Differences between the physiological disorders of intumescences and edemata

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Abstract

The physiological disorders of intumescences and edemata are characterized by blister-like protrusions of leaf cells that form lesions and become necrotic as they senesce. The two disorders are frequently confused or viewed as the same disorder in the literature, but light and scanning electron microscopy of lesions on five plant species, as well as environmental factors involved in their occurrence, suggest that the disorders are different. Lesions on tomato (*Solanum lycopersicum* 'Maxifort'), ornamental sweet potato (*Ipomoea batatas* 'Blackie'), and bat-faced cuphea (*Cuphea llavea* 'Tiny Mice') all began with one to a few epidermal cells undergoing hypertrophy, and the affected area expanded as lesions developed. Lesions in interspecific geranium (*Pelargonium* 'Caliente Coral') and ivy geranium (*P. peltatum* 'Amethyst 96') did not involve epidermal cell hypertrophy, but instead, a large group of mesophyll cells hypertrophied to form a blister-like lesion until the swelling tore the epidermal layer and a wound response that included cell suberization occurred. In different experiments with these crops, we studied whether the environmental factors of root medium water status or presence of ultraviolet B (UVB) light were related to the occurrence of the disorders. Development of intumescences in sweet potato and tomato 'Maxifort' were abated, but not completely prevented, when plants were exposed to UVB light; however, light quality did not affect occurrence of lesions in geranium. Others' research has linked plant water relations to the occurrence of lesions in geranium. Therefore, we propose that edema is the appropriate term to describe the lesions in *Pelargonium* sp., and intumescence should be used for the disorders of ornamental tomato, sweet potato, and cuphea.

Keywords: *Cuphea* sp., geranium, *Ipomoea* sp., oedema, scanning electron microscopy, *Solanum* sp., tomato

INTRODUCTION

Many plant species are prone to physiological disorders in which lesions develop on leaf tissue (Pinkard et al., 2006). Terms including intumescences and edemata have been used interchangeably to describe such disorders on several plant species produced in protected environments. Intumescences have been described as abnormal, translucent outgrowths on the leaf surface with a gall or wart-like appearance (Morrow and Tibbitts, 1988; Wetzstein and Frett, 1984) and edemata have been defined as a 'watery swelling of plant organs or parts' (Lang and Tibbitts, 1983). The lack of clarity in use of these terms creates confusion as to whether the names refer to the same or different disorders.

In order to evaluate differences and similarities in abnormal lesion development across a range of plant species produced in greenhouses, our research characterized stages of development of the physiological disorders that occur on cuphea, interspecific geranium, ivy geranium, ornamental sweet potato, and tomato using light microscopy, field emission scanning electron microscopy (FESEM), and scanning electron microscopy (SEM). By determining cellular layers involved in lesion development (epidermal and/or mesophyll) and lesion development patterns (hypertrophy, or cell enlargement, versus hyperplasia, or an abnormal increase in cell number), we were able to propose categorization of the disorders and recommend appropriate terminology associated with them. We found it also important to consider environmental factors involved in the occurrence of the disorders to categorize them.

MATERIALS AND METHODS

Lesions were characterized on five plant species: ornamental sweet potato (*Ipomoea batatas* 'Blackie'), tomato (*Solanum lycopersicum* 'Maxifort'), interspecific geranium (*Pelargonium* × 'Caliente Coral'), ivy geranium (*P. peltatum* 'Amethyst 96'), and bat-faced cuphea (*Cuphea llavea* 'Tiny Mice'). For all species but cuphea, plants were propagated from seed (i.e., tomato) or cuttings and grown in the glass greenhouse range at Kansas State University's Throckmorton Plant Sciences Center (Manhattan, Kansas, USA) until lesions developed under ambient conditions. Cuphea 'Tiny Mice' plugs with lesions at the initial stage of development were collected from a local commercial greenhouse covered with UV-blocked polyethylene and grown on in the glass greenhouse range of Throckmorton Plant Sciences Center.

Three stages of lesion development were evaluated: early (initial lesion development and early expansion), intermediate (full expansion of the lesions and beginning stages of senescence), and late (complete senescence of the lesion and surrounding tissue). Six plants of each species with representative lesions were sampled for microscopic evaluation from stands of >30 plants.

For light microscopy, leaf sections of about 0.5-cm diameter with developing lesions were excised from plant tissue. These sections were fixed in a solution of 10% formalin for two hours and then subjected to an ethanol dehydration series before being mounted in paraffin. Ten-µm thick cross-sections were mounted and stained with 0.5% Toluidine blue O (TBO). Images were collected using a light microscope (Nikon Eclipse E600; Nikon, Melville, NY, USA).

All species except ivy geranium were also imaged using FESEM, as follows. Tissue displaying a specific stage of lesion development was excised into ~0.5-cm-diameter sections and fixed using 2% paraformaldehyde/2% glutaraldehyde solution in a 0.2 M phosphate buffered saline solution (PBS), pH 7.2. After two hours, the tissue was transferred to PBS solution until imaging was conducted. Tissue sections of all species were mounted on carbon tape and imaged using FESEM (Nova NanoSEM 430; FEI Co., Hillsboro, OR, USA). Ivy geranium and tomato were imaged with scanning electron microscopy. Leaf sections of ~0.5-cm-diameter were glued onto a SEM slide using graphite emulsion. Slides were placed in the SEM (S-3500N Hitachi Science Systems Ltd., Hitachinaka, Japan) and rapid-cooled using liquid nitrogen to fix the samples. Micrographs were taken under high vacuum using a backscatter detector (Robinson Detector ETP-USA/Electron Detectors Inc., Rocklin, CA, USA).

RESULTS AND DISCUSSION

Epidermal cell hypertrophy

Epidermal cell hypertrophy did not occur in geranium (Figures 1A, 2E, F). Instead, a large group of mesophyll cells hypertrophied to form a blister-like lesion until the swelling stretched the epidermal layer to the point of tear (Figure 2G) and a wound response that included cell suberization occurred. In the case of ornamental sweet potato, FESEM indicated that a lesion began with the hypertrophy of a few epidermal cells (Figure 2A), but light microscopy images did not clearly delineate the inclusion of epidermal cells in the hypertrophy (Figure 1B). Based on our light microscopy imaging, we suggest that after lesion initiation in epidermal cells (Figure 2A), palisade parenchyma cells expanded rapidly and may have pushed aside other epidermal cells as they rose above the laminar surface (Figure 1B). For tomato (Figures 1C and 2B) and cuphea (Figure 1D), light microscopy clearly showed involvement of hypertrophied epidermal cells in lesion development. As lesions in ornamental sweet potato, tomato (Figure 2C) and cuphea (Figure 2H) progressed, epidermal cells in the center of the lesion collapsed or ruptured and the lesion grew to include more surrounding cells (Figure 2D).

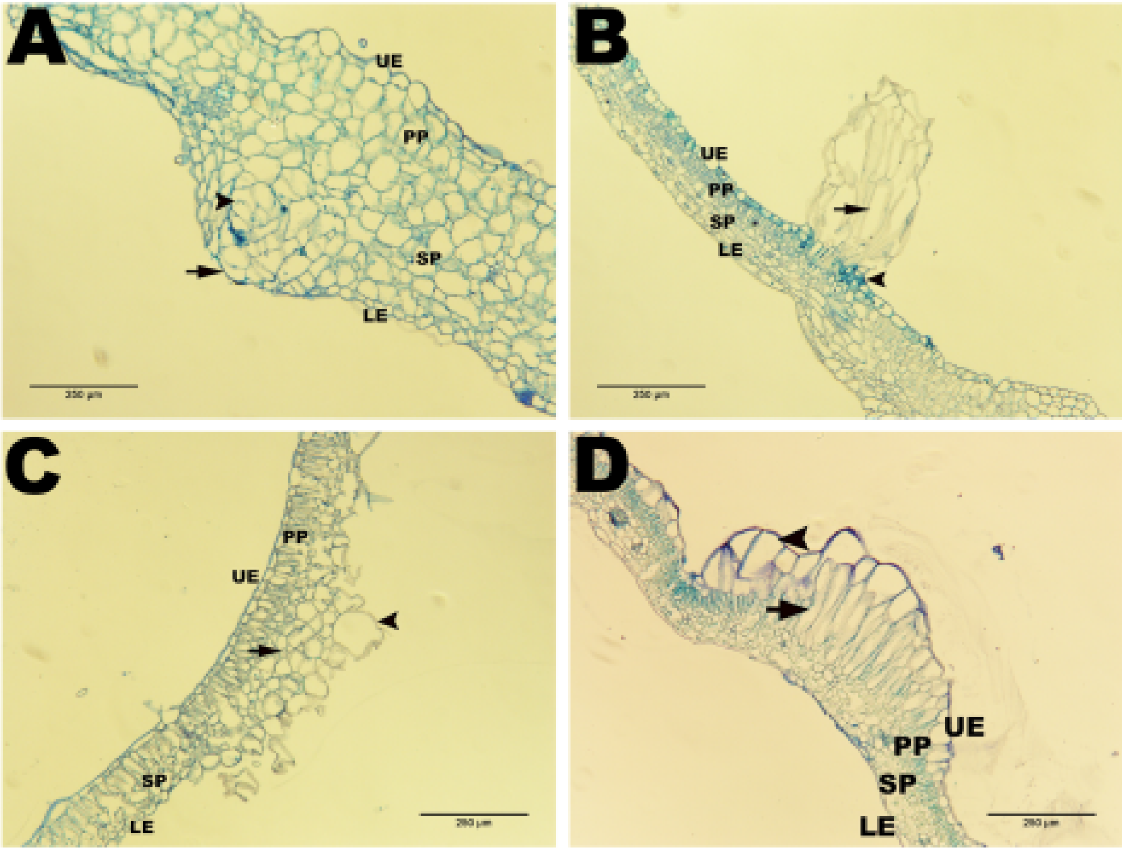

Figure 1. A) Geranium 'Caliente Coral' image shows that epidermal cells do not undergo hypertrophy (arrow). B) Ornamental sweet potato 'Blackie' image shows cell hypertrophy of unknown layer origin (arrow); it is unclear if epidermal cells (point) are incorporated into the hypertrophied area. C) Tomato 'Maxifort' image shows epidermal cell hypertrophy (point) compared to normal mesophyll cells (arrow). D) Bat-faced cuphea 'Tiny Mice' image shows epidermal cell hypertrophy (point) and mesophyll cell hypertrophy (arrow). Additional labelling includes the lower epidermis (LE), palisade parenchyma (PP), spongy parenchyma (SP), and upper epidermis (UE). Arrows and points indicate epidermal and mesophyll cells of interest.

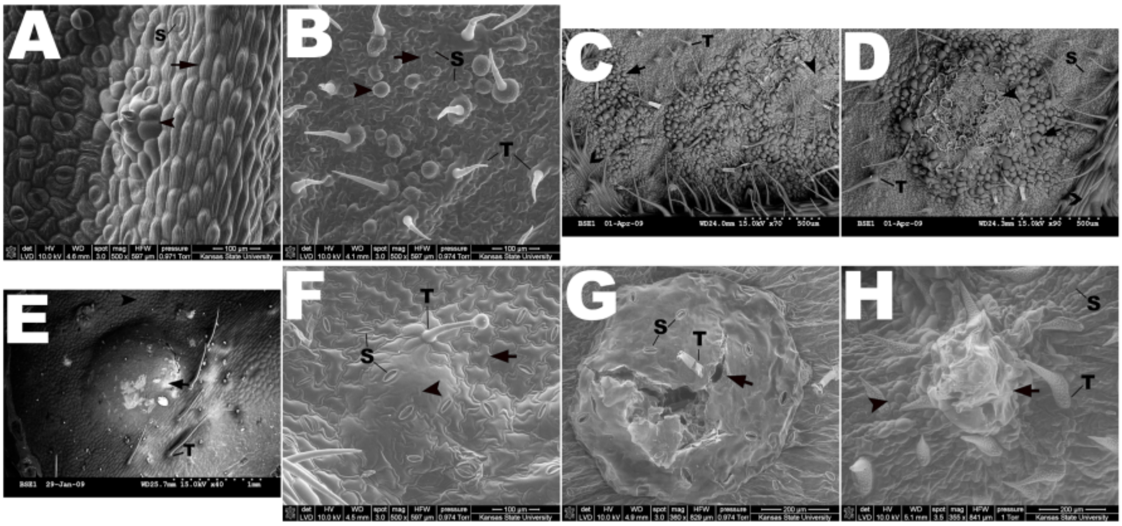

Figure 2. Images A, B, F, G, and H were taken with FESEM; images C, D, and E were taken with SEM. A) Ornamental sweet potato 'Blackie' image shows a small group of epidermal cells (point) beginning to undergo hypertrophy compared to a normal epidermal cell (arrow). B, C, and D) Tomato 'Maxifort'; image B shows individual epidermal cells beginning to undergo hypertrophy (point) compared to normal cells (arrow), image C shows a lesion in the intermediate stage before epidermal cells have begun to rupture/collapse, and image D shows cell collapse (point) in the center of a senescing lesion. E) Ivy geranium 'Amethyst 96' image shows swelling under the epidermis of a large group of mesophyll cells to the point of tear as indicated by loss of cell definition in the epidermal cell layer (arrow). F) Interspecific geranium 'Caliente Coral' image shows lower epidermal cells stretched (point) due to the hypertrophy of underlying mesophyll cells. A loss in epidermal cell definition was apparent when compared to the surrounding unaffected cells (arrow). G) Interspecific geranium image shows that lower epidermis was torn (arrow) across the lesion surface due to pressure from underlying mesophyll expansion. H) Bat-faced cuphea 'Tiny Mice' image shows collapse of a coalesced region of cells (arrow) during lesion senescence. Additional labelling includes stomata (S) and trichomes (T).

Environmental factors associated with lesion development

While the occurrence of hypertrophy of various cell layers was apparent in lesion development on all five species, the cause and mechanism by which these abnormal lesions occurred on each species was not necessarily similar. Therefore, it is useful to consider environmental factors involved in occurrence of the disorder alongside morphological and anatomical aspects of lesion development to most accurately determine the appropriate terminology.

Ultraviolet B (UVB) radiation has been shown to mitigate, if not prevent, the physiological disorder in tomato (Lang and Tibbitts, 1983; Rud, 2009) and ornamental sweet potato (Craver et al., 2014). Presence of UVB does not influence lesion occurrence in geranium species (Rud, 2009). Others have established that plant water relations are associated with the occurrence of the physiological disorder in *Pelargonium* (e.g., Balge et al., 1969; Metwally et al., 1970). Research has not yet been undertaken to establish whether UVB radiation is associated with lesion development in cuphea, but this connection is expected based on lesion anatomy on this species and their occurrence in under protected environment cultivation where glazing materials screen UVB radiation.

CONCLUSIONS

Both involvement of epidermal cell hypertrophy and environmental factors involved in the occurrence of the physiological disorder can be used to categorize lesions based on terminology. As such, we suggest edema or oedema as the appropriate term for the disorder in *Pelargonium* sp., and intumescence for use with ornamental sweet potato and tomato. If the disorder in cuphea is also proved to be mitigated by UVB radiation as it is in ornamental sweet potato and tomato, intumescence would be the appropriate term for the disorder in cuphea, as well.

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