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Carbohydrates, pollinators, and cycads

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Cycad biology, ecology, and horticulture decisions are not supported by adequate research, and experiments in cycad physiology in particular have been deficient. Our recent report on free sugar content in a range of cycad taxa and tissues sets the stage for developing continued carbohydrate research. Growth and development of cycad pollen, mediation of the herbivory traits of specialist pollinators, and support of expensive strobilus behavioral traits are areas of cycad pollination biology that would benefit from a greater understanding of the role of carbohydrate relations.

Cycads represent the most threatened group of plants worldwide.[1] Global research trends have been heavily focused on disciplines such as taxonomy and phylogeny, and research into cycad physiology has not received adequate attention.[2] As a result, we studied various aspects of free sugar content and reported that fructose, glucose, and sucrose were abundant in tissues from species representing every described cycad genera, and sugar content and stoichiometry varied greatly among organs.[3] The importance of these carbohydrates as forms of carbon storage, components of signaling, or regulators of cycad metabolism have not been determined to date.

Cycads and insects have developed sophisticated pollination mutualisms and specialist antagonistic relationships. A greater understanding of how non-structural carbohydrates influence these relationships may improve conservation efforts for the many threatened cycad species. Here we focus on pollination biology and how the inclusion of carbohydrate studies could aid in efforts to reach that goal.

Pollen

Carbohydrates play critical roles during pollen growth and development, then in the final phase prior to dispersal carbohydrates change to prepare pollen for dispersal.[4] Following dispersal and during pollen storage, the various metabolites comprising the carbohydrate pool change in relative proportion, a behavior that is understood as an adaptation for sustaining pollen viability in time.[5] Sucrose in particular may be of particular importance during pollen storage, as variations among species for pollen desiccation tolerance have been linked to sucrose content.[6] Sucrose may replace water to preserve native protein structures and spacing between phospholipids in the plasma-membrane during dehydration.[7] The role of starch and sugars in pollen development, dispersal, and maintenance of viability has not been determined for any member of the Cycadales, and is therefore a focus of needed research.

Several authors have conducted robust surveys and noted that starchy pollen occurred disproportionately among anemophilous species.[8-10] Representatives from the Cycadales were absent from these surveys and this should be corrected, as cycad pollination studies have only recently illuminated the sophisticated pollination syndromes that characterize the threatened plant group. Although arthropods were collected from reproductive structures on various cycad taxa in the past, the scientific community largely ignored their potential role as pollinators due to the established belief that all cycads

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were anemophilous.[11] The pendulum swung in the 1980s and 1990s when extensive experimental evidence confirmed mutualisms between cycad species and specialist insect pollinators.[2,12,13] More recently, ambophily has been confirmed or proposed for several *Cycas* species where wind has been shown to augment insect pollination.[14-17] Furthermore, variations in aggregation tendencies and settling velocity have been reported for pollen from various cycad species.[14,15] This is an opportune time to add members of the Cycadales to studies that have linked the relationship of pollen carbohydrates to insect versus wind pollination strategies.

Herbivory

The insect pollinators of cycads gather on male cones, where the adults socialize, mate, and use the post-dispersal male strobilus tissue as larvae food (**Fig. 1**).[2,13,14,18] The study of idioblasts within cycad sporophyll tissues has illuminated intricacies in how general tissue chemistry may interact with pollinator feeding behaviors. For *Zamia furfuracea*, these idioblasts remained intact in the male sporophyll tissue but appeared to release their contents into female sporophyll tissue prior to ovule receptivity.[19,20] The authors concluded that toxins remained sequestered within the idioblasts for male tissue only, such that the pollinators could feed on parenchyma tissue around the idioblasts or consume intact idioblasts which protected insect metabolism from the toxins after consumption. Many but not all cycad species studied in this context exhibited similar idioblast traits.[21] For example, *Cycas rumphii* and *Stangeria eriopus* strobili did not contain identifiable idioblasts. In contrast, *Macrozamia lucida* and *Microcycas calocoma* exhibited idioblast breakdown prior to pollination stage for both male and female sporophyll tissues. The interactions among carbohydrates, nutrients, and toxins within cycad strobili tissues in relation to these idioblasts may prove to be of crucial importance for developing a full understanding of how pollinators feed on the plant's reproductive tissues while enacting effectual pollination. Conservationists in need of improved knowledge of various pollination syndromes within the Cycadales will require more studies within this context.

The majority of cycad pollinators consume strobilus tissue, but direct consumption of cycad pollen may occur for some cycad pollinators.[22,23] Cumulative research on pollen digestion has not progressed enough to discern generalities from idiosyncracies.[24] The addition of cycads to studies that determine carbohydrate and nutritional quality of pollen will greatly improve this line of research whether the mutualisms represent ancient associations that pre-dated angiosperms or examples where the contemporary mutualisms were recently derived from an initial antagonistic relationship.[14,25,26]

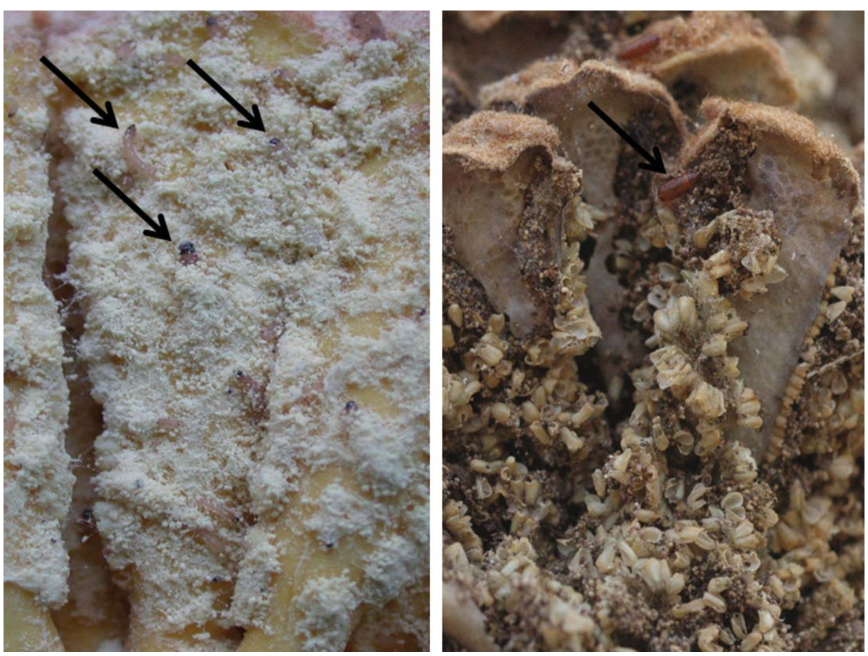

Figure 1. The microlepidopteran *Anatrachyntis* sp. is a specialist pollinator of *Cycas micronesica*. Left: microstrobilus tissue is tunneled and consumed by larvae (arrows) immediately after pollen dispersal. Right: within days the entire microstrobilus is reduced to frass and pupation (arrow) heralds in a new generation of pollinators. The role of cycad sugars in mediating this mutualism is unknown.

Strobilus Behavior

The reproductive structures of cycad species exhibit thermogenesis and volatile emissions at the time of pollination, traits that mediate pollinator behavior and maintain pollinator specificity.[13,27,28] Research is accumulating in areas such as modeling the plant behavior [29,30] and parsing the influence of specific volatiles on insect behavior.[31,32] Enacting synchronized thermogenesis and volatile biosynthesis is an exceedingly expensive plant behavior, and no studies have determined the tissues of residence, quantities, and stoichiometric relations of the carbohydrate reserves that are mobilized to fund those activities.

Cycad ovules are large structures that greatly exceed the size of their pollinators. Droplets have been documented at the micropyle location on cycad ovules, and these droplets contain metabolites that may provide a reward for pollinators.[33] Studies that marked pollinators then used deposition of the markers to track pollinator behavior have confirmed that the pollen they vector is trapped by the ovule droplets.[23,25,34] This remarkable female strobilus behavior represents a system that would be interesting to study in relation to specificity of pollinator attraction, diversity of sugar rewards, and cycad phylogeny. Droplets may exhibit taxonspecific carbohydrates that are suited to attracting and nourishing specific specialist pollinators.

Conclusions

Our recent report of fructose, glucose, and sucrose content in cycad tissues[3] sets the stage for designing continued research on how non-structural carbohydrates are involved in cycad pollination biology. Moreover, cycad horticulturists routinely harvest, store, and ship pollen prior to its use for successful pollination. Improved understanding of how carbohydrates and other factors influence pollen viability and longevity would improve protocols for artificial pollination.[35] Finally, the risks associated with coextinctions are very real during this phase of the Anthropocene, and species with complex life history traits, such as cycads, appear to be at greater risk for direct involvement in coextinctions.[36] An increase in knowledge of how cycad carbohydrates influence successful pollination relationships may help reduce the risks of coextinctions in these mutualisms that support contemporary cycad biology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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