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Histological changes during maturation in male and female cones of the cycad *Zamia furfuracea* and their significance in relation to pollination biology

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VOVIDES, A. P., NORSTOG, K. J. FAWCETT, P. K. S., DUNCAN, M. W., NASH, R. J. & MOLSEN, D. V., 1993. **Histological changes during maturation in male and female cones of the cycad *Zamia furfuracea* and their significance in relation to pollination biology.** The cycad *Zamia furfuracea* L. Fil. is pollinated by a curculionid beetle, *Rhopalotria mollis* Sharp which completes its life cycle in male cones of the cycad, and effectively pollinates female cones. Idioblasts within parenchyma in both male and female cones appear to contain toxic compounds, including at least one neurotoxin, 2-amino-3-(methylamino) propanoic acid (BMAA), and a toxic glycoside, methylazoxymethanol-β-primeveroside (macrozamin). Idioblasts appear structurally unmodified in male cones throughout the period of pollen maturation, and feeding weevils consume much of the

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starch-rich microsporophyll parenchyma tissue, including idioblasts. During this activity no appreciable change in morphology or staining reactions of male-cone idioblasts is detectable. Prior to pollen receptivity, female-cone idioblasts resemble those of male cones. Thereafter, many female-cone idioblasts show marked changes in morphology and content not caused by the weevils themselves. Idioblast changes in female cones are probably associated with the defence of female-cone resources against predation by animals, including pollinating weevils, and may relate to mobilization of toxins. Absence of similar morphological changes in male-cone idioblasts is correlated with toxin sequestration, enabling the pollinator to breed and feed without intoxication.

ADDITIONAL KEY WORDS:—Idioblasts – macrozamin – toxicity – weevils.

CONTENTS

INTRODUCTION

The curculionid beetle (snout weevil), *Rhopalotria mollis* Sharp, is an obligate parasite and an effective pollinator of the cycad, *Zamia furfuracea* L. fil. The entire reproductive cycle of the weevil, including year-long dormancy of certain lipid-rich larvae, occurs in tissues of the cycad male cones (Norstog, Stevenson & Niklas, 1986; Norstog & Fawcett, 1989). The initial visitations by weevils occur only during a brief 1–2 day period when microsporangia are mature and near dehiscence. Weevils swarm upon the male cones, bore into the microsporophylls and feed on their starch-rich tissues before, during and after copulation, and the females lay eggs in the subepidermal microsporophyll tissues. The eggs hatch into larvae which rapidly consume the parenchyma of microsporophylls then pupate. Two pathways of further metamorphosis occur: first, during most of the reproductive season of host and insect, pupae undergo a final metamorphosis and emerge as adults, visit another newly maturing male cone and repeat the breeding cycle once again. The entire cycle from copulation to emergence of the next generation is completed in 7–10 days. Second, toward the end of the breeding season, some larvae develop thick, waxy cuticles and construct thick-walled pupa cases. They remain in diapause within these cases until the next reproductive season of the cycad, some nine to ten months later.

In an earlier study, Norstog & Fawcett (1989) focused their attention on differences in male and female sporophyll tissues that might account for restriction of feeding to male cones of the pollinator and the complete avoidance of female-cone tissues as feeding and breeding sites. In addition to variation in starch content, high in male and low in female cones, an immediately obvious difference is the nature of certain parenchymal cells referred to as idioblasts.

Idioblasts are common in most cycad genera (Vovides, 1991). Those in FAA-fixed but unstained microsporophylls have very large vacuoles that are gold in colour, and are called the 'gold cell' type by Dennis Stevenson (personal communication). Norstog & Fawcett (1989) reported that gold-cell contents in male cones are ninhydrin positive (NIN+), when stained with ninhydrin–Schiff (NIN) reagent (Jensen, 1962), but are unstained by periodic acid–Schiff (PAS) reagent. Although these staining reactions do not identify specific molecules, they show the presence of protein and amino acids (NIN) or carbohydrate (PAS). Conversely, although some idioblasts in female-cone tissues exhibit gold-cell characteristics, many others are stained only to a limited degree with NIN but are PAS+, that is, staining deeply throughout the entire cell with periodic acid–Schiff reagent (Jensen, 1962). This idioblast type also often exhibits vacuolar trabeculae (referred to here as 'trabecular idioblasts'). Norstog & Fawcett (1989) proposed that differential development of male- and female-cone idioblasts may relate to differences in their toxin content and/or toxin mobility, and thereby influence the feeding preferences of weevils. These authors considered that food and a breeding site attract the pollinator preferentially to male cones. Male-cone selection probably depends upon the high starch content of microsporophylls and conversely, absence of feeding upon female tissues relates to the low starch content of megasporophyll tissue at the time of pollination. However, these differences do not entirely explain the feeding/breeding preferences of *R. mollis*.

Weevils are strongly attracted to female cones and effectively pollinate them, but do not feed on them. Male and female cones have similar shape and colour at the time of pollination and female cones are only slightly larger at this time. Visits by weevils may be a consequence only of visual attraction but it also is possible that the major attractants are volatile emissions, in that similar or identical fragrances are emitted by both sexes (Pellmyr *et al.*, 1991). Norstog & Fawcett (1989) proposed that feeding on female-cone tissue is precluded because toxins in some way repel insect predators, including weevils, and they reported instances of attempted but undamaging weevil feeding.

The present study continues the investigation of this unique plant/insect-pollinator relationship, focusing particular attention on factors that may be responsible for the differential development of male- and female-cone idioblasts, and possible roles of cycad toxins in affecting the behaviour of the pollinating weevil.

The literature concerned with links between cycad toxins and insect predators is sparse. Teas (1967) described the glucosylation of the cycad toxin, methylazoxymethanol (MAM), to form a non-toxic MAM-glycoside (in this case cycasin), and noted its sequestration in the haemolymph of a moth, *Seirarctica echo*. The moth is a predator upon cycad leaves. Rothschild, Nash & Bell (1986) described a similar sequestration of cycasin by larvae of the butterfly, *Eumaeus atala florida*, also a cycad-leaf predator. Both studies suggest that each insect avoids intoxication by sequestering toxin, a concept in which sensitivity to toxin is implicit though not proven. However, it has been shown that MAM, produced by glycolysis of cycasin, is a mutagen for *Drosophila* (Teas & Dyson, 1967; Smith, 1967). Although we have no direct evidence that *R. mollis* is repelled by free toxins present in female cones of *Z. furfuracea*, indirect evidence leads us to suspect that toxins may be a factor in the pollination biology of this

cycad. We focused our attention on the amino acid, 2-amino-3-(methylamino)propanoic acid (BMAA) because it is a known neurotoxin present in cycads although it has not been shown to be toxic to insects. However, by using BMAA as an example, we have found evidence consistent with the hypothesis that a toxic constituent (unidentified but perhaps BMAA) plays a role in the feeding/reproductive cycle of the weevil and its host relationship.

BMAA is a low-density excitotoxin which has been implicated in human neurodegenerative disorders. Studies in neuronal-cell-culture testing indicate that it is toxic at concentrations in the low millimolar range (Ross, Seelig & Spencer, 1987; Weiss & Chol, 1988). These concentrations are difficult to achieve in animal studies, but they are not unrealistic when considering insects feeding exclusively on cycads. The low body weight of weevils, combined with their high food intake, could lead to significant levels of BMAA in their tissues and/or gut contents and dung. Although we focused attention on BMAA as a putative factor in influencing weevil behaviour in female vs. male cones of *Z. furfuracea*, we also have considered the possibility that a MAM-glycoside, specifically macrozamin (methylazoxymethanol-β-primeveroside), may also be important in protecting female-cone resources both from generalized animal predators and from *R. mollis*.

MATERIAL AND METHODS

Histology, histochemistry and microscopy

Male and female cones were collected at intervals from individuals of *Zamia furfuracea* in the vicinity of the Robbins Research Center of Fairchild Tropical Garden prior to, during, and after pollination from late May to mid-August 1989. They were fixed in FAA, sectioned, stained and examined by light microscopy (LM) or fixed in glutaraldehyde–OsO_4, epoxy-bedded, sectioned, and stained for LM and electron microscopy (EM). Most of the samples were obtained from the same population described in Norstog & Fawcett (1989); a few were collected from an adjacent planting. Representative microscope sections of micro- and megasporophylls of pre-, at- and post-pollination cones were examined, photographed, and measurements taken from randomly selected idioblasts ($N = 25$) from each developmental interval. The resulting data were subjected to a one-way analysis of variance and the SNK multiple-range test (Schefler, 1979; Sokal & Rohlf, 1981) (Table 1).

Some female cones were enclosed in plastic bags prior to pollen receptivity and remained enclosed past the stage at which they normally would have been pollinated (as determined by megasporophyll separation (Norstog *et al.*, 1986)). The enclosed cones were examined periodically to ensure they had not been visited by weevils. Representative megasporophylls were prepared for microscopy as outlined above. The purpose of this experiment was to uncouple any possible relationship between female-cone idioblast development and weevil visits in order to determine whether or not weevil activity of any kind, including pollination, induces changes in morphology and/or stainability of idioblasts in female-cone tissues. Normally, weevils enter unprotected female cones, carry pollen to ovules, nibble the inner surfaces of the cone axes and megasporophylls, but do not feed or oviposit (Norstog & Fawcett, 1989).

TABLE 1. Measurements (μm) of idioblast diameter at different developmental stages of male and female cones of *Zamia furfuracea* ($N = 25$)

	Developmental stage			
	Early	Dehiscence	Receptive	Post-pollination
Male	47.0±2.6	69.5±3.7	—*	—*
Female	37.0±2.4	—†	58.7±2.2	120.7±6.7

*Following dehiscence, parenchyma is consumed by weevil larvae or becomes senescent.
†Female cones are receptive at the time male cones are dehiscent.

BMAA analyses

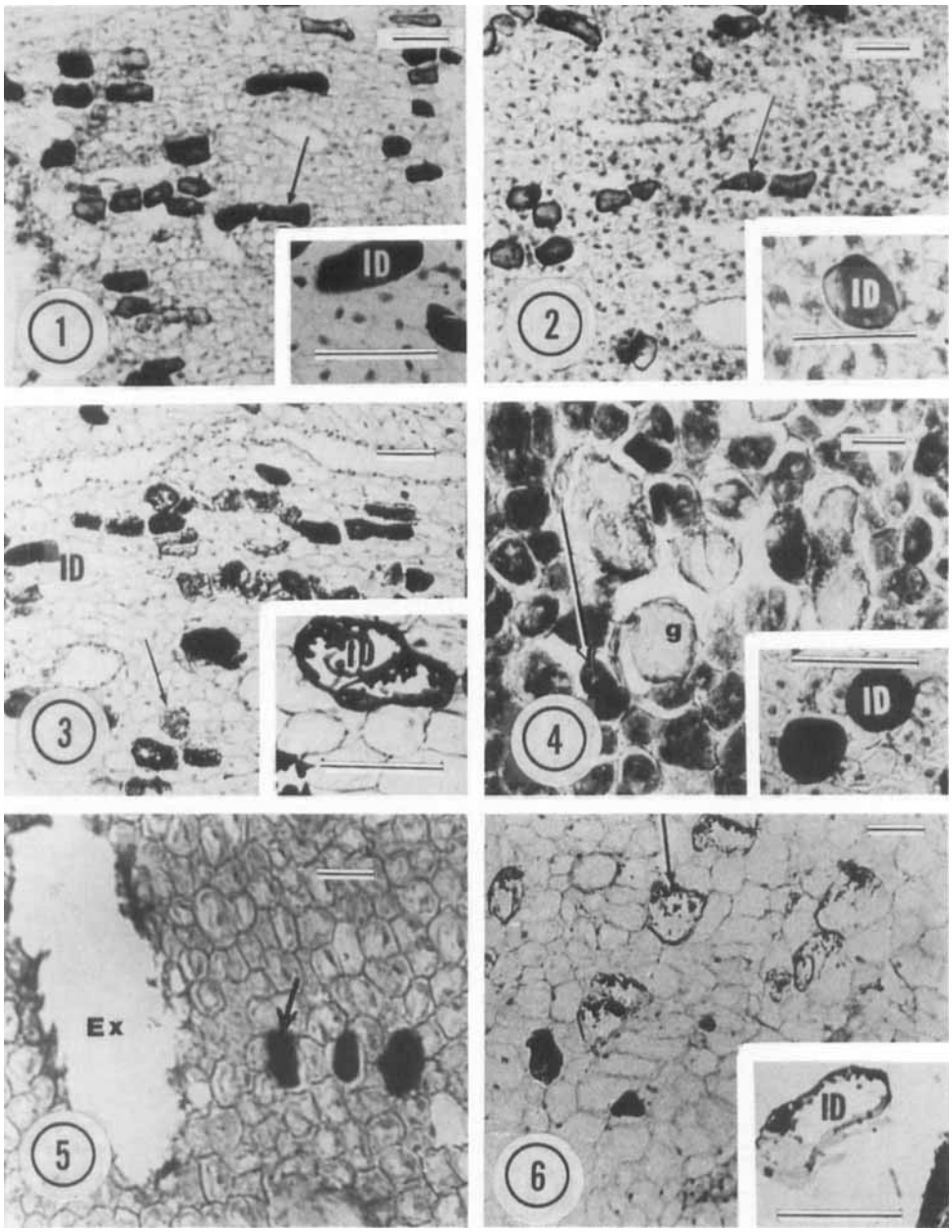
Cone and cone-axis tissues, together with weevil larvae and pupae from female cones were stored at −70°C until assayed. Immediately on thawing, samples were weighed, internal standard added, and the BMAA extracted and derivatized according to the method described in Duncan *et al.* (1989). BMAA content was determined by combined gas chromatography–mass spectrometry. The analytical procedure was performed on a Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5970 mass selective detector. Chromatography was performed with a split/splitless capillary inlet on a fused-silica capillary column (12 m × 0.22 mm i.d.) with a cross-linked methyl silicone stationary phase (HP Ultra 1, 0.33 μm film thickness, Hewlett-Packard) as described in Duncan *et al.* (1989). The results are presented in Table 2.

Idioblasts were extracted by grinding with a little sand separately chopped micro- and megasporophylls from fresh cones using a mortar and pestle in ice-cold phosphate buffer (pH 7.0). A crude idioblast fraction was obtained by filtering the entire mortar content through cheesecloth to separate off sporophyll fibres and sand. The filtrate was allowed to settle or spun down in a bench centrifuge to obtain a pellet. Microscopic observation on similarly ground FAA-fixed sporophyll tissues showed that the majority of idioblasts (gold cells) in the filtrate remained intact. The reason for this is not fully understood; but there may be a difference in toughness between the walls of parenchyma cells and those

TABLE 2. 2-Amino-3-(methylamino) propanoic acid (BMAA) content of tissues of *Zamia furfuracea* and of larvae and pupa cases of *Rhopalotria mollis*, a faithful pollinator of *Z. furfuracea*

Sample name	BMAA (μg g^{-1}) (wet wt)	BMAA (μg g^{-1}) (dry wt)
Male cone, 1	9.2	—
Male cone, 2	5.7	—
Female cone	11.2	—
R. mollis, larvae	9.0±1.4 ($N = 5$)	—
R. mollis, pupa cases	—	34.2±21.7 ($N = 3$)
Idioblasts	Pellet BMAA (μg g^{-1})	Supernatant BMAA (μg g^{-1})
Male 1*	252.0	186.0
Male 2†	207.0	189.0
Female‡	362.0	193.0

*Male 1, predehiscent stage.
†Male 2, late predehiscent cone.
‡Female receptive cone.



Figures 1–6. Male and female cone tissues of *Zamia furfuracea*. Fig. 1. Megasporophyll collected 26 May and stained with I_2KI–fast green. Note idioblasts (arrow) which are mostly of the gold-cell type. Inset, same material stained with NIN, gold-cell idioblast (ID) stains brown-purple. Fig. 2. Microsporophyll collected 26 May and stained with I_2KI–fast green. Idioblasts (arrow) are gold cells; little starch is present (cf. Fig. 4). Inset, same specimen stained with NIN; idioblast (ID) stains brown-purple. Fig. 3. Pre-pollination megasporophyll collected 8 June and stained with I_2KI–fast green shows continued low starch content and variable idioblast morphology, some unmodified gold cells, others (arrow) with modified vacuolar content. Inset, same material stained with NIN shows NIN– idioblast having vacuolar trabeculae. Fig. 4. Microsporophyll collected 13 June, prior to but approaching pollen maturation. With I_2KI staining it shows parenchyma cells loaded with starch (arrow), unstained gold cells (G). Inset, same material stained with NIN shows densely stained idioblasts (ID). Fig. 5. Microsporophyll collected in mid-July and stained with NIN, contains

of idioblasts due to the absence of plasmodesmata and pits in the latter. Pellets and supernatant were analysed for BMAA content by the procedure described above.

Macrozamin analyses

Norstog & Fawcett (1989) reported that contents of female-cone idioblasts stain a deep purple-red with PAS, which is indicative of a carbohydrate content (see Norstog & Fawcett, 1989: figs 10, 11). In contrast, idioblasts in male cones of this species remain unstained with PAS. Suspecting that the PAS+ staining contents of female-cone idioblasts might include toxic glycosides such as cycasin or macrozamin, we tested a small sample of macrozamin with PAS for the presence of reducing sugar. Samples of cone tissues, weevils and their pupa cases were also analysed by gas chromatography (GC) for cycasin content as described by Rothschild *et al.* (1986), and for macrozamin content by a combination of thin-layer chromatography and GC analysis of acid-hydrolysed macrozamin.

RESULTS

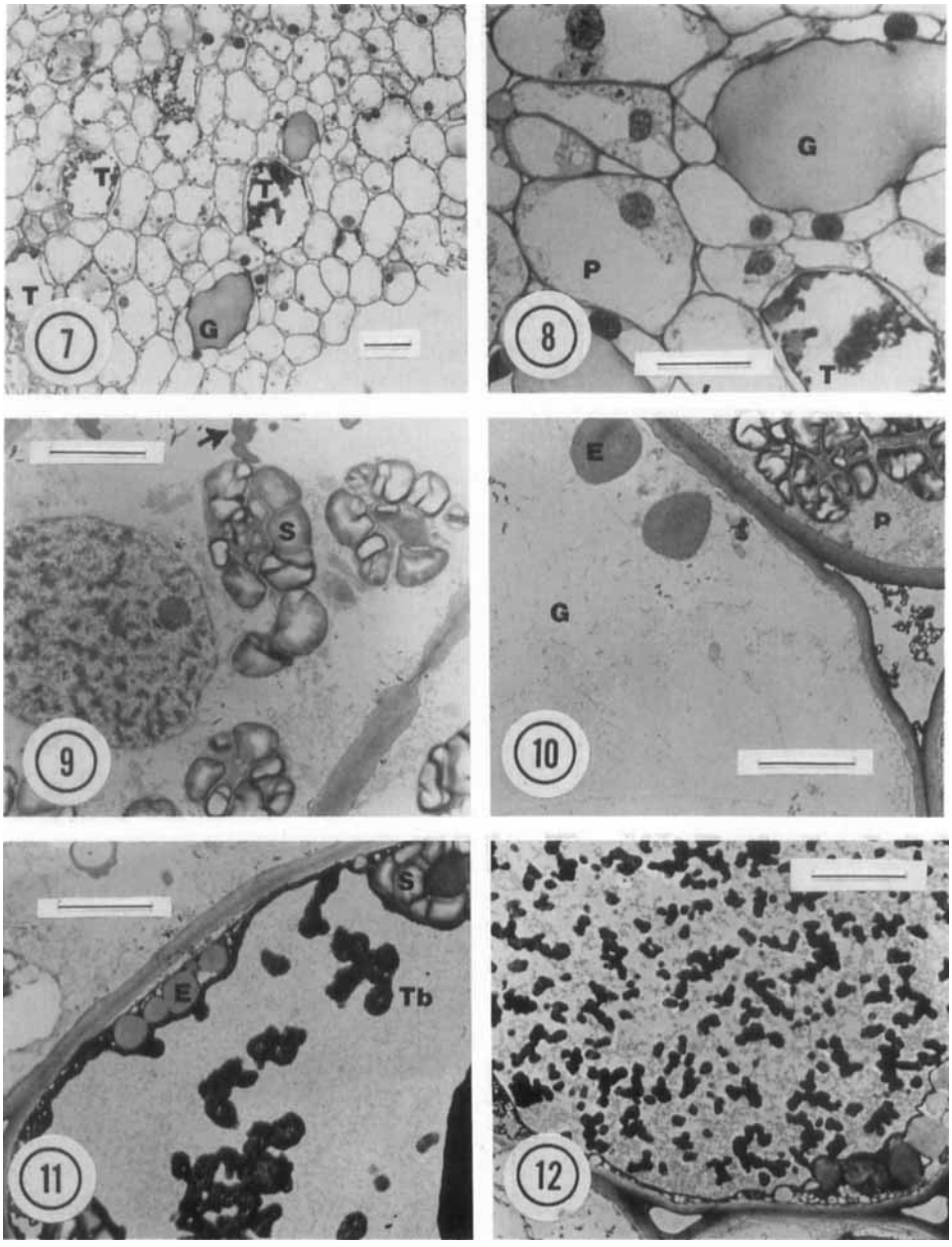
Early cones

Micrographs of sectioned and stained sporophyll tissue of cones of *Z. furfuracea* collected on 26 May 1989 are shown in Fig. 1 (female) and Fig. 2 (male). Sporophyll sections of both sexes contained idioblasts that stained an intense brown-purple with NIN (insets Figs 1, 2). This reaction with NIN is similar to that of BMAA isolated from tissues of *Cycas circinalis* (Vega & Bell, 1967). Starch grains were absent in both male- and female-cone parenchyma stained with iodine, potassium iodide reagent (I_2KI).

Older prepollination cones

As cones approach the period of pollination (8–13 June), marked differences are apparent between male- and female-cone parenchyma. Megasporophyll sections, stained with I_2KI or NIN (Fig. 3 and inset), contain little starch, show partial modification of idioblast contents; some exhibit gold-cell characteristics and stain NIN+, others have trabecular-idioblast morphology. Norstog & Fawcett (1989: figs 9, 10, 13) showed that such idioblasts stained PAS+. They also stated that such trabecular idioblasts are unstained with NIN, but our re-examination of their slides and colour micrographs leads us to modify this diagnosis. Although the vacuoles of these cells stain intensely with PAS, and are unstained with NIN, their trabeculae stain deeply with NIN (as in insets in Figs 3, 6). Microsporophyll sections from prepollination cones which were near to harbouring weevil activity, when stained with I_2KI show parenchyma cells

unmodified idioblasts (gold cells); excavation (Ex) by weevil larvae has not induced any changes in idioblast morphology. Fig. 6. Megasporophylls collected on 8 August from a post-pollination, ripening cone. Stained with I_2KI– fast green, it shows continuing low starch level and trabecular idioblasts (arrow). Inset, same material, but stained with NIN, shows idioblast (ID) with NIN– vacuole but NIN+ staining. Scale bars = 100 μm.



Figures 7–12. Megasporophylls of pollen-receptive female cones of *Zamia furfuracea* fixed in glutaraldehyde–OsO_4, embedded in epoxy resin, sectioned and stained either with toluidine blue (Figs 7, 8) for LM, or with lead citrate and uranyl acetate for EM (Figs 9–12). Fig. 7. Megasporophyll section from at-pollination megasporophyll. Note trabecular idioblasts with densely stained trabeculae and unstained vacuoles. The few gold cells are lightly stained. Scale bar = 10 μm. Fig. 8. Enlarged view of Fig. 7. Note contrast between parenchyma cells and idioblasts and absence of nuclei in the latter. Scale bar = 8 μm. Fig. 9. Parenchyma cell containing a few compound starch grains. Small inclusions of irregular, electron-dense particles (arrow) suggest presence of substance(s) similar to that composing trabeculae (cf. Figs 9, 11). Scale bar = 8 μm. Fig. 10. Part of a gold cell showing electron-lucent vacuole and partially electron-dense, peripheral globules. Examination of idioblast cell walls revealed absence of pits. Scale bar = 8 μm. Fig. 11. Part of a trabecular idioblast with peripheral globules and starch. Except for irregular, electron-

loaded with starch (Fig. 4) and unstained idioblasts, but when stained with NIN, show intact, intensely NIN+ idioblasts (inset, Fig. 4). We did not stain microsporophylls with PAS in this study but an earlier report states that none of their idioblasts are PAS+ (see Norstog & Fawcett, 1989: figs 8, 10).

At-pollination and post-pollination cones

Only gold-cell idioblasts are seen in microsporophyll sections from male cones collected in mid-July, at the peak of weevil activity as demonstrated by weevil-larvae excavations (Fig. 5). Those idioblasts that have escaped predation remain NIN+. Unpredated parenchyma cells in such cones still contain extensive starch deposits. On the other hand, megasporophyll sections from post-pollination female cones, collected on 8 August and stained with I_2KI (inset, Fig. 6), show both a continued low level of starch per cell, NIN-stained gold cells, and some trabecular idioblasts.

Toluidine-blue-stained, plastic embedded, megasporophyll sections also show both idioblast types when examined with LM (Figs 7, 8). With EM it can be seen that the gold cells have large electron-lucent vacuoles plus a few starch grains and partially electron-lucent globules in the peripheral cytoplasm (Fig. 10). We have not observed nuclei in any gold cells and pit connections, and plasmodesmata appear to be absent (Figs 7, 8). Trabecular idioblasts (Figs 9, 10) have faintly fibrillar, electron-lucent vacuolar contents, and, in addition, display electron-dense, vacuolar trabeculae (Figs 9, 10). These take various forms (cf. Figs 11, 12), and may be artifacts. A few peripheral starch grains are present as are scattered partially electron-dense globules, and adjacent parenchyma cells are seen to contain a few starch grains (Figs 8–10). In toluidine blue-stained, plastic embedded microsporophylls, thin sections show only gold-cell-type idioblasts and no trabecular idioblasts are seen.

Bagged, unpollinated female cones

Megasporophyll tissues of female cones from which weevils had been excluded at the time of readiness for pollination (i.e. megasporophylls separated) were, with respect to morphology and staining of idioblasts, no different than those of unbagged, normally weevil-pollinated female cones. Two kinds of idioblasts were present: NIN+ gold cells and trabecular idioblasts.

Idioblast dimensions in male and female cones

There is a significant ($P < 0.01$) increase in idioblast diameter with maturity of both male and female cones, but, at the time of pollination, idioblasts of male vs. female cones do not differ significantly from each other in mean diameter (Table 1). After pollination, male-cone tissues either are consumed by weevil larvae or become senescent. Therefore, we have no additional data on their idioblasts. Post-pollination female-cone idioblasts show a further, significant

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dense trabecula, vacuolar content consists of finely fibrillar material Scale bar = 8 μm. Fig. 12. Trabecular idioblast showing variable trabecular morphology. In this cell, trabeculae are smaller and more numerous than those in Fig. 11. Scale bar = 8 μm. E, Partially electron-lucent globule; G, gold cell; P, parenchyma cell; S, starch; T, trabecular idioblast; Tb, trabecula.

increase in diameter ($P < 0.01$), probably a consequence of generalized cell growth also evident in surrounding cells, but perhaps also evidence of ongoing idioblast differentiation and functioning (Fig. 6 and inset).

BMAA content of samples

Samples of male- and female-cone tissues, weevil larvae and pupa cases, as well as extracted idioblasts, all were found to contain BMAA (Table 2). It should be noted that the intent of our BMAA assays was limited to showing whether or not BMAA was present in the samples; our procedures were not precise enough to enable us to draw inferences concerning relative quantities of BMAA per sample type. Idioblast fractions were qualitatively useful but quantitatively inconclusive, which is not surprising given the simplicity of the extraction procedure. It is significant that pellets derived from both male-cone tissues and female-cone tissues were composed of intact idioblasts which contained BMAA.

Macrozamin content of samples

Some idioblasts in megasporophyll sections reacted positively with PAS, unlike any of the idioblasts in microsporophylls which were all PAS− (this study and Norstog & Fawcett, 1989). Because this test indicates presence or absence of reducing sugars, or macromolecules composed wholly or in part of such sugars, it is possible that a MAM-glycoside may be present in PAS+, female-cone idioblasts. This supposition is strengthened by our finding that *Z. furfuracea* tissues and weevil specimens (including adults, larvae and pupae) contain a MAM-glycoside, specifically macrozamin, and that a sample of pure macrozamin was PAS+. Macrozamin is a common toxic constituent in a number of cycad species (Moretti, Sabato & Siniscalco Gigliano, 1983).

DISCUSSION

In sections of early male cones stained with I_2KI, we note that starch content is quite low in comparison with that of near-pollination male cones. Idioblasts, on the other hand, are NIN+ to the same degree as those in later (older) cones. We have not found weevils on or in early cones, but this may be because they are immature and did not emit insect-attracting fragrances which characterize cycad cones at pollination time (Tang, Sternberg & Price, 1987; Pellmyr *et al.*, 1991). It is also likely that at this time few weevils in diapause had emerged from long-term dormancy (larvae of *R. mollis* remain in diapause from one cycad reproductive season to the next (Norstog & Fawcett, 1989)). Subsequent accumulation of starch in male cones as they near pollination time probably is associated both with presenting a reward to the pollinator and with fuelling thermogenesis, cone elongation, and odour volatilization in cycads (Tang *et al.*, 1987) and in *Zamia furfuracea* (Tang, 1987).

Idioblast development in female cones prior to, at the time of pollination, and afterwards, is so markedly different from that in male cones that we think it may correlate with the symbiotic pollination interactions between *R. mollis* and *Z. furfuracea*. We note in older, post-pollination female-cone tissues that a majority of idioblasts are enlarged, trabecular idioblasts. This may be associated with

continuing production (and liberation?) of contents (product?). Irregular profiles of electron-dense substances in thin sections fixed for EM suggest that vacuolar contents of trabecular idioblasts have undergone some degree of degradation (Figs 7–9). This may be an artifact of chemical fixation, although procedures used for EM are generally thought not to greatly alter cell structures. Moreover, the same fixation methods do not produce trabeculae in idioblasts of microsporophylls. Whether or not a reasonably accurate image of idioblast structure is presented in our micrographs, staining reactions suggest that during their differentiation, trabecular idioblasts have come to contain at least somewhat compartmentalized proteinaceous (NIN+ and electron-dense trabeculae) and carbohydrate (PAS+, faintly fibrillar) components. Because a macrozamin sample also was PAS+, we think it not unlikely that in tissues of this cycad this toxin is localized in trabecular idioblasts. It should be noted that in no studies of cycad toxins, other than our own, have attempts been made to localize toxins in any cell type; all such investigations are based on whole-plant or whole-organ extracts (e.g. Teas, 1967; Vega & Bell, 1967; Morretti *et al.*, 1983; Rothschild *et al.*, 1986; Duncan *et al.*, 1989).

As far as the plant is concerned, the differential development of female-cone idioblasts, *vis-à-vis* those of male cones, is self-induced. Weevil predation on male cones induces no change in idioblast morphology, but idioblast development in female cones protected from weevils features not only marked morphological changes, but also biochemical modifications of idioblast contents. It is clear, therefore, that changes in female-cone idioblast development are not responses to generalized or specific insect predation. Instead they are a manifestation of a different developmental pathway. We interpret these pathways in terms of differential mobilization of toxin in females vs. males. If BMAA and, possibly, macrozamin is released by female-cone idioblasts of changed morphology and stainability, then such changes may relate to protection of female-cone resources from (a) general predation by animals, and (b) specific predation by *R. mollis*.

Zamia furfuracea seed cones, both in cultivation and in nature, are remarkably free of animal predation (Norstog, personal observation), but pollen cones of this species are, as we note, quite susceptible to predation by *R. mollis*. We wondered if *R. mollis* had acquired the ability to avoid intoxication by sequestering toxins in some manner analogous to that of *Seirarctica* and/or *Eumaeus* (Rothschild *et al.*, 1986; Teas, 1967). Norstog & Fawcett (1989) speculated that male-cone idioblasts that are ingested by weevils may not be digested but instead pass through the weevil gut and are excreted. They based this supposition mainly on the presence of NIN+ particles throughout the larval gut and especially concentrated in the hind gut. Since larvae of *R. mollis* construct their pupa cases from their own dung, the authors speculated that idioblast contents, such as BMAA, would be present in the pupa case and might even protect pupating weevils from predation by other insects. Because some weevil larvae in diapause remain dormant in male-cone debris and in soil for many months and even for several years, such toxic pupa cases could be important in long-term survival of the weevil population. In our study, the above concept is supported by the data which show that idioblasts do in fact contain BMAA, and that pupa cases also contain relatively high concentrations of this toxin.

In conclusion, differential development of idioblasts in male and female cones, as well as high starch levels in male cones vs. low levels in female cones, can be

interpreted as evidence of a coevolutionary pollination syndrome analogous with those of some angiosperm flowers and their pollinators. The cycad seems to have evolved sex-specific mechanisms for handling toxins (e.g. sequestration in male-cone idioblasts vs. release by female-cone idioblasts). Such sequestration in male cones would permit the insect pollinator to complete its reproductive cycle without being subject to intoxication. It also is noteworthy that the weevil does not damage microsporangia and that pollen development and distribution is not interfered with. The pollinator is, in effect, non-destructive, and this is all the more remarkable when one considers that female-cone resources are not preyed upon by the pollinator.

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