J. Aquat. Plant Manage. 21: 49-53

Phenol-Storing Cells in Waterhyacinth Leaves[1]

R. D. MARTYN, D. A. SAMUELSON, AND T. E. FREEMAN[2]

ABSTRACT

Phenolic compounds in waterhyacinth leaves are localized in specialized idioblasts (phenol-storing cells) in the palisade layer on both sides of the leaf and also in close association with the vascular tissue. Light and electron micrographs indicated that the phenol cells are of two distinct morphologies. Those occurring in the palisade cell layers are 100-200 μm in length and the phenolic substances appeared as discrete, circular globules. Phenol cells near the vascular tissue were more isodiametric, averaging 50 μm in diameter and the phenolic substances appeared as an amorphous mass. There were no major morphological differences in phenol cells of the same type among the three plant sizes examined (small, medium, and large plants). There was, however, a significant difference in the spatial distribution of phenol cells among the three plant sizes. The concentration of subepidermal phenol cells increased significantly ($P=0.05$) from a mean of 33.6/mm^2 leaf area in small plants to 48.7/mm^2 leaf area in large plants. Histochemical staining suggested that both phenol cell types in waterhyacinth leaves were composed chiefly of o-dihydroxy phenols.

Key words: tannin cells, aquatic weeds, disease resistance, electron microscopy, histochemistry.

INTRODUCTION

Phenolic compounds are among the most widespread and varied substances in plants. Perhaps the best known role for plant phenolics is their assimilation into the anthocyanin and flavone pigments (18). However, as many authors have indicated, phenolic compounds have nearly unlimited potential in accounting for the many differences that occur in disease resistance (3,4,5,7,9).

Phenolic compounds in plants may be present in individual cells or in specialized idioblasts termed tannin sacs (6) or phenol-storing cells (13). Recent studies have shown that specialized phenol-storing cells occur in several plant species (1,2,10,11,12,14,15), although their exact role remains poorly understood.

This paper describes two morphologically distinct types of phenol-storing cells in waterhyacinth leaves and differences in their concentration among three plant morphotypes (sizes).

MATERIALS AND METHODS

Plant material. Waterhyacinth plants [*Eichhornia crassipes* (Mart.) Solms] were collected from naturally infested waterways in south Florida, trimmed of any necrotic or senescent tissue, and maintained under greenhouse conditions in Gainesville. Plants were separated into three size categroies based upon their leaf surface area (13): (i) small plants with a leaf surface area of 15 cm^2; (ii) medium plants with a leaf surface area of 15 to 40 cm^2; and (iii) large plants with a leaf surface area of 40 cm^2. Subsequent tests were conducted on plants from each size category.

Light microscopy. Cross sections (12 to 24μm) of fresh, healthy leaf tissue from each plant morphotype were made with a Hooker plant microtome, tested for phenols by the nitroso reaction (17), and observed with the light microscope. The spatial distribution of the subepidermal phenol cells was determined from tangential sections made along the vascular bundles. Approximately 50 leaf sections (10 x 15 mm) were taken at random from each plant size and the epidermal surfaces separated from each other with a razor blade. Each half was stained for phenols by the nitroso reaction method and observed with the light microscope. The mean number of phenol cells/mm^2 leaf tissue was calculated for the top and bottom surfaces of each plant size.

Electron microscopy. Fresh, healthy leaf tissue was placed in a buffered (0.2 M sodium cacodylate, pH 7.2) solution of Karnovsky's fixative (8) and cut into 3 to 5 mm pieces. The sections were fixed for two hours at room temperature, washed in half-strength (0.1 M) sodium cacodylate buffer, pH 7.2, for 30 minutes and post-fixed in 1.0% osmium tetroxide for one hour at 22 C. Sections were then rinsed several times in the half-strength cacodylate buffer and passed through a graded ethanol dehydration series at 25% increments and finally into 100% acetone. After dehydration, the sections were infiltrated with a graded acetone-plastic series and embedded in a 100% low viscosity epoxy resin (19). The embedded sections were placed under vaccum for five minutes and the resin polymerized overnight at 60 C. Thin sections were cut on a Sorvall MT-2 ultramicrotome with a diamond knife and placed on single-hole Formvar-coated copper grids. The sections were post-stained in 0.5% uranyl acetate and 1.0% lead citrate and examined with a Hitachi HU-11E electron microscope.

RESULTS

Light microscopy. Phenolic compounds in waterhyacinth leaves appeared bright red when stained by the nitroso reaction method (Figure 1). These compounds were localized in large, specialized idioblasts or phenol cells in the palisade cell layer immediately under both epidermal sur-

[1]Approved as Journal Series No. 14674, Texas Agricultural Experiment Station and No. 1545, Florida Agricultural Experiment Station. This research supported in part by the U.S. Army Corps of Engineers Florida Department of Natural Resources, U.S. Department of Interior, Office of Water Resources and Research Act (as amended) and by the University of Florida Center for Environmental Programs.

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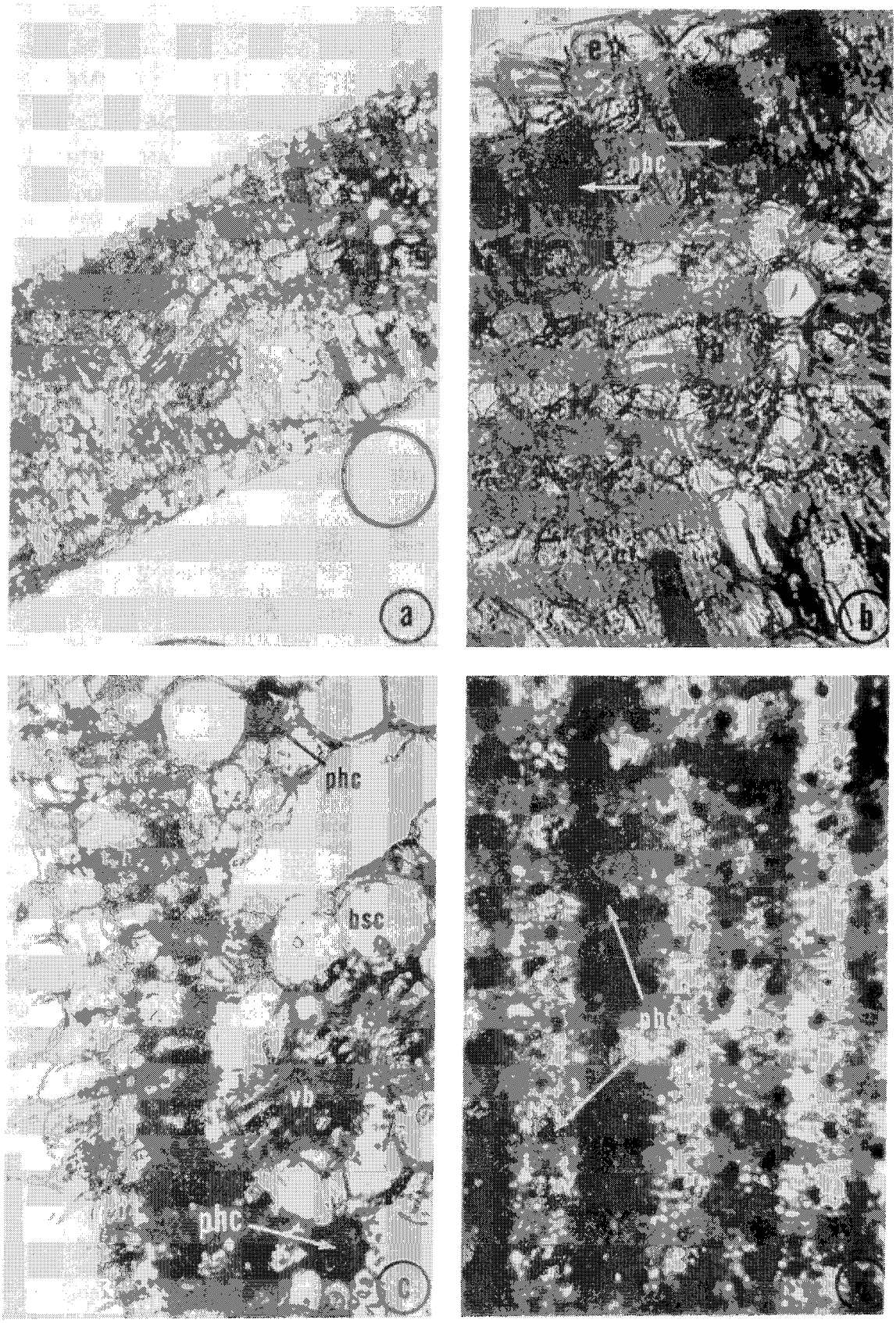

Figure 1. Waterhyacinth leaf sections (fresh tissue) stained for phenols by the nitroso method. Phenol-storing cells (phc) appear red. (a and b) Cross sections showing linear arrangement of phenol cells in the palisade cell layer immediately under both epidermal surfaces, e = epidemis, a) X200, b) X800, c) Cross section arrangement of phenol-storing cells near the vascular tissue; X800, vb = vascular bundle, bsc = bundle sheath cell; d) Tangential section of the adaxial surface of a small leaf showing spatial arrangement of the supepidermal phenol cells; X200.

faces (Figures 1a and 1b) and in cells closely associated with the vascular tissue (Figure 1c). The size of the phenol cells in the palisade layer varied considerably, often exceeding several hundred μm in length and extending down to the vascular elements. Those phenol cells near the vascular tissue were much more isodiametric and varied much less in size, averaging about 50μm in diameter (Figure 1c). No significant difference was observed at the light microscope level in the morphology of phenol cells of the same type among the three plant sizes examined.

Phenol cells were observed in all plants examined. There was a significantly greater (P = 0.05, Duncan's multiple range) number of phenol cells on the adaxial leaf surface (40.6/mm^2) than on the abaxial surface (26.6/mm^2) in small plants but the reverse was true for medium and large plants (Figure 2). Medium size plants had 31.8 phenol cells/mm^2 on the top surface of the leaf and 52/mm^2 on the bottom. Large plants had a more equal distribution of phenol cells between the top and bottom leaf surfaces but there were still more on the bottom than on top (43.5/mm^2 and 59.1/mm^2, respectively). The total number of phenol cells per mm^2 leaf area of both adaxial and abaxial surfaces significantly increased as the leaf size increased with a mean of 33.6/mm^2 for small, 41.8/mm^2 for medium, and 48.7/mm^2 for large plants.

Electron microscopy. Electron micrographs of healthy waterhyacinth leaf sections confirmed the presence of two morphologically distinct phenol cells (Figures 3a and 3b).

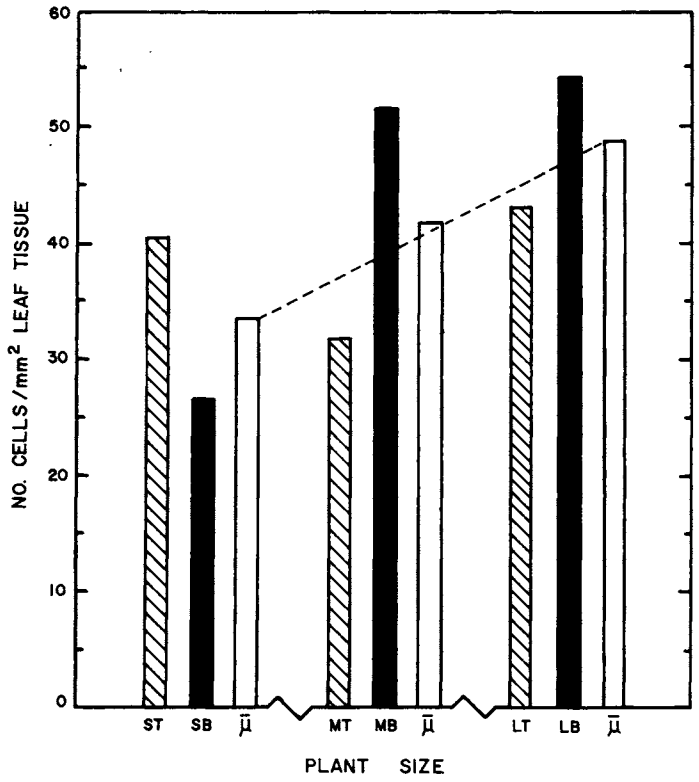

Figure 2. Mean number of subepidermal phenol-storing cells/mm^2 leaf area in small, medium, and large waterhyacinth leaves. ST = small plants, top surface of leaf; SB = small plants, bottom surface of leaf; MT = medium plants, top surface; MB = medium plant, bottom surface; LT = large plants, top surface; LB = large plants, bottom surface; u = mean number of phenol cells/mm^2 leaf (both surfaces).

In most cases, the subepidermal phenol cells were two to three times longer than the adjacent palisade cells (Figure 3a). The phenolic compounds appeared as discrete, individual, and generally circular globules within a large vacoule and in close association with the tonoplast. In some cells there were no discernible organelles while others contained a nucleus and several mitochondria and plastids.

In contrast, the phenol cells near the level of the vascular tissue (Figure 3b) were much more circular, had a much thicker cell wall, and the phenolic compounds appeared as an amorphous mass, probably due to the coalescing of smaller globules. Cellular organelles were observed less frequently in this cell type, indicating perhaps that maturation in these cells was attained much earlier than in the subepidermal phenol cells. No significant ultrastructural differences were observed between phenol cells of the same type in any of the three morphotypes examined.

DISCUSSION

Recent studies have indicated that specialized phenol-storing cells occur in several plant species (1,2,10,11,14,15). These cells are often called "tannin cells" when the nature of the phenolic substances is not known or the substances have become decompartmented, oxidized, and polymerized to various degrees (15). Several common, nonspecific histochemical techniques for the localization of tannins have been reported but appear to be of little value in determining the exact nature of the phenolic substance. The nitroso reaction (17), however, forms a cherry-red nitroso derivative with o-dihydroxy phenols and appears to be a generally reliable means of differentiating these compounds from other phenols (10). Using this technique in conjunction with chromatographic and ultraviolet absorption data, Mace (10) was able to identify 3-hydroxytyramine (dopamine) as the major o-dihydroxy phenol in the cells of banana roots. The phenol cells in waterhyacinth leaves stained bright red with the nitroso method and therefore probably contain one or more o-dihydroxy phenols. Additional data from our laboratory (11) indicate that waterhyacinth leaf tissue contains several o-dihydroxy phenols, including protocatechuic, caffeic, and chlorogenic acids.

Several papers have appeared recently on the ultrastructure of phenol cells in plants (14,15). In each case, the cells described were in root tissue. The data presented here are the first reported from an ultrastructural investigation of phenol-storing cells as they occur in angiosperm leaf tissue. Two morphologically distinct phenol cells occur in waterhyacinth leaves. At certain stages of development, both types contain nuclei, mitochondria, and pastids and are presumably metabolically active. The phenol cells near the vascular tissue more closely resemble those described in the root tissue of cotton (15) and banana (14). Phenol cells in the palisade cell layers of waterhyacinth, however, are quite different morphologically in size, shape, and the appearance of the phenolic compounds. To our knowledge, this is the first report of this type of phenol-storing cell.

The spatial distribution of foliar phenol cells in waterhyacinth varied with the highest concentrations occurring in the leaves of larger plants. Greatest numbers of phenol

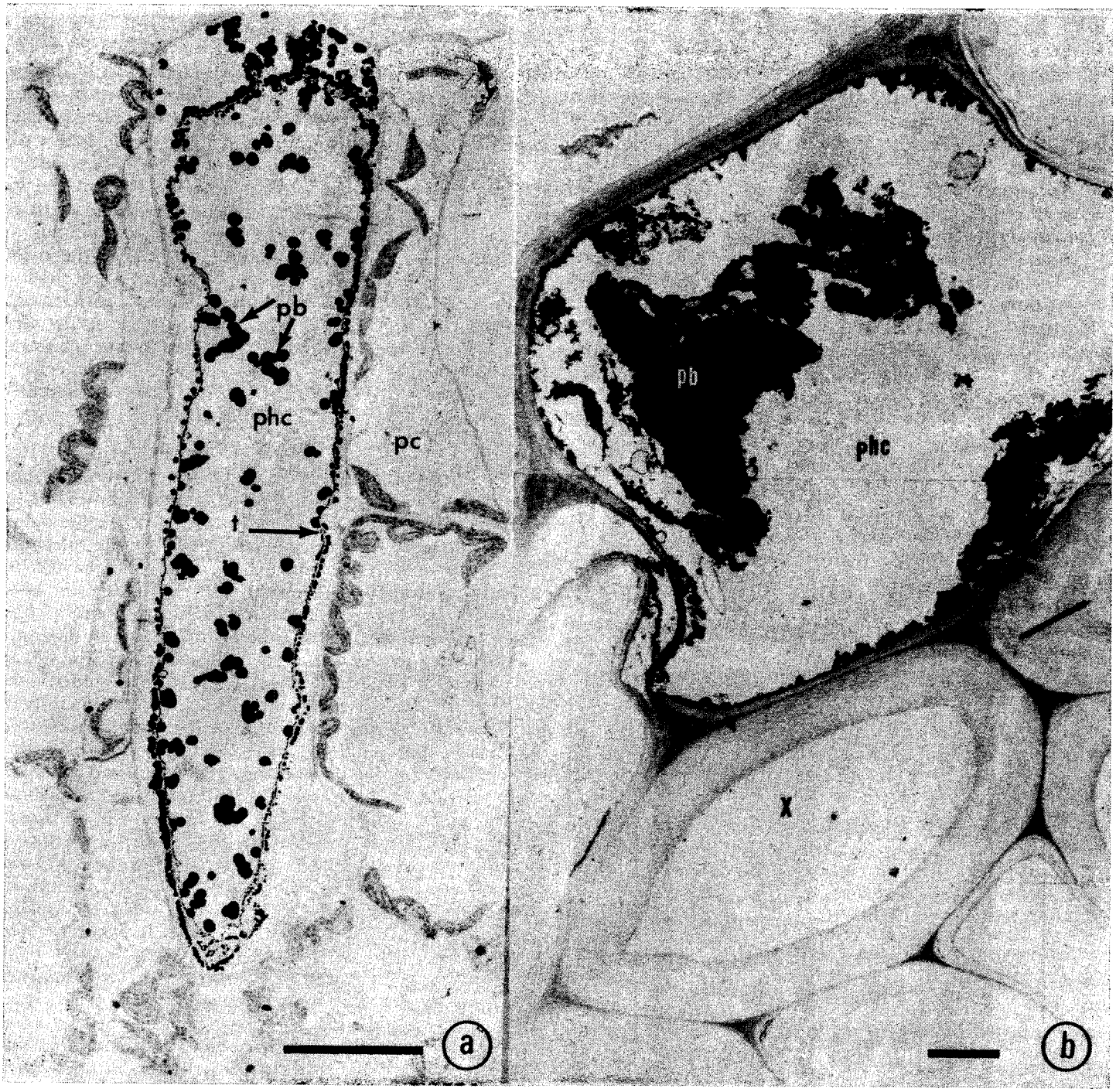

Figure 3. Electron micrographs of the two types of phenol-storing cells in waterhyacinth leaves. a) subepidermal phenol cell (phc) in palisade cell layer showing phenol bodies (pb) as discrete, circular bodies within the vacuole and in association with the tonoplast (t). Phenol cell is several times longer than the adjacent palisade cells (pc). Scale bar = 20 μm; b) Phenol-storing cell (phc) near the xylem (x) showing phenol bodies (pb) as an amorphous, electron-dense mass. Phenol cells in this area of the leaf are much smaller and more isodiametric than those in the palisade layer. Scale bar = 2 μm.

cells occur on the top surface of small leaves but the opposite is true for medium and large leaves. The reason for this distribution is unknown and any explanation at this time would only be speculation. It is possible, however, that their distribution pattern is an adaptive trait peculiar to the plant's aquatic environment. Since waterhyacinths have an almost equal distribution of stomata on both adaxial and abaxial leaf surfaces, (16) correlation of phenol cell occurrence with the presence or absence of stomata can be ruled out.

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J. Aquat. Plant Manage. 21: 53-58

Electron Microscopy of the Penetration and Colonization of Waterhyacinth by *Acremonium Zonatum*[1]

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ABSTRACT

The penetration and colonization of waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] by *Acremonium zonatum* (Sawada) Gams was examined ultrastructurally to aid in the evaluation of the pathogen as a biocontrol agent for this aquatic weed. Penetration of waterhyacinth leaves by the fungus occurred either through the stomata or directly through the unbroken cuticle. An inward displacement of the cuticle and papillae formation were observed in micrographs of infected cells which is suggestive of mechanical pressure exerted by the fungus during penetration. Electron micrographs indicated that penetration was apparently enhanced by the localized secretion of fungal pectinases and cellulases. Penetration of the host's specialized phenol cells resulted in the apparent death of the invading hyphae and these phenol cells may have limited infection and spread. Cytological changes noted in infected cells included the disappearance of starch granules from the chloroplasts, an increase in size and number of plastoglobuli in the chloroplasts, and an increase in the number of microbodies in the cytoplasma.

Key words: phenol cells, disease resistance, papillae, plastoglobuli, biocontrol, ultrastructure, pathogen.

INTRODUCTION

Acremonium zonatum (Sawada) Gams [= *Cephalosporium zonatum* Sawada], the casual agent of a zonate leafspot disease of waterhyacinth [*Eichhornia crassipes* (Mart.) Solms], was first isolated by Rintz in 1973 (16) and further evaluated as a biocontrol agent by Martyn and Freeman (12). The disease is first evident as small sunken lesions on both upper and lower leaf surfaces and the petiole. Infection is favored by high humidity and under conditions of prolonged high humidity, severe spotting and death of the plants may occur (12). *A. zonatum* produces conidia in muscilagenous heads (16) and is, therefore, not conducive wind dissemination. Under natural conditions, insects appear to be important in both the dissemination of the conidia and inoculation of the plant (7,8). Little is known about the infection process and subsequent tissue colonization of waterhyacinth by *A. zonatum*. Martyn (11) and Martyn *et al.*, (15) described specialized phenol-storing cells in waterhyacinth leaves and implicated them (11) in disease resistance by limiting the spread of the pathogen. The purpose of this investigation was to report the infection process and subsequent colonization of waterhyacinth leaves by *A. zonatum* under greenhouse conditions.

MATERIALS AND METHODS

Waterhyacinths were collected from natural infestations

[1]This research supported in part by the U.S. Army Corps of Engineers, Florida Department of Natural Resources, U.S. Department of Interior, Office of Water Resources and Research Act (as amended) and by the University of Florida Center for Environmental Programs.

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