterophorus* eher unter Feuchte-Streß leidet, wenn Gallen gebildet werden. Die durch die Larven hervorgerufene Wasseransammlung im Gewebe scheint bei der Kontrolle von *P hysterophorus* durch *C albocinereus* der ausschlaggebende Vorteil zu sein.

Schlüsselbegriffe: *Conotrachelus albocinereus* Fiedler 1940 - *Parthenium hysterophorus* Linnaeus - Feuchte-Streß - Gallenbildung - Trocken-Streß - Wirtspflanzen-Reaktionen

1 Introduction

Parthenium hysterophorus Linnaeus is a native of Gulf of Mexico and Central-South America and has invaded North America, southern parts of South America, the Caribbean, and many parts of Asia, Africa, and Australia [EVANS 1997, NAVIE et al 1996]. In Australia, *P hysterophorus* occurs mainly in the state of Queensland occupying about 170,000 km^2 of prime pasture land, causing economic loss up to Au$16.5 m per year [CHIPPENDALE & PANETTA 1994]. *P hysterophorus* causes health problems to humans [MCFADYEN 1995] and total change in the native vegetation, besides contaminating seed produce [CHIPPENDALE & PANETTA 1994].

P hysterophorus is aggressive plant in non-native environments, such as the drier and warmer parts of Australia. A mature plant produces up to 15,000 florets each bearing 4–5 fertile seeds. Seeds of *P hysterophorus* persist and remain viable in soil for reasonably long periods, up to 6 years [NAVIE et al 1996]. With good rainfall and warm temperature, *P hysterophorus* seeds germinate and grow at any time of the year. Use of herbicides has proved uneconomical; therefore, biological control methods are being tried as a viable alternative to regulate *P hysterophorus* populations in Central Queensland [MCFADYEN 1992, DHILEEPAN et al 1996, RAMAN & DHILEEPAN 1999]. Among them, *Conotrachelus albocinereus* Fiedler 1940 is recognized as an agent that shows signs of promise [MCFADYEN 2000].

Gall-inducing insects have been implicated to play a useful role in weed management [HARRIS & SHORTHOUSE 1996, JULIEN & GRIFFITHS 1998, FLORENTINE et al 2001], because they [*i*] remain specific to a particular host-plant species, [*ii*] prefer to attack specific plant organs, [*iii*] create a nutrient sink at the galled region, and [*iv*] weaken the overall host-plant metabolism.

The weevil *C albocinereus* has been found to be highly specific to *P hysterophorus* and to one of its close relatives, the common ragweed, *Ambrosia artemisiifolia* Linneaus [MCFADYEN 2000]. The objective of the present study is to clarify the responses of *P hysterophorus* to *C albocinereus*, keeping the specialized and subtle dimensions of interactions between the plant and the insect, by addressing the following questions: How does the weevil induce the gall? What kind of tissue and consequent physiological changes occurs during gall development? How can the weevil-induced changes in the gall enable better regulation of *P hysterophorus*?

2 Material and methods

Seedling Propagation and Gall Induction: Seeds of *P hysterophorus* were collected in the neighbourhood of the town of Charters Towers [20°55S and 145°16E] in Queensland from January to March 1999. Air-dried and cleaned seeds were stored in airtight glass containers at -5°C until use. In April 1999 the seeds were thawed and sown in sterilized coarse sand in seedling trays maintained in the greenhouse at the Alan Fletcher Research Station, Brisbane [L:D 12:12; mean day temperature 32°C and mean night temperature 15°C; relative humidity 65–80%].

One hundred and fifty seedlings [1-2 cm tall] were transplanted into dark plastic pots [20x20cm^2] containing commercial soil mixture [loam, bagasse, and millmud in the ratio of 2:1:2] in May 1999. All the pots were maintained in a greenhouse. One hundred vigorously growing young plants [ca 30 cm tall] were selected and placed in 10 insect-proof cages [88x54x90cm^3] in June 1999. All cages with the seedlings were maintained in the same greenhouse of earlier-described greenhouse conditions.

Twenty adult ♂♂ and ♀♀ were released into each cage that contained 10 *P hysterophorus* seedlings and they were watched every day for the next 7 d. Whenever mating pairs were sighted, the seedling that harboured them was isolated to a separate cage for closer observations. A hand lens [20x magnification] was used to locate the oviposition scars and to sight egg locations. Sites of egg insertion by the ♀♀ were marked using narrow and short strips of masking tape. Sequence of gall development was pursued following the external response of the host plant. Samples [n=50] of determined age were sacrificed for observing larval behaviour.

Following those observations, gall development could be divided into galls of following age categories: young: 7–14 d, mature: 15–30 d, and old: 45–60 d. The host shoots that housed the eggs [1–6 d galls] did not show any distinct change in the external morphology. Because a limited number of those samples showed patterns of gross structural changes similar to those reported in the stem galls of *Brassica napus* Linnaeus var *oleifera* DeCandolle [Brassicaceae] induced by the weevil *Ceuthorrhynchus napi* Gyllenhal [LE PAPE & BRONNER 1987], only the subsequent gall stages housing developing larvae were examined by microscopy.

Light Microscopy: Young, mature, and old gall samples [n=60] were fixed in formal-acetic-alcohol [FAA: 70% ethanol: 90 ml, 40% Formalin: 5 ml, and Glacial Acetic Acid: 5 ml] for 72 h at the Alan Fletcher Research Station [Brisbane] and were shipped to the Tropical Weeds Research Centre [Charters Towers]. Prior to fixation in FAA, to facilitate rapid infiltration of the fluid into gall tissues, the galls were trimmed at both ends in transverse and vertical axes. Galls segregated into the developmental categories were placed separately in labelled glass vials containing 90% ethanol. The specimens were dehydrated in an ascending alcohol series and imbedded in wax. Transverse and longitudinal sections of the galls were made at 6 µm thickness in a rotary microtome [R Jung-Wetzlar™, Heidelberg]. Sections were contrasted in safranin and fast green, and mounted in DPX. Photographs were made using a Nikon™ [Model SMZ-2B] stereoscopic zoom and an Olympus™ [Model CHT] compound microscopes.

Histochemical Assays: Fresh galls of the determined stages [n=60] were couriered from Alan Fletcher Research Station in a dry-ice chest to the Histochemistry Laboratory of James Cook University [Townsville]. Galls were trimmed to 0.5 cm thickness and mounted in the appropriate axis on to one or more copper stages using the tissue freezing medium TBS™, and frozen for 10 min. In a pre-cooled [-5°C for 24 h] cryotome [Damon/IEC™, Needham Minitome® HTS] the frozen specimens were sectioned at 8–10 µm thickness. Sections were collected using a fine-tipped bamboo stick and were fixed to clean glass slides with Haupt's adhesive. They were then contrasted with appropriate histochemical dyes [JENSEN 1962: IKI reaction for starches, PCA reaction for carbohydrates, and the Oil o'Red reaction for lipids].

Transmission Electron Microscopy: Clean-cut pieces [>2 mm^2] of young, mature, and old galls [n=24] were spot-fixed in 2% gluteraldehyde solution in phosphate buffer [pH 6.9] and were retained at room temperature [ca 22°C] for 24 h. Before post-fixation, the materials were trimmed further, by floating them simultaneously in fresh phosphate buffer [pH 6.9]. The cut pieces were then post-fixed in 1% OsO_4 solution in phosphate buffer [pH 6.9] for 3–6 h, dehydrated in ethanol series, and imbedded in epoxy resin. Sections were cut with glass knives in an ultratome [Leica AG: Reichert™, Ultracut® 701704] and were contrasted with 2% uranyl acetate

and 1% lead citrate. Semi-fine sections [1-2 μm] were contrasted in 1% toluidine blue [in 1% aqueous borax solution], prepared as temporary mounts, and viewed under the light microscope, after the dissolution of the resin with sodium methoxide. Fine sections [0.25-0.4 μm] were viewed in a Transmission Electron Microscope [Philips™, CM-12].

Scanning Electron Microscopy. Young, mature, and old galls from the same sample pool used for TEM studies, and larval stages of the weevil [n=18] were spot-fixed and post-fixed, as described in the TEM procedures. The materials were washed repeatedly in distilled water, and then dehydrated in ethanol series, and critical-point dried [BAL-TEC™; CPD 030]. Determined samples of host-plant tissue and larval stages of the weevil were mounted separately on SEM stubs. They were coated with gold [Edwards™ Vacuum System; # E306A]. Mounted specimens were viewed in a Scanning Electron Microscope [Philips™, XL Series, 30-CP] at an acceleration voltage of 10kV and photographs were obtained.

3 Results

Adults of *C albocinereus* [4–5 mm] live up to 3 months and remain active at night. The gravid ♀ uses her rostrum to chew a hole, more often at the axillary areas closer to the nodal points, and rarely on the internodal regions. Oviposited eggs are pale-white and oval-cylindrical. The ♀ lays one egg in each hole and pushes them into deeper regions of the host plant with the ovipositor. It subsequently covers the oviposition site with its frass, which is mixed with the host-plant's epidermal cells and trichomes. In 4–5 d, the channel created by the insertion of ovipositor for oviposition gets obliterated with the regeneration of wounded parenchyma cells of cortical and vascular regions.

The apodous larva chews the central pith parenchyma, moves in a positively geotropic direction by tunneling the shoot column and initiates the gall. Young larvae feed on the pith parenchyma cells, and as they grow older they feed on the vascular tissues. The larvae deposit frass in their former tunnel path. Developmental stages of the larva remain within the gall for about 60 d. The final larval instar cuts a hole at the lower end of the tunnel and emerges through that to move to the soil for pupation. The adult emerges in the next 3 weeks. Newly emerged adults possibly feed on the leaves and young vegetative shoot terminals. In 1–2 weeks, they mate and commence oviposition.

In the first week after eclosion within the host-plant tissue, the neonate larva behaves essentially like a stem borer. Modest swelling of the internodal stem becomes apparent only in 8–10 d. Fifteen to 28 d old galls are 2±0.5 cm long and elliptical in outline, and include one linear gall chamber. Details of the biology of *C albocinereus* have been provided by DHILEEPAN et al [2001]. Samples raised within the glasshouse show that size and numbers of galls per plant vary, though within a modest range. Five out of the 100 tested population housed 2–3 larvae in the same gall. This could be due to incitement of more than one gall in close proximity and the their subsequent coalescence to result in one long gall chamber. Galls grow for 7–9 weeks.

Early Phase of Gall Development [7 –14 d]: Upon hatching from the egg, the larva starts feeding on the surrounding pith parenchyma cells. A few cells lying close to the feeding site show abnormal patterns of wall thickening and signs of necrosis. Cells immediately following the innermost layer of parenchyma cells enlarge and accumulate cytoplasm, turn metaplastic, and soon develop into the nutritive tissue. A few other cells in the neighbourhood have unevenly thick walls and those cells turn necrotic and die subsequently [**Fig 1**]. Larval feeding activity at this stage occurs, generally, at all sides of the larval cavity. This results in the pith parenchyma responding actively by regenerating layers of newly differentiated cells that contain activated cytoplasm and prominent nuclei and grow towards the larva [**Fig 2**].

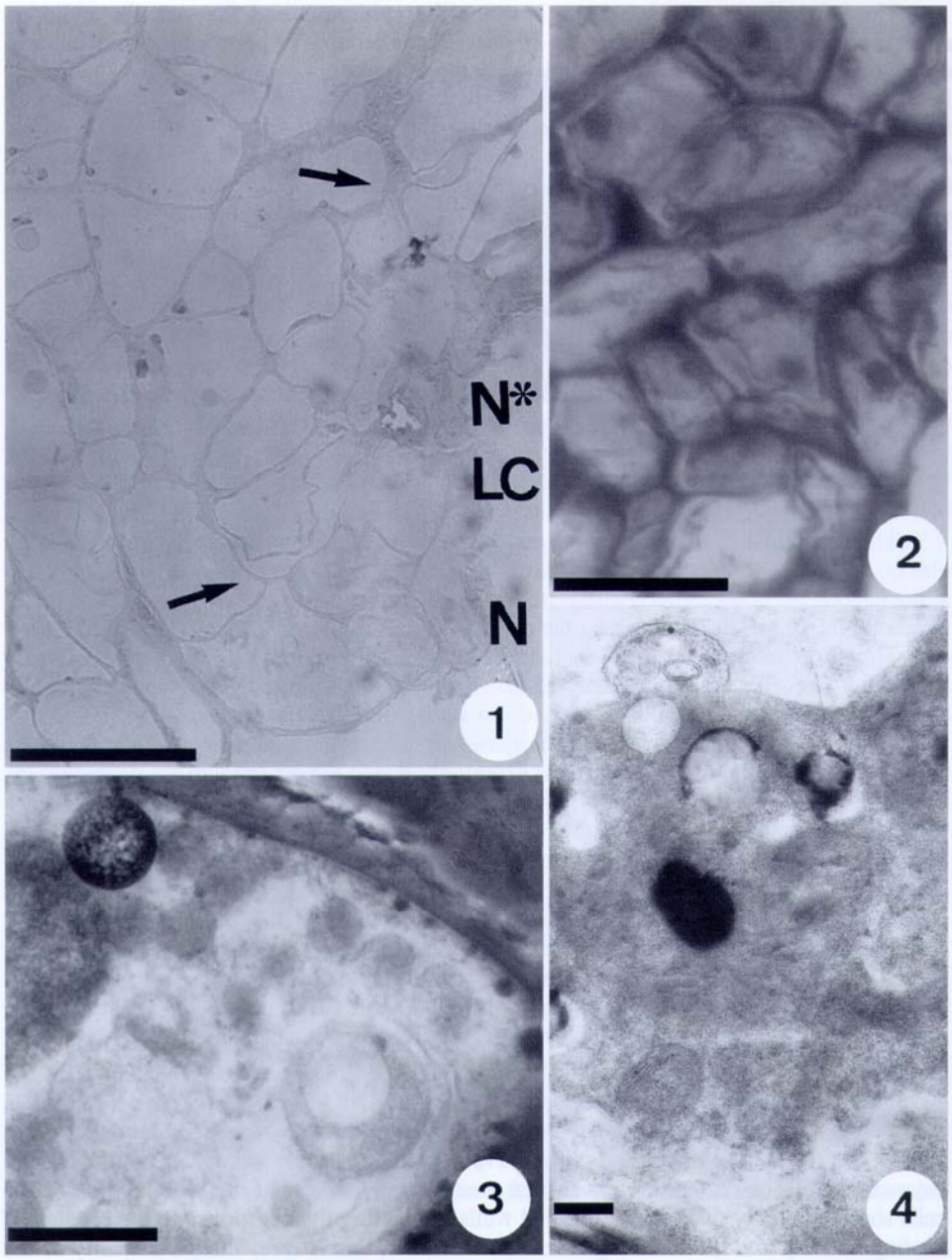

Fig 1-4: Young gall [7–14 d] induced in the stems of *Parthenium hysterophorus* [Asteraceae] by the weevil *Conotrachelus albocinereus* Fiedler 1940 [Coleoptera: Curculionidae].- **1** Response of the pith parenchyma cells to the initial feeding action of the weevil. Some of the cells bordering the larval chamber [**LC**] turn into nutritive cells [**N**] while others turn necrotic [**N***]. →→→ irregularly thickened walls (cs view). —— 100 μm). **2** Metaplasied pith parenchyma cells turning into nutritive cells (cs view). (—— 100 μm). **3** Nutritive cell with circular mitochondria and peroxisomes, the latter lying close to the nucleus (—— 100 μm). **4** Nutritive cell with rich ergastoplasm and several multi-vesicular structures close to the vacuoles. (—— 100 μm).

The cortical parenchyma tissue between the epidermis and the vascular cylinder also responds actively to the feeding impact. They become hypertrophied and include active cytoplasm, and often show division activity, and thus contribute to the increment in size of the gall. The larva feeds vigorously and indiscriminately, not confining itself to the pith parenchyma alone. Very often, feeding extends to the vascular tissues. Because of the rapid division activity in the xylary parenchyma, vascular traces dissociate and that process complements the tunneling activity of the larva on the one hand and the expansive growth the cortical tissues display on the other. As the larva moves downward, it places the frass at the points it fed earlier.

Pith parenchyma cells lying close to the vascular strand differentiate into the tissue of nutrition through cellular metaplasia. These cells include hypertrophied nuclei with electron-dense chromatin material dispersed unevenly close to the nuclear envelope. The cytoplasm is dense spreading almost evenly throughout the cell. Numerous abnormal and vesiculated chloroplasts occur around the nucleus. The mitochondria, like the plastids, occur close to the nucleus. They appear round and do not display well-defined cristae [**Fig 3**]. Closer to the nucleus, peroxisomes bound by a single, well-contrasted membrane, enclosing granular material occur. Several osmiophilic globules exist close to the cell membrane [**Fig 4**].

The ergastoplasm is rich in rough endoplasmic reticulum and lipidic inclusions. Vacuoles occur fragmented. Multi-vesicular structures appear closer to the vacuolar membranes. Autophagous vacuoles including digested sub-cellular materials exist infrequently. Intense concentration of starch occurs along the cortical regions of the very young galls [7 d]. However, slightly older galls [*ca* 14 d] include starch in cells bordering the larval chamber. Carbohydrates occur in greater intensity in 12–14 d old galls and in cells closer to the larval chamber. Lipids in young galls [7–10 d] occur abundantly in tissues closer to the larva.

Maturing Phase [15–30 d]: The stem part that includes the larva swells up at least twice the width of the ungalled portions essentially due to hypertrophy of fascicular parenchyma resulting in asymmetrical growth in the gall [**Fig 5**]. The critical aspect of this developmental stage is the accumulation of frass at the fractured sites within the gall [**Fig 6**]. Because of that action and, possibly due to toxicity of the excretory material, cells at these points turn necrotic and die. Vascular parenchyma and phloem elements collapse due to degeneration of walls and result in breaking the continuity of the vascular trace [**Fig 7**]. However, at regions where the larva feeds actively as it tunnels further, the vascular parenchyma and the pith parenchyma continue to respond by dedifferentiating into active cells of nutrition. Scanning electron micrograph of this region illustrates the regenerative nutritive cells and their tangential walls facing the gall's interior consumed by the larva [**Fig 8**].

Senescent Phase [45– 60 d]. Concurrent with the larval increment in size, the larval chamber also becomes considerably wide. Radial files of actively dividing cells continue to differentiate and nourish the feeding larva. Due to tunnelling behaviour and feeding all around the head region, the larva is able to activate intense growth of parenchymatous cells of both pith and vascular areas [**Fig 9**]. A few of the peripheral nutritive cells show unevenly thickened, lignified walls, indicating their response to the ceasing of feeding activity and the resulting stimulus. However, this is rare, since a majority of the cells in the neighbourhood respond actively and have thinner walls. The nutritive cells within in galls close to 60 d of age die and become sclerified elements.

Older galls [c 55 d] include hypertrophied, erstwhile nutritive cells that show relatively hyaline cytoplasm and enlarged nuclei. The nucleus appears exhausted with the heterochormatin less dense than those of the younger phase. Chromatin material occurs concentrated generally along the parts of nuclear membrane. Hypertrophied plastids including prominent starch inclusions and vesiculated lamellar systems normally occur closer to the nucleus.

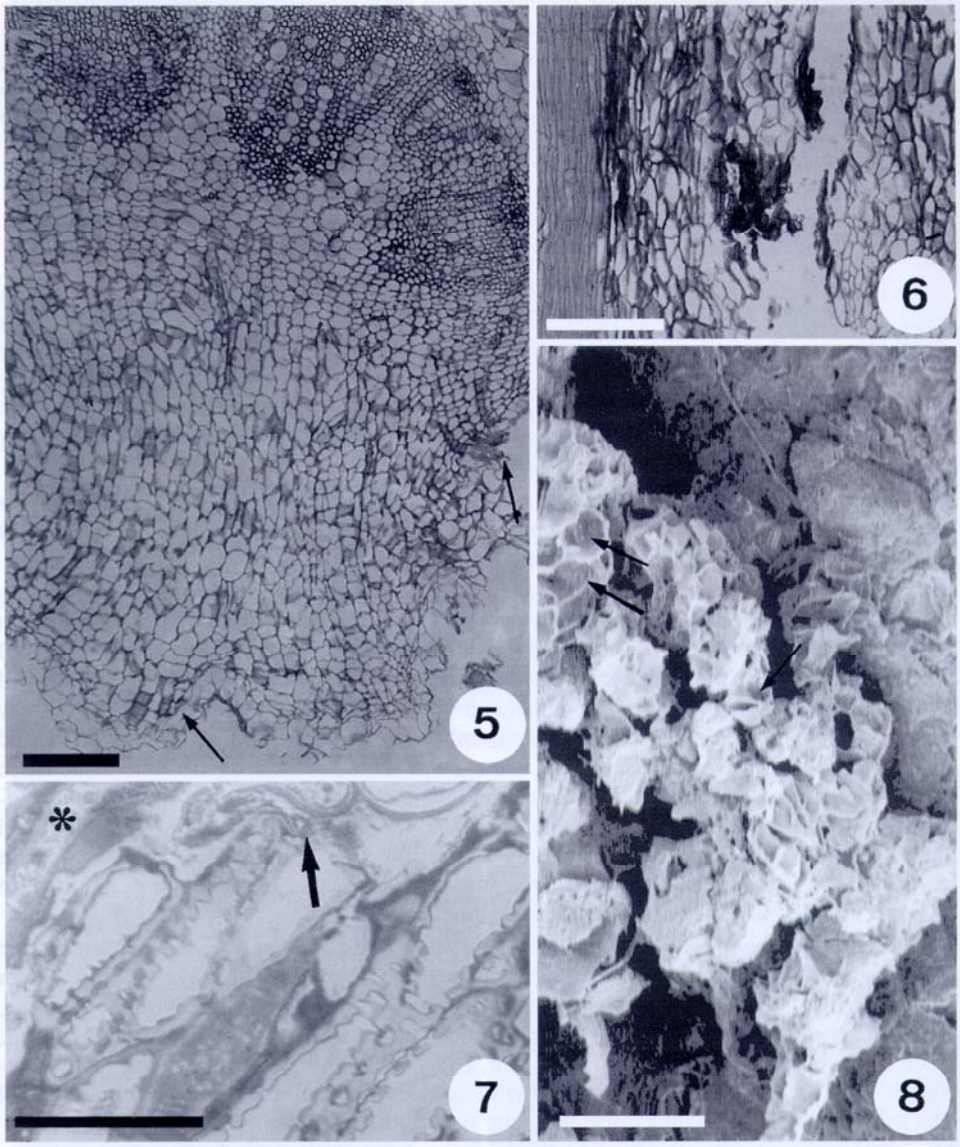

Fig 5-8: Young gall [7–14 d] induced in the stems of *Parthenium hysterophorus* [Asteraceae] by the weevil *Conotrachelus albocinereus* Fiedler 1940 [Coleoptera: Curculionidae] [continued].- **5** Regenerative activity in the pith, →→→ vascular and cortical parenchyma (cs view). (—— 100 μm). **6** Tunnel path of the weevil showing degenerating pith parenchyma cells in the hinder regions of the gall (ls view). (—— 100 μm). **7** Coagulation and break down of cytoplasm in vascular parenchyma [*] and dissolution of walls →→→ in vascular cells (ls view). (——100 μm). **8** Parenchymatous nutritive tissue with their broken radial walls. (——1 μm) [*SEM*].

Mitochondria are numerous, but are distinctly smaller in their dimensions. Numerically more osmiophilic globules exist closer to the cell wall [**Fig 10**]. Several autophagous vacuolar systems appear prominent and they include sub-cellular debris. Hyaline spots in the cell wall are apparent [**Fig 11**].

As the galls enter into the senescent phase, changes occur in the epidermal system. The epidermal cells and stomata of the external gall turn hypertrophied and the substomatal chambers occur abnormally inflated. The stomatal pores remain permanently closed and the stomata appear dysfunctional. [**Fig 12**].

4 Discussion

Gall-inducing arthropods are favoured presently in weed management programs since they have a narrow host range [HARRIS & SHORTHOUSE 1996]. Their capability to re-canalize the host plant's morphogenetic processes and metabolic activity [RAMAN 1994], to place their host plants under stress by draining nutrients and key metabolic products to the larva [RAMAN 1996, RAMAN & ABRAHAMSON 1995], and to exist in adverse environmental conditions [MARTEL 1995] renders them candidates of choice for application in biological control campaigns.

Among the 10 insect species introduced to manage populations of *P hysterophorus* in Australia, the tortricid moth *Epiblema strenuana* (Walker) and the curculionid weevil *C albocinereus* are gall inducers [FLORENTINE et al 2000].

C albocinereus's capability to induce a gall and consequently redirect the host-plant's tissue differentiation and manipulate the metabolic processes for its survival are decisive in the interaction between *C albocinereus* and *P hysterophorus*. As shown in several gall models [ROHFRITSCH 1992], *P hysterophorus* responds by generating 'new' tissues and activating 'new' metabolic pathways to neutralize the weevil's impact. One of the early actions of the weevil larva is to feed on the soft parenchymatous tissue, inflict wound, and eventually kill several of those cells. Such an action entails a rapid regenerative response in the host plant and one of the immediate consequences is that a majority of the newly differentiated cells turn into nutritive cells. However, the ability of the weevil larva to tunnel through the shoot column, to feed not only on the pith parenchyma but also on the vascular tissues, and to place frass at the fractured vascular sites is crucial in enabling the larva to overcome better than the host plant's efforts limiting larval action.

C albocinereus induces water-stress in the galled shoots of *P hysterophorus*, more by inundation than by deficit. Fracturing of xylary conduits contributes to this. Under normal circumstances, the xylary parenchymatous elements regenerate after wounding, repairing the wounded site [MEYER & MARESQUELLE 1983]. In *P hysterophorus* galls, such a regenerative activity does not occur, because the larva places the excretory material at the fractured sites and the possible toxicity of that material kills the living cells of the xylary complex and thus preventing any healing activity.

Parenchymatous cells close to the larva in young galls include abundant carbohydrates and starch. Besides serving the feeding larva as a nutritional source, these sugars, after the movement of the larva further down in the gall, facilitate the transformation of the living, thin-walled parenchyma elements into non-living, thick-walled sclereids [RAMAN 1991]. The thick-walled sclereid elements add to the deteriorating water movement mechanism in an aging gall.

Incidence of distorted and vesiculated plastids, numerous 'weak', round mitochondria, and several osmiophilic globules within the organelles and in the peripheral cytoplasm along the cell wall indicate stress due to water logging [GAFF 1980].

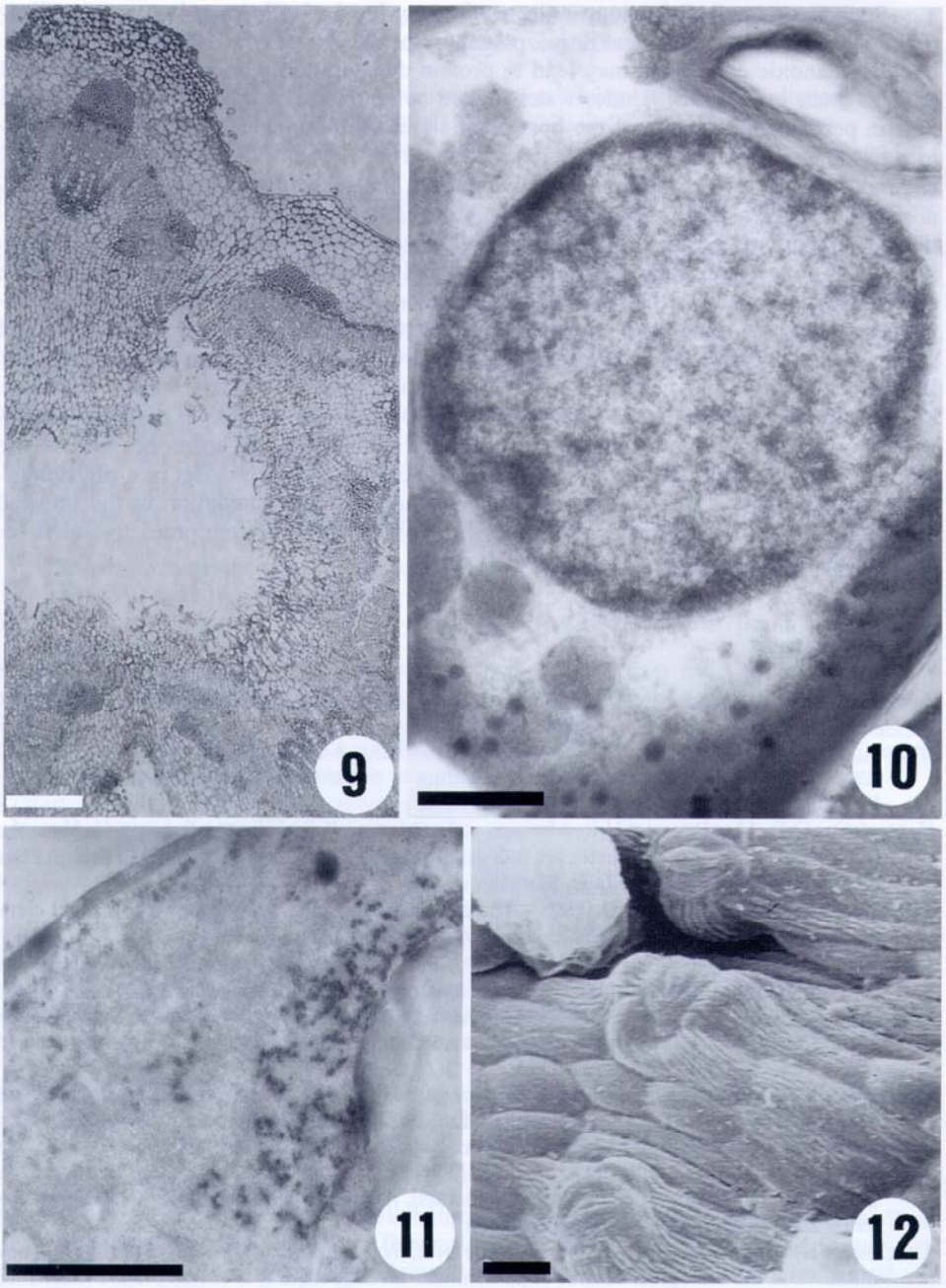

Fig 9–12: Old stem gall [30–60 d] induced in the stems of *Parthenium hysterophorus* [Asteraceae] by the weevil *Conotrachelus albocinereus* Fiedler 1940 [Coleoptera: Curculionidae].-
9 Gall wall showing incremental growth and larval chamber (c s view). (—— 100 μm). **10** Nutritive cell with less dense heterochromatin and hypertrophied plastids (—— 100 μm) [*TEM*]. **11** Nutritive cell with autophagous vacuoles and hyaline spots in the cell wall (—— 100 μm) [*TEM*]. **12** Epidermis showing closed stomatal aperture and abnormally inflated substomatal chambers. (—— 100 μm) [*SEM*].

Such an organelle behaviour indicates the poor level of efficiency in oxygen utilization by the cells and consequent development of anoxia [BLOM et al 1990] in gall tissues. Localized anoxic conditions may lead to protein denaturation within plant cells as well [CARYSTINOS et al 1995]. Under water-logged conditions the stomatal complex should remain permanently open and the host plant should differentiate more of aerenchymatous tissue to nullify oxygen insufficiency [ARMSTRONG et al 1995]. In the *C albocinereus*-induced galls of *P hysterophorus*, by contrast, the substomatal chamber remains abnormally inflated and the stomata remain permanently closed. No impressive differentiation of aerenchyma occurs either. The weevil places the host plant in a metabolic situation causing moisture inundation on the one hand and closed stomatal apertures on the other, eventually leaving the host plant under stress. Fractured xylary ducts and the dysfunctional stomata demonstrate a functional correlation. Sap within stressed xylem and gall tissues is known to include elevated levels of the hormone abscisic acid [ABA] [DE BRUYN et al 1998] and ABA's role in regulating guard cell movements is known [ZEEVAART & CREELMAN 1997]. Larvae of *C albocinereus* are able to dislodge the entrained water and nutrients, and consequently that of the levels of hormones such as the abscisic acid, through the physical process of fracturing xylary vessels in a physiologically stressed gall. *C albocinereus*'s ability to induce water inundation in the infested areas and restrict the stomatal movements in *P hysterophorus* reinforces its value for application in *P hysterophorus* control campaigns.

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