

*THE EFFECTS OF UVB RADIATION ON INTUMESCENCE DEVELOPMENT AND THE CHARACTERIZATION OF LESIONS FROM PHYSIOLOGICAL DISORDERS ON ORNAMENTAL SWEET POTATO (IPOMOEA BATATAS), TOMATO (SOLANUM LYCOPERSICUM), AND INTERSPECIFIC GERANIUM (PELARGONIUM SPP.)*

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## Abstract

Intumescences are a physiological disorder characterized by hypertrophy and possibly hyperplasia of plant cells. Many plant species are susceptible to intumescence development, but the specific causative factors remain uncertain. Ultimately, this disorder results in the death of the affected cells. Previous observations and research suggest that the quality and quantity of light to which plants are exposed may be a factor in development of the disorder. The purpose of the first study was to assess the preventive effect of UVB radiation on intumescence development in ornamental sweet potato (*Ipomoea batatas*). Two sweet potato cultivars, 'Sidekick Black' and 'Ace of Spades,' were grown under four light treatments of 1) Normal; 2) UVB; 3) UVB-Blocked; 4) Full-Spectrum. The 'Ace of Spades' cultivar was highly susceptible to intumescence development, while 'Sidekick Black' was much less susceptible to the disorder. For 'Ace of Spades,' the addition of UVB radiation significantly reduced the number of leaves affected with intumescences when compared to plants grown under the other light treatments. This study indicates a cultivar-specific effect of UVB light in minimizing intumescence development on ornamental sweet potato, therefore suggesting a potential genetic component in intumescence susceptibility.

Many plant species are prone to similar physiological disorders in which lesions develop on the leaf tissue. Nomenclature for such lesions has varied significantly in the literature. Interchangeably using these terms causes confusion as to whether these names refer to the same or different disorders. The objective of the second study was to characterize the development of lesions on ornamental sweet potato (*Ipomoea batatas* 'Blackie'), tomato (*Solanum lycopersicum* 'Maxifort') and interspecific geranium (*Pelargonium* × 'Caliente Coral'). Light microscopy, field emission scanning electron microscopy (FESEM), and digital photography were used to observe lesion development on each species. Lesions on ornamental sweet potato predominately involved the hypertrophy of the palisade parenchyma through the upper epidermis, while geranium lesions involved the hypertrophy of spongy parenchyma cells restricted by the lower epidermis. Tomato lesions involved both the hypertrophy and hyperplasia of the lower epidermis and spongy parenchyma. Thus, different species possess varied cellular responses when developing lesions due to physiological causes.

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## Chapter 1 - Literature Review

Intumescence is a physiological disorder that develops sporadically on the leaf tissue of many plant species, including some varieties of tomato (*Solanum lycopersicum*; Rud, 2009), sweet potato (*Ipomoea batatas*; Wetzstein and Frett, 1984), and *Cuphea* spp. (Jaworski et al., 1988). Reportedly, intumescences have been observed on mono- and dicotyledonous angiosperms, gymnosperms, and ferns (La Rue, 1932). This disorder is often described as abnormal, translucent outgrowths on the leaf surface, that present a gall or wart-like appearance (Morrow and Tibbitts, 1988; Wetzstein and Frett, 1984). This disorder was first observed and named as intumescence by Sorauer in 1899, and was found at that time to develop on numerous plant species (La Rue, 1932). In 2006, Pinkard et al. published a review summarizing much of what has been documented concerning intumescences. Within this review, a list of the common and interchangeably used nomenclature was comprised which included excrescences, neoplasms, galls, genetic tumours, leaf lesions, enations and oedemata (Pinkard et al., 2006). For the sake of this review, the term lesion will be used unless cited literature refers to the disorder otherwise.

Lesions can have a substantial impact on both the economic and aesthetic value of affected plants (Balge et al., 1969; Rangarajan and Tibbitts, 1994). With many of the most susceptible species being grown solely for ornamental purposes, these growths can greatly impair the overall aesthetics of the crop. Additionally, severe cases of lesion development can result in impaired photosynthesis (Pinkard et al., 2006; Roloff and Scherm, 2004). Many of these factors will be discussed in detail later, but it is important to establish the need to better understand and ultimately prevent this disorder.

### Anatomy and Physiology

Lesions will develop on a variety of plant tissue types. Most commonly, the disorder is found on the leaves of affected plants. These growths have been observed on the adaxial leaf surface (Trotter, 1904), abaxial leaf surface (Jaworski et al., 1988), or a combination of the two (Kirkham, 1974; Lang et al., 1983; La Rue, 1932; Wetzstein and Frett, 1984). The leaves most commonly affected are those approaching maturity or full expansion (La Rue, 1932; Morrow and Tibbitts, 1987; Petite and Ormrod, 1986; Wetzstein and Frett, 1984). However, fully matured leaves have also been found subject to lesion development (Kirkham and Keeney, 1974;



Metwally et al., 1971). Other plant tissues affected include petioles (Eisa and Dobrenz, 1971; Lang et al., 1983; La Rue, 1932; Wetzstein and Frett, 1984), stems (Lang et al., 1983; Wallace, 1928), and buds (Wallace, 1928). The localization of lesions on the plant tissue appears to vary among species. On ivy geranium (*Pelargonium peltatum* ‘Yale’), Rangarajan and Tibbitts (1994) reported that oedema injury formed predominantly at the base of the leaf near the petiole. However, La Rue (1932) documented intumescences forming on all segments of poplar (*Populus tremuloides* and *P. grandidentata*) leaves, and that development appeared most abundant along the midrib and larger veins. While many accounts have shown that lesions will predominately form along the veins of the leaf, interveinal growths are also commonly seen (Eisa and Dobrenz, 1971; Kirkham and Keeney, 1974; Wetzstein and Frett, 1984).

In 1951, White defined an intumescence as “a random arrangement of cells.” While vague, this definition is relatively appropriate, as lesions are believed to be groups of cells that have undergone significant hypertrophy (Eisa and Dobrenz, 1971; Lang et al., 1983; La Rue, 1932; Metwally et al., 1970a; Petite and Ormrod, 1986; Trotter, 1904) or a combination of hypertrophy and hyperplasia (Wallace, 1928; Wetzstein and Frett, 1984). From this list of sources, it is apparent that hypertrophy, or cellular swelling, is the most often cited cause for this disorder.

The cellular layers involved in the development of lesions appear to vary among species and documented accounts by researchers. In 1984, Wetzstein and Frett described intumescences on tissue-cultured sweet potato (*Ipomoea batatas* ‘Jewel’) as involving both the epidermal and mesophyll layers. They stated that the inclusion of the epidermal cells with these growths allows for this cellular layer to remain continuous regardless of extensive swelling and growth (Wetzstein and Frett, 1984). Other reports have stated only the mesophyll is involved in development, which results in extensive pressure on the epidermis. As pressure from the growth of underlying cells increases, there is potential that the epidermis will rupture (Douglas, 1907; Eisa and Dobrenz, 1971; La Rue, 1932). La Rue (1932) described this specific phenomenon in the palisade and spongy parenchyma cells of poplar leaves. He stated that the mesophyll cells closest to the epidermis underwent the most expansion, while the epidermis was then forced to stretch as these cells swelled outward. This outward growth was due to the very limited space for lateral expansion among the palisade and spongy parenchyma layers. In this specific case, the epidermis was never involved in the hypertrophic response. Rather, the epidermis was eventually

torn and turned back after ample pressure from the swelling mesophyll cells was supplied (La Rue, 1932). Schrenk (1905) described a similar phenomenon in cauliflower (*Brassica oleracea*) leaves where mesophyll cells, both spongy parenchyma and palisade, would enlarge until eventually breaking through the epidermis. Lang et al. (1983) reported hypertrophy of epidermal cells in tomato (*Solanum lycopersicum* var. *hirsutum* and *Solanum lycopersicum* var. *esculentum* ‘Oxheart’) leaves that would potentially lead to rupture. While the cell layers involved may be different from those recorded by La Rue (1932), the end result of cell rupture is the same. Rud (2009) found the opposite to be true for oedema on ivy geranium (*Pelargonium peltatum*), in which she stated that, rather than rupture, the cells appeared to collapse. She hypothesized that the necrosis following this collapse may have been due to the separation of intracellular pathways which inhibited water and nutrient movement to affected cells (Rud, 2009).

The swelling of these cell layers has also been documented to have an effect on the physiological processes within the leaf tissue. In 1969, Balge et al. found that the hypertrophy of spongy parenchyma cells would inhibit transpiration due to stomata blockage in geranium (*Pelargonium ×hortorum*). In 1970a, Metwally et al. further validated these claims when they found that both epidermal and spongy parenchyma cells would undergo hypertrophy, and that this hypertrophy would lead to pressure on guard cells and the potential collapse of the stomata. Extreme cases of abnormal cellular development have also been documented, such as an account by Wallace (1928) where separation of the epidermal tissue and the liberation of individual protoplasts occurred in apple (*Pyrus malus* var. *transparent*) tissue.

A deviation from the previously documented anatomy of intumescences can be found in research conducted by Pinkard et al. (2006) on eucalyptus (*Eucalyptus nitens* and *E. globulus*) seedlings. They stated that intumescences that formed on eucalypt leaves were actually lenticel-like structures that formed due to high relative humidity conditions. It was believed that the epidermis would rupture under these conditions, allowing for greater aeration of the leaf tissue (Pinkard et al., 2006). These findings are quite different from what has been previously reported in literature, especially considering these lenticel-like structures may be viewed to benefit the plant.

## Causative Factors

One key finding concerning lesion development is that these disorders seem to predominantly occur on plants being produced in controlled environments (Jaworski et al., 1988; Lang and Tibbitts, 1983; Petite and Ormrod, 1986; Wetzstein and Frett, 1984). Defining the factor(s) that are lost and/or gained in the transition from natural to controlled environment production has proved to be a difficult task. This issue becomes evident in the review compiled by Pinkard et al. (2006) where a substantial list of reported causative factors was given. The causes for lesion development cited include mechanical injury, chemical injury, nutrient status, hormones, genetics, insect injury, fungal infection, air quality, light quality, light availability, temperature, and excess water. For the most part, there seems to be no pathogen involved in development, which leads most to agree that this is a physiological disorder (Rangarajan and Tibbitts, 1994).

However, there has been an occasional pathogenic cause documented. Pinkard et al. (2006) cited previous mentions of both insect and fungal causative factors. The issue that arises with claiming a pathogenic cause is that, the damage done by a pest or fungus can look very similar to intumescence damage, but can be completely unrelated. An example of this can be seen in photos taken by Yan Chen of western flower thrips damage and oedema on *Pelargonium peltatum* 'Sybil Holmes' (Fig. 1.1). Additionally, damage observed from oedema on ivy geranium has also been stated to look similar to injury caused by two-spotted spider mite (Burns, 2002). The Association of Applied Biologists has also listed enations as being the product of pea enation mosaic virus on pea (*Pisum sativum*), with growths on the leaves looking very similar to those typically associated with these disorders (Shepherd, 1970).

One of the only instances where lesions have been observed in nature was documented by La Rue in 1932. The intumescences, in this case, would develop on the leaves of poplar under very specific environmental conditions. They found that an abundance of moisture in the air was critical for intumescence development, which is very uncommon in outdoor settings. However, there were cases where insects would roll the leaves of these plants tightly together to create chambers for reproduction and feeding. In these scenarios, the moisture in the air was assumed to be much greater as the chamber would act to block out the dry outer air. Under these conditions, intumescences would develop on the leaf and larvae could be found feeding on the growths. In a similar way, other insects would make webs and fasten two leaves together to create a larval

chamber. Conditions in this chamber were favorable to intumescence development, and the outcome was ultimately the same as the above scenario. However, La Rue stated that other than these couple of documented cases with insect interaction, no other occurrence of intumescence development on poplar leaves in nature had been found (La Rue, 1932). Again, this is one of only a few instances where lesions have been observed in a natural environment.

The remainder of this section will review many of the most commonly proposed causative factors for the development of lesions. There is much discrepancy amongst the literature concerning this disorder and, as such, there is no definitive cause that has been ultimately identified or agreed upon. With the seemingly elusive nature of this disorder, La Rue (1932) appropriately stated, “There seems to be no law as to the development of intumescences within groups of plants.”

### ***Carbohydrates***

While it may not be an explicit causative factor, carbohydrates are believed to play a large role in the development of lesions. Douglas (1907) believed that the hypertrophied cells typical of lesion development are probably caused by the osmotic action of glucose. He stated that the tubers of potato (*Solanum tuberosum*) plants used in the study are full of starch and likely providing ample amounts of glucose to the leaf. With this same idea in mind, Pettite and Ormrod (1986) conducted an experiment to determine if there were differences in intumescence susceptibility between the potato cultivars ‘Superior’, ‘Norchip’, ‘Kennebec’, and ‘Russet Burbank’ propagated from tubers versus rooted cuttings. They found that the plants propagated from tubers showed much greater incidence of intumescence injury compared to those propagated from cuttings. Thus, they believed that the large carbohydrate source found in the tubers of the potato plants may play a large role in the development of the disorder (Pettite and Ormrod, 1986).

### ***Chemical Applications***

There have been a few accounts of chemical applications also contributing to the development of lesions. In 1905, Schrenk discussed the occurrence of these growths on cauliflower leaves after various fungicide applications. The author stated that moisture, heat and light did not appear to be involved in the development of the growths. Rather, applications of copper ammonium carbonate, copper chloride, copper acetate, copper nitrate and copper sulfate

produced numerous intumescences on the leaf surface. He believed that the intumescences were produced due to the copper and ammonium salts in these applications, and proposed that these salts were affecting the cells in one of two ways. The first option was that the salts were stimulating the formation of compounds within the protoplast that have high osmotic coefficients. The second option was that these salts may have stimulated the formation of organic acids in large amounts within the protoplast, but it was uncertain what this stimulation would actually be. Interestingly, this phenomenon seems to be limited to cauliflower leaves, as applications made by the author to grape (*Vitis*), violet (*Viola*), radish (*Raphanus*), beet (*Beta*), and *Mesembryanthemum* sp. produced no intumescences (Schrenk, 1905).

### ***Excess Water***

One of the most popular theories of what causes these swollen growths involves excess water being supplied to the plant. This proposed cause is most commonly cited when referring to geranium (*Pelargonium*) species. In most of these papers, these growths were referred to as oedema. The idea is that when water is supplied in excess to these plants under high humidity conditions, water levels in the plant tissue build faster than transpiration can occur (Morrow and Tibbitts, 1988). Thus, cellular swelling occurs as plants are unable to remove this water quickly enough. In a paper concerning drought tolerance in geraniums, Hassanein and Dorion (2006) stated that plants responded to water stress by the means of both morphological and physiological modifications. While this paper primarily focuses on drought stress, it can be assumed that excess water may also lead to significant modifications in the plant tissue.

In 1969, Balge et al. described oedema on five cultivars of zonal geranium (*Pelargonium* × *hortorum*). These cultivars were ‘Dark Red Irene’, ‘Princess Irene’, ‘Hawkeye Pink Cloud’, ‘Pink Camellia’, and ‘Radio Red’. They described these growths as being the result of hypertrophic spongy parenchyma cells. They stated that cells may eventually rupture and form water-soaked blisters, which further develop into necrotic spots. This occurrence is thought to be due to excessive water absorption by the plant (Balge et al., 1969).

Similar results were observed by Metwally et al. (1970b), in which the occurrence of oedema on zonal geraniums ‘Dark Red Irene’ and ‘Princess Irene’ was dependent on the moisture content of both the air and soil. They discovered this cause by applying various moisture regimes to plants being grown in a greenhouse. In a related paper by the same authors,

they suggested that high moisture levels in the soil may be resulting in an increase of xylem elements in the geraniums. This increase in xylem elements was also proposed to lead to an increase in water movement to the leaf, which the authors suggested was a requirement for oedema development in geraniums (Metwally et al., 1970a). In a paper published the following year, these same authors looked at the density and behavior of stomata on zonal geranium cultivars ‘Dark Red Irene’ and ‘Princess Irene’ (Metwally et al., 1971). They found that the lower stomatal density and higher diffusion resistance of ‘Dark Red Irene’ made this cultivar more susceptible to oedema under high moisture conditions when compared to ‘Princess Irene’ (Metwally et al., 1971). Thus, water would build up in the leaf and, without sufficient means for removal or utilization, cause extensive cellular swelling.

On the contrary, Lang et al. (1983) suggested that “watersoaking” did not appear to be the cause of intumescence development on tomatoes ‘Oxheart’ and var. *hirsutum*. The basis for their statement was that cellular hypertrophy only seemed to occur in certain tissues. If excess water were indeed the cause, it would be assumed that all plant tissues where water was transported would be affected. Additionally, the authors found that tyloses developed in the xylem of intumescent petioles. These tyloses were assumed to prevent the transport of water, which may have led to the wilting and abscission of leaves that was reported (Lang et al., 1983). Rud (2009) also found that water did not seem to play a dominant role in the development of oedema on ivy geranium. Regardless of various treatments involving root medium water content, she found that there were no differences in the occurrence or severity of oedema of ‘Amethyst 96’, ‘Lambada’, and ‘Sybil Holmes’.

In 2004, Roloff and Scherm stated that edema-like growths on blueberry (*Vaccinium ashei* ‘Premier’ and ‘Climax’ and *V. corymbosum* ‘Bluecrisp’) may result from drought conditions followed by an over-irrigation of the plants during the summer. Similar experimental conditions were studied on burley tobacco (*Nicotiana*) by Ligon and Benoit (1966). While intumescence development did not occur, they did find that increased soil moisture tension resulted in reduced growth rate, delayed maturity, and lower quality plants. Related to this idea, Jonas (2000) found that high irrigation frequency of ivy geranium ‘Amethyst 96’ resulted in less occurrence of oedema than when plants were allowed to dry down severely and then rewatered.

### ***Air Contamination***

Air contamination has been suggested as a potential cause for lesion development, but no concrete evidence as to what specific factors in the air may be attributing to the growths has ever been found. Lang and Tibbits (1983) conducted a study showing the effects of air quality on intumescence development in tomato (*Solanum lycopersicum* var. *esculentum* 'Oxheart' and *S. lycopersicum* var. *hirsutum*). They found that tomato plants exposed to toxic (pressurized laboratory) air produced more intumescences than those exposed to outdoor air. Their findings suggest that some factor in this toxic air was related to intumescence development, and that with increasing amounts of toxic air the plants displayed more severe symptoms.

A similar incidence was found in an experiment conducted by Kirkham and Keeney (1974) evaluating the role of air pollutants in enation development on potato. Assuming air pollution as the causative factor, air purifiers were placed inside of growth chambers, which effectively halted the development of the disorder. Again, while assumptions regarding specific constituents of the air that may be contributing to development can be made, currently, no solid evidence has been found.

### ***Hormones***

In 1986, Petite and Ormrod suggested that hormones may play a role in the development of enations. This account, along with many others, have proposed that plant hormones, such as auxin, abscisic acid, cytokinin, and ethylene, are likely to have a dominant role in the cellular hypertrophy and hyperplasia observed in plant tissues affected by this disorder. Unfortunately, most of the literature merely suggests that hormones are involved and provide little quantification and analysis.

Auxin is commonly cited as being involved in lesion development due to its role in cell expansion (Morrow and Tibbits, 1988). One of the earliest accounts regarding the involvement of auxin is found in a review concerning neoplastic growths written by White in 1951. In this review, he discussed a variety of documented accounts and potential causative factors for intumescence-like disorders. At the end of the paper, he discussed the peculiar finding that many of the accounts documenting these growths have suggested that auxin was a potential factor in development. Due to multiple accounts suggesting this role, he believed that auxins must play at least some part in the formation of neoplastic growths (White, 1951). Alongside auxin, Lang and

Tibbitts (1983) suggested that abscisic acid may be playing a role in intumescence development on tomato, and that UV radiation may be regulating auxin in this response. Morrow and Tibbitts (1988) also suggested that auxin may be playing a dominant role in this disorder. Specifically, they believed that the inhibitory action of UV radiation on intumescence development may be due to the ability of these wavelengths to degrade auxins. This theory of auxin inactivation or degradation by UV light was further supported in a review published by Springer (1978). In addition to auxins, Morrow and Tibbitts (1988) also stated that cytokinins may be involved in the occurrence of hyperplasia, as they are often involved in cell division.

One of the first documentations of ethylene as a causative factor in lesion development can be found in research conducted by Wallace (1928) on apple (*Pyrus malus* var. *transparent*). In this particular study, plants were subjected to various ethylene gas treatments. In short, this resulted in a disorganization of tissues exhibited by the buds and stems of the apple cuttings and ultimately led to swollen, functionless cankers, which Wallace then described as intumescences. The connection with ethylene is that this hormone is known to cause abscission. This means that cell wall digestion along with hypertrophy and hyperplasia of cells may potentially be induced or upregulated by the presence of this hormone (Wallace, 1928). Another mention of ethylene is found in research conducted by Kirkham and Keeney (1974), mentioned previously under the air contamination section. It was assumed by these authors that ethylene may be playing a dominant role in development of enations due to the typical ethylene damage symptoms observed on the potato plants. In terms of how ethylene production may be occurring in the greenhouse, Wills and Patterson (1970) found that this gas was produced from the ballast chokes in fluorescent lights, which affected the growth of peas (*Pisum sativum*) in their study. The release of ethylene through these lighting systems would certainly explain how this hormone might be produced in various controlled environments. However, it is also important to keep in mind that most of these fluorescent lights will not produce ultraviolet (UV) wavelengths, unless specifically stated, which are thought to prevent lesion development. So while ethylene may indeed be produced from fluorescent lights, it is important to decipher what specific factor may be causing or preventing the disorder to occur. Lighting will be discussed in more detail later in this section.



## *Humidity*

One of the first experiments regarding the effects of humidity on lesion development was conducted by Douglas (1907). In that study, potato plants were placed inside bell jars with various other environmental parameters being adjusted and controlled. He found that a high relative humidity and an ample water supply to the roots were necessary for intumescence development. He stated that transpiration under these conditions was inhibited, and that this saturated air inside the bell jars along with a high rate of water absorption led to the disorder.

Eisa and Dobrenz (1971) claimed that high relative humidity was necessary for oedema development on eggplant (*Solanum melongena*) leaves. Specifically looking at the cultivars 'Hybrid No. 4', 'Hybrid No. 1', 'Hybrid No. 25', 'Hybrid No. 23', 'Jersey King Hybrid', 'Black Magic Hybrid', and 'CV', they found that warm moist soil, cool nights, and high levels of relative humidity were conditions typical of oedema development on eggplant. This is one of many accounts that claim high humidity plays a dominant role in the development of lesions. This explanation supports the idea that excess water promotes development of this disorder, specifically when high humidity levels inhibit adequate transpiration by the plant. With the ability for transpiration reduced, excess water in the plant tissue is believed to cause hypertrophy of the cells.

However, much of the research concerning the role of humidity on lesion development has shown that it does not play a primary role. One of the first of these accounts was research conducted by La Rue (1932) in which he attempted to recreate the conditions for intumescence development on poplar leaves in a controlled environment. The goal was to see if plants from other families and genera would also develop intumescences under high air moisture conditions. To achieve these conditions, plants were placed in enclosed damp chambers and evaluated for intumescence symptoms. With multiple genera from 67 families included in the test, La Rue was surprised to find that only one species, *Thuja occidentalis*, developed intumescences. Even in the case of this species, only a single intumescence was ever observed (La Rue, 1932).

Additionally, Lang and Tibbits (1983) determined that humidity was not a factor in intumescence development on tomato. Rather than discrediting previous research, they concluded that the relationship of humidity with the development of this disorder must differ between species (Lang and Tibbits, 1983). Rud (2009) also found that differences in vapor pressure deficit had virtually no effect on the occurrence of oedema on ivy geranium.

## *Light*

### *Ultra-violet*

Light quality is a strong candidate as a causative factor or potential preventative measure for lesion development. Specifically, ultra-violet (UV) light, which includes UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm) is commonly cited and will be discussed in the following section. UV radiation, in particular, is thought to play a role in the development of the disorder because many greenhouse-glazing materials block UV wavelengths and lesions occur in protected culture. For example, all greenhouse polyethylene includes a UV-block additive to slow degradation and extend its useful life.

However, while there is much effort to block this UV light in the growing environment, there has been recent research regarding specific benefits. Specifically, it has recently been suggested as a means to manage plant growth by Frantz et al. (2012). With a potential increase in grower interest regarding UV light, the hypothesis that a lack of UV radiation may be the missing link in explaining what causes intumescence development has become an increasingly relevant topic.

Research conducted by Kirkham and Keeney (1974) concerning the role of air pollution in the development of enations on potato plants grown in growth chambers, showed that UV light may be a factor in development. However, they hypothesized that this disorder was more directly related to air pollutants, and that stomata may have opened more widely, due to filtering out UV radiation. These wider openings would have allowed for more pollutants to be taken up, and increased the occurrence of the disorder. They further stated “ultraviolet light may alleviate, but not prevent, the symptoms” (1974). However, research conducted by Lang and Tibbits (1983) suggests that the idea of stomata regulating intumescence injury may not be a sound explanation. They found that tomato plants (*S. lycopersicum* var. *hirsutum* and *S. lycopersicum* var. *esculentum* ‘Oxheart’) exposed to toxic air in both dark and light conditions displayed equal amounts of intumescence injury. Through measurements of stomatal resistance, they found that significantly more stomata were open on the leaves under light conditions compared to dark. Thus, it would be expected that the plants subjected to the light conditions would have the

greater incidence of injury due to the open stomata allowing greater exposure to the toxic air. However, this outcome was not observed in their studies.

In the same study by Lang and Tibbitts (1983), it was also found that UVB radiation effectively prevented intumescence development on tomato. In their study, exposure boxes were constructed using Plexiglas G (blocks wavelengths below 330nm) and Plexiglas G II-UVT (transmits wavelengths above 230nm). They found that the tomato plants grown in the Plexiglas G-II-UVT boxes were free from injury, while those plants grown in the Plexiglas G boxes displayed severe symptoms. This research showed the ability of UV radiation to effectively prevent intumescence development. Thus, it was “assumed that if greenhouses were constructed of UV-B-transmitting glass or plastic, intumescence injury would be effectively prevented in these structures”.

Continuing with the UV radiation research, Morrow and Tibbitts (1987) looked at intumescence development on tomato plantlet disks. The theory behind using the disks was that variations among whole plant leaves could be minimized to achieve more accurate results. Leaf disks were induced with intumescences by blocking out the UV radiation emitted from cool-white fluorescent lamps using UV-absorbing Plexiglas. They found that intumescences formed readily under these conditions on both the abaxial and adaxial surface of the leaf disk, with the greatest injury occurring on the surface directly facing the radiation. Additionally, no intumescence development was observed on leaf disks subjected to darkness, which further suggested that irradiance plays a dominant role in development. Due to the leaf disks being floated on water, they also found that the injury seemed to be stimulated by contact with water. They believe that these leaf disks were obtaining water through the laminar surface, and that this water was directly affecting cell expansion.

Rud (2009) found similar results in that UVB light greatly reduced the occurrence of intumescences on tomato (*Solanum lycopersicum* ‘Maxifort’). She hypothesized that the development of intumescences under UVB light was due to either shading from the upper canopy of leaves reducing UVB exposure or naturally cloudy days, which reduced the overall UV levels in the treatments. Further, she suggested that there may be a threshold mechanism involved with this response. That is, “if a plant susceptible to the development of intumescences receives a certain amount or intensity of UV light, intumescence development may be prevented” (Rud, 2009). Therefore, based on that hypothesis, leaves subjected to shading by upper canopy

leaves would not receive the necessary levels of UV exposure to prevent intumescence development.

A more practical application of UV radiation being used as a prevention method for lesion development was presented by Wheeler (2010). While discussing the potential of crop production for life support systems in space, Wheeler directly addresses many physiological disorders that can occur under these conditions. Intumescences are one of the disorders that were addressed, and he stated that UV light can effectively prevent this disorder from occurring in these controlled environment scenarios. This discussion not only provides greater insight that UV radiation is a viable means for prevention, but also displays the direct negative impact of this disorder, which will be discussed later in this review.

### ***Far-red and Red***

UV light has not been the sole focus of studies involving the relationship of light and lesion development. Research by Morrow and Tibbitts (1988) looked at the potential involvement of phytochrome in tumor development of *Solanum lycopersicum* var. *hirsutum* plantlets. The floating leaf disk method was used, and various radiation spectra were created by using different lamps and filters. They found that red light induced tumors on the disks, and that increased irradiance of red light resulted in more severe injury. However, when disks were also subjected to far-red light, the effects of the red light on intumescence development seemed to diminish. It was discovered that if ample amounts of far-red light were made available, intumescence injury was effectively inhibited. The authors stated that this inhibitory action by far-red wavelengths suggested the involvement of phytochrome in this disorder. They further explained this hypothesis by proposing that there were two irradiance responses involved, a prolonged red response and a reversible red/far-red response. In this case, the prolonged red response would control induction of this disorder on the plant, while the red/far-red response would directly control expression (Morrow and Tibbitts, 1988). Additionally, Rangarajan and Tibbitts (1994) conducted research based on the suggestion by Morrow and Tibbitts (1988) that intumescence development is controlled by the phytochrome system. They investigated whether or not exposure to far-red light would inhibit oedema development on ivy geraniums, and concluded that far-red light had no direct effect on prevention; however they recommended that future research in the ratio of far-red:red photon flux may provide different results.

### ***Blue and Green***

In 1998, Seabrook and Douglass found that intumescences were reduced in potato (*Solanum tuberosum* ‘AC Brador’ and ‘Shepody’) plantlets grown under a yellow filter. This yellow filter was stated to eliminate the blue-green (380-525 nm) portion of the spectrum. It was implied that blue-green light was essential for the formation of intumescences on the potato plantlets, at least within their controlled experiment (Seabrook and Douglass, 1998). On the contrary, Morrow and Tibbitts (1988) found that blue and green wavelengths had little to no effect on the induction of intumescences occurring on tomato leaf disks. However, blue and green light have been found to have an inhibitory effect on intumescence development according to Wallaeger and Runkle (2014). They found that tomato plants grown under at least 50% blue light were nearly void of any intumescence development, and that plants exposed to green light also showed control to a lesser extent.

### ***Temperature***

Temperature is another environmental factor that has been proposed to be involved in the development of lesions. However, conflicting results lead one to question whether or not temperature truly plays a role. In 1969, Balge et al. found that warmer soil temperatures increased the severity of oedema on zonal geranium (*Pelargonium ×hortorum*). However, they indicated that this increase in soil temperature may ultimately be linked to an increase in water absorption by the plant (Balge et al., 1969). Thus, these high temperatures are not directly causing the oedema but acting to increase the severity and rate at which they occur. Further, Eisa and Dobrenz (1971) reported that high temperatures were necessary for oedema development on eggplant (*Solanum melongena*) leaves. Conversely, Lang and Tibbitts (1983) found that intumescence development was more severe on tomatoes grown at 20° and 25° C than at 30° C, suggesting that cooler temperatures increased severity. These conflicting results propose that temperature may indeed play a role in development for some species, but is most likely not the definitive causative factor.

### ***Genetics***

Differences in resistance and susceptibility to lesion development are found among cultivars of a single species such as potato (*Solanum tuberosum*; Pettite and Ormrod, 1986; Seabrooke and Douglass, 1998), eggplant (*Solanum melongena*; Eisa and Dobrenz, 1971),

*Cuphea* spp. (Jaworski et al., 1988), tomato (*Pelargonium ×hortorum*; Metwally et al., 1970b), and geranium (*Pelargonium ×hortorum*; Balge et al., 1969). This fact leads to the assumption that genetic differences play at least some role in the development of this disorder (Eisa and Dobrenz, 1971). One example of this occurrence was seen in research conducted by Petite and Ormrod on potato plants (1986). They found that two early to midseason cultivars ('Norchip' and 'Superior') were resistant to the disorder, while two late-maturing cultivars ('Kennebec' and 'Russet Burbank') were susceptible. Another example can be seen in a trial conducted by Jaworski et al. on *Cuphea* spp. in 1988. Upon completion of the trial, it was stated that intumescences could be avoided in *C. wrightii* by selecting accession numbers of plants that were not susceptible to the disorder (Jaworski et al., 1988). Additionally, Metwally et al. (1970b) suggested that susceptibility differences in two zonal geranium (*Pelargonium ×hortorum*) cultivars ('Dark Red Irene' and 'Princess Irene') were due to genetic variability in the vascular elements and stomata. With the great amount of uncertainty involved in uncovering a specific causative factor, Rud (2009) suggested that perhaps one of the best options for control of this disorder was the selection of resistant cultivars.

### **Intumescence vs. Oedema**

In a review concerning neoplastic growths on plant tissue, White (1951) covered a range of documented observations and proposed causes. In his discussion, he concluded that, "there exists a considerable range of such growths, of a variety of origins." In other words, much of the controversy concerning nomenclature and causation of lesion development may be due to cases of varying disorders. Two of the most commonly used names when referring to these growths are oedema and intumescence. Oedema seems to commonly be found on geranium (*Pelargonium* spp.), while the term intumescence is more often associated with growths on sweet potato (*Ipomoea batatas*) and tomato (*Solanum* spp.).

In 1905, Dale suggested that the first name for the disorder was "intumescence", while American authors later adopted the term "oedemata". This implies that the disorders are equivalent and is the theory that has been adopted by many researchers involved in this area. However, Lang and Tibbits (1983) suggested that the names oedema and intumescence should instead refer to completely different disorders. They stated that oedema is not the appropriate term for the disorder found in their experiment, and that the name intumescence should be used

instead. According to the authors, oedema can best be defined as “a ‘watery swelling of plant organs or parts,’ resulting from water congestion in plant tissue” (Lang and Tibbits, 1983). On the other hand, what was observed on tomatoes in their study was suggested to be called intumescence, due to the plants showing symptoms when the relative humidity “was low and there was no water congestion in the tissue” (Lang and Tibbits, 1983). Morrow and Tibbits (1988) added to this differentiation by stating that “edema” typically forms under conditions where excess water and high humidity prevent sufficient transpiration by the plant.

Further suggestion of differences between oedema and intumescence can be found in the research of Rangarajan and Tibbits (1994). Based on the results obtained in their study involving the failure of far-red light to inhibit oedema injury on ivy geranium, they suggested that the causative factors and physiological systems that regulate oedema on geranium and intumescence on solanaceous species were different. They supported this statement by claiming that UV radiation aids in the prevention of intumescence on solanaceous species (Morrow and Tibbits 1988), but has little to no effect regarding the development of oedema on geranium (Rangarajan and Tibbits, 1994).

## **Impact**

It is also important to discuss the impact that lesion development can have on affected plants. This matter is crucial as not only as a means of establishing the relevance to the industry, but also in better understanding the potential mechanism by which this disorder develops.

### ***Whole plant.***

The negative impacts of lesions on plant tissue include chlorosis (Rangarajan and Tibbits, 1994; Wetzstein and Frett, 1984), senescence (Rangarajan and Tibbits, 1994; Wetzstein and Frett, 1984), leaf abscission (Rangarajan and Tibbits, 1994), the downward curling of leaves (Eisa and Dobrenz, 1971; Kirkham and Keeney, 1974), and impaired photosynthesis (Eisa and Dobrenz, 1971; Lang et al., 1983; Pinkard et al., 2006; Roloff and Scherm, 2004).

### ***Aesthetic Value.***

For the grower, a loss in aesthetic value can be detrimental for crops produced solely for ornamental purposes. As leaves begin to undergo chlorosis, droop, and eventually fall off, the overall value of the plant may decrease dramatically (Anonymous, 2011). Issues such as

chlorosis and senescence (Wetzstein and Frett, 1984) can be detrimental to overall plant quality, but can also lead to other issues. For instance, as previously mentioned, damage observed from oedema has also been stated to look similar to injury caused by two-spotted spider mite (Burns, 2002). This can be a major issue when plants are nearing the shipment date and appear to be infested with pests.

### ***Photosynthesis.***

One of the most detrimental impacts is the impairment of photosynthesis. Eisa and Dobrenz (1971) document a reduced number of chloroplasts within hypertrophied cells of eggplant (*Solanum melongena*) leaves. It was suggested that this reduction in chloroplasts would lead to a decrease in photosynthetic activity. Likewise, Lang et al. (1983) found that hypertrophied palisade and chlorenchyma cells had few or no chloroplasts when studying intumescence development on tomato (*S. lycopersicum*) plants. La Rue (1932) stated that poplar (*Populus tremuloides* and *P. grandidentata*) leaf cells, which had undergone hypertrophy, did not increase the number of chloroplasts contained within the cell. Thus, these chloroplasts were spaced more widely apart and the cells appeared translucent rather than green. Douglas (1907) also described the fate of chloroplasts in hypertrophied cells and stated that the granules gradually lose their green color, eventually disappearing completely. However, the most concrete evidence of photosynthesis impairment by this disorder was observed in a study by Roloff and Scherm (2004). Edema-like damage was documented on blueberry (*Vaccinium ashei* ‘Premier’ and ‘Climax’ and *V. corymbosum* ‘Bluecrisp’) plants, and photosynthesis was measured by net CO<sub>2</sub> assimilation rate (NAR) at varying degrees of leaf area affected by the disorder. They found that as the severity of the disorder increased, NAR significantly decreased (Roloff and Scherm, 2004). Pinkard et al. (2006) furthered this idea of impaired photosynthesis by stating that intumescences may reduce the amount tissue available for light absorption.

### ***Conclusion***

Ultimately, these negative effects lead to a loss in both the aesthetic and economic value of the crop (Balge et al., 1969; Rangarajan and Tibbitts, 1994). Additionally, a reduction in the yield of crops, such as blueberry, has also been noted (Roloff and Scherm, 2004). While discussing future research, Jaworski et al. noted that cultivars with severe intumescence susceptibility would be very difficult to work with (1988). Thus, it is apparent that



intumescences not only impair the physiological processes of the plant, but can also be detrimental to the quality and salability of a crop grown for commercial use.

## **Ornamental Sweet Potato**

*Ipomoea batatas*, or ornamental sweet potato, is an annual ornamental crop commonly produced in greenhouses during the spring season. It is a popular species because of its trailing habit and striking foliage colors, with several new cultivar introductions in recent years. However, the species is highly prone to lesion development when produced in greenhouses, as indicated in a recent patent application for a new variety (Yencho et al., 2012).

Lesions on sweet potato have not been extensively studied. Currently, there are only two published research articles specifically concerning development of this disorder on the crop. In 1984, Wetzstein and Frett published one of the two papers; while the other paper, “Intumeszenze fogliari di ‘*Ipomoea batatas*,’” was published by A. Trotter in 1904. Wetzstein and Frett cited Trotter (1904), stating the claim that the intumescences he observed were due to hypertrophy of the cells on the adaxial surface of the leaf. Specifically, that there was a vertical elongation of palisade cells (Wetzstein and Frett, 1984).

Through the research conducted by Wetzstein and Frett, it was discovered that intumescences on sweet potato appeared to be the result of not only cell hypertrophy, but also hyperplasia. Additionally, these authors found that both the abaxial and adaxial surfaces of the leaf were affected by the disorder. They explained that these differences from Trotter’s findings were most likely due to environmental conditions resulting in differences in cellular differentiation.

In the case of intumescence on sweet potato, the epidermal layer has been found to remain continuous regardless of extensive cellular swelling. Many of these hypertrophic cells grew several times in size; Wetzstein and Frett (1984) referred to these elongated cells as protuberances. The intumescences were also shown to be highly turgid and translucent. Within a matter of weeks, these outgrowths supposedly began to desiccate and potentially collapse, with the ultimate result being leaf senescence and abscission.

## **Microscopy and Intumescences**

Microcopy methods have been used in multiple instances to assist in better defining these disorders. Light microcopy has been used to evaluate intumescences on tomato (*S. lycopersicum*;

Lang et al., 1983), sweet potato (*Ipomoea batatas*; Wetzstein and Frett, 1984), and eucalyptus (*E. nitens* and *E. globulus*; Pinkard et al., 2009); and oedema on geranium (*Pelargonium ×hortorum*; Metwally et al., 1970a) and eggplant (*Solanum melongena*; Eisa and Dobrenz, 1971). Stains, such as Toluidine Blue O (tolonium chloride; TBO), are commonly used to more clearly depict the individual cell layers in a cross section. Specifically, TBO will selectively stain acidic tissue components and has a high affinity for nucleic acids (Sridharan and Shankar, 2012). Thus, it will bind very effectively to material that has a high DNA and RNA content. In addition to light microscopy, electron microscopy was used by Wetzstein and Frett (1984) to further analyze intumescences on sweet potato leaves, and by Rud (2009) on tomato leaves.

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## Figures and Tables

**Figure 1.1** Western Flower Thrips (*Frankliniella occidentalis*) feeding damage and oedema on the abaxial leaf surface of a *Pelargonium peltatum* 'Sybil Holmes'. Photo credit: Yan Chen (2003).



## Chapter 2 - UVB Radiation Affects Intumescence Development in Ornamental Sweet Potato (*Ipomoea batatas*)

### Introduction

Intumescences are a physiological disorder that develop sporadically on the leaf tissue of many plant species, including some varieties of tomato (*Solanum lycopersicum* L. 'Maxifort' [an interspecific tomato cultivar bred as a grafting rootstock that includes var. *hirsutum* in its parentage]; Rud, 2009), sweet potato (*Ipomoea batatas* (L.) Lam.; Wetzstein and Frett, 1984), and *Cuphea* P. Br. spp. (Jaworski et al., 1988). This disorder is often described as abnormal, translucent outgrowths on the leaf surface with a gall or wart-like appearance (Morrow and Tibbitts, 1988; Wetzstein and Frett, 1984). This disorder was first observed and named as intumescence by Sorauer (1899) and was found at that time to develop on numerous plant species (La Rue, 1932). While the term intumescence is commonly used to describe this disorder, other common and interchangeably used nomenclature in the published literature includes excrescences, neoplasms, galls, genetic tumours, leaf lesions, enations and oedemata (Pinkard et al., 2006).

Intumescences can have a substantial impact on both the economic and aesthetic value of affected plants (Balge et al., 1969; Rangarajan and Tibbitts, 1994). Many of the species most susceptible to this disorder are grown solely for ornamental purposes, and intumescences can greatly impair the overall aesthetics of the crop. Additionally, severe cases of intumescence development can result in impaired photosynthesis (Pinkard et al., 2006; Roloff and Scherm, 2004). Thus, this physiological disorder presents a substantial problem for growers attempting to produce crops that may be prone to its development.

The causative factors related to intumescence development remain somewhat elusive. One key finding has been that this disorder predominantly occurs on plants being produced in controlled environments (Jaworski et al., 1988; Lang and Tibbitts, 1983; Petite and Ormrod, 1986; Wetzstein and Frett, 1984). For the most part, no pathogen has been found to be involved in intumescence development, which leads most to agree that this is a physiological disorder (Rangarajan and Tibbitts, 1994). Some of the most commonly proposed causative factors include air contamination (Kirkham and Keeney, 1974; Lang and Tibbitts, 1983), hormones and hormone concentrations (Kirkham and Keeney, 1974; Lang and Tibbitts, 1983; Morrow and Tibbitts, 1988;



Pettite and Ormrod, 1986; White, 1951), humidity (Douglas, 1907; Eisa and Dobrenz, 1971), temperature (Balge et al., 1969; Eisa and Dobrenz, 1971), and light (Lang and Tibbits, 1983; Morrow and Tibbits, 1987; Rud, 2009; Wheeler, 2010). Excess water has also been cited as a potential causative factor, and is commonly found when referring to the development of oedema on geranium (*Pelargonium* (L.) L'Hér. ex Aiton spp.; Balge et al., 1969; Metwally et al., 1970b; Metwally et al., 1971).

Light quality is one of the strongest candidates as a causative factor or, conversely, as potential preventative measure for intumescence development. Ultra-violet (UV) light, in particular, is thought to be connected to the disorder because many greenhouse-glazing materials block UV light wavelengths (100-400 nm) and intumescences occur in protected culture. Lang and Tibbits (1983) found that UV light, specifically UVB, effectively prevented intumescence development on tomato (*Solanum lycopersicum* L. var. *hirsutum* and *S. lycopersicum* L. var. *esculentum* 'Oxheart'). Similar results have been observed by Rud (2009) on *Solanum lycopersicum* L. 'Maxifort' and Morrow and Tibbits (1987) on *Solanum lycopersicum* L. var. *hirsutum*. However, the exact mechanism by which this UV light effectively inhibits intumescence development remains uncertain.

Ornamental sweet potato is an annual ornamental crop commonly produced in greenhouses during the spring season; it is a popular species because of its trailing habit and striking foliage colors, with several new cultivar introductions in recent years. However, the species is highly prone to intumescence development when produced in greenhouses, as indicated in a recent patent application for a new variety (Yencho et al., 2012).

Intumescences on sweet potato have not been extensively studied. Currently, only two published research articles discuss the development of this disorder on the crop. The first paper, "Intumeszenze fogliari di 'Ipomoea batatas,'" was published by A. Trotter in 1904. This research was cited by Wetzstein and Frett (1984) as they evaluated intumescence anatomy on sweet potato leaves based on light and electron microscopy. However, very little is known about specific causative factors that may contribute to the occurrence of this disorder on ornamental sweet potato. Therefore, the goal of this study was to assess the effect of UVB radiation in the prevention of intumescence development on two cultivars of ornamental sweet potato (*Ipomoea batatas*).

## Materials and Methods

Rooted cuttings of two ornamental sweet potato cultivars, ‘Ace of Spades’ and ‘Sidekick Black’, were obtained from a commercial supplier. The cuttings were potted on 1 Mar. 2013 in 11.43 cm diameter (465 ml volume) pots using a peat-based media (Fafard #2; Conrad Fafard Inc.; Agawam, MA) and were grown in a glass greenhouse of Kansas State University’s Throckmorton Plant Sciences Center (Manhattan, KS). The greenhouse temperature was set at 22° C day and 20° C night. Data loggers (Onset Computer Corporation; Bourne, MA) were placed within each treatment to monitor for any differences in temperature or relative humidity between treatments. Plants were fertigated with a 200 mg·L<sup>-1</sup> N constant liquid feed using 20N-4.4P-16.6K (Peters Professional Peat-Lite Special; Everris NA Inc.; Dublin, OH) when greater than half of the plants reached ~ 45% container capacity due to water loss. Supplemental lighting, using full spectrum lamps (Verilux, Inc.; Waitsfield, VT) providing a spectrum similar to that provided by solar radiation, was provided to achieve a 13-hr photoperiod prior to treatment initiation. These lamps provided control lighting that simulated the light quality to which plants would be exposed in outdoor production where intumescences are not observed (as adapted from Morrow and Tibbitts, 1988). A greenhouse experiment using both sweet potato cultivars was conducted using a 2-way factorial treatment structure in a replicated row-orthogonal experimental design with subsampling. Treatment factors consisted of light treatments (described later) and cultivars (i.e. ‘Ace of Spades’ and ‘Sidekick Black’). Within the greenhouse where the experiment was conducted, benches (total of 3, each measuring 1.2 x 6.4 m) constituted complete blocks and defined the rows of the design. On each bench, four 1.2 x 1.5 m separate sections were designated, so that all light treatments could be represented. Distance from the fans (total of 4) constituted balanced incomplete blocks and defined the columns of the design. A total of 3 individual plants from each cultivar were randomly assigned to a light treatment on each bench. Since each combination of cultivar and light treatment occurred on each bench (row), the treatments were orthogonal to the rows. Within each light treatment on a bench, individual plants of each cultivar represented the observational units and thus, the level of subsampling. The experiment was repeated, thus defining 2 experimental runs.

The first experimental run was conducted 4.5 weeks after potting, while the second run followed at 7.5 weeks after potting; thus plant age was different for each run. In each



experimental run, plants were subjected to light treatments for two weeks, during which data and observations were recorded.

### ***Light Treatments***

The four light treatments evaluated included 1) Normal: typical glass-glazed greenhouse growing conditions (no supplemental lighting); 2) UVB: supplemental UVB lighting (280-315 nm) provided with UVB-313 lamps (Q-Lab Corporation; Westlake, OH); 3) UVB-Blocked: the same as treatment 2 with the addition of Mylar® sleeves (North Solar Screen; Andover, MA) placed over the lamps to block UVB light; and 4) Full-spectrum: control lighting using full spectrum lamps, as previously described. Supplemental lighting for Treatments UVB, UVB-Blocked, and Full-Spectrum was provided using a single 121 cm long light ballast (American Fluorescent; Waukegan, IL) suspended 72 cm from the surface of the bench within each treatment. The light periods were timed to mimic the natural day length with a 13-hr photoperiod. For treatments UVB and UVB-Blocked, economy grade polystyrene lighting panels (Plaskolite Inc.; Columbus, OH) were suspended 7.5 cm from the base of the ballast to prevent extensive plant damage from the high levels of UVB emitted. Due to degradation of the diffusion panels, the panels used in treatment UVB were replaced every two days to maintain consistent levels of UVB light. To prevent cross-contamination between light treatments across adjacent bench sections, UV-blocking plastic film (DuraGreen Marketing USA; Mount Dora, FL) was used to isolate treatment sections on each bench. The total amount of UVB radiation ( $W \cdot m^{-2}$ ) within each of the treatments was measured in each bench section every three days using a portable UV-VIS spectroradiometer (BLACK-Comet, StellarNet; Tampa, FL). Measurements of UVB radiation were later used to validate light treatments.

### ***Data collection***

During each experimental run, plants were observed every three days for intumescence development. At each observation, leaves displaying any sign of intumescence development were individually tagged. After two weeks, the total number of tagged leaves continuing to display intumescence development was recorded, as some of the previously tagged leaves no longer showed signs of the disorder. Total leaf count per plant was also obtained at the end of each run. In order to quantify potential plant growth differences that may have occurred under different light treatments, plant widths were measured at initiation and completion of each

experimental run. Plant width was obtained by averaging the linear measurement of the plant at the greatest width and a second linear measurement perpendicular to the first. In addition, fresh and dry weights were recorded for the group of 3 plants of each cultivar under the same light treatment at the end of each experimental run. Also, at the end of each run, one representative plant of each cultivar was selected from each bench section and rated qualitatively on a one to five scale (Table 2.1) to evaluate salability. The salability ratings considered two criteria, 1) the severity of intumescence development and 2) the severity of UVB side effects (e.g. leaf curling and discoloration) due to UVB light.

### *Statistical Analysis*

Response variables of interest subjected to statistical analyses included fresh and dry weight, plant width, affected leaves per plant and plant ratings, as well as daily temperature, relative humidity and UVB measurements. General or generalized linear mixed models were fitted to each of these response variables, depending on whether the responses were continuous or categorical in nature, respectively. In general, the linear predictor for these statistical models included the fixed effects of experimental run (i.e. 2 runs), light treatment (4 levels: Normal, UVB, UVB-Blocked and Full spectrum) and cultivar (2 levels: ‘Ace of Spades’ or ‘Sidekick Black’), as well as all 2-, and 3-way interactions. More specifically, the statistical model for plant width also included time (i.e. start vs. end of the experimental run), and all interactions with remaining fixed effects. For daily temperature and relative humidity, the model included the fixed effects of run, light, temperature and time, as well as all interactions. For the response on number of affected leaves per plant, separate analyses were conducted for each cultivar due to problems with quasi-complete separation of datapoints under conditions of higher order interactions. The logit and cumulative logit link functions were used to connect the binomial probability of intumescence-affected leaf and the ordered categorical probability of plant rating with their respective linear predictors.

For all variables, the random effects of bench nested within run and also its cross-products with light treatment were considered in the linear predictor in order to recognize the appropriate experimental unit for each of the fixed effect factors. For this same reason, random effects for the model on plant width also included cross-products with treatment-cultivar combinations and with plant nested within treatment-cultivar combinations.

Whenever necessary, as dictated by the model fit Bayesian Information Criteria, heterogeneous residual variances were fitted to ensure that model assumptions were properly met. All variance components were estimates using residual (pseudo) likelihood. Degrees of freedom were estimated using Kenward-Roger's procedure and then fine-tuned, as needed, to accommodate zero-estimates for some of the variance components, whenever necessary. For general linear mixed models, model assumptions were evaluated using externally studentized residuals and were considered to be appropriately met. For generalized linear mixed models, overdispersion was evaluated using the maximum-likelihood-based fit statistic Pearson Chi-Square over degrees of freedom. No evidence for overdispersion was apparent in any case.

All statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.2, SAS Institute, Cary, NC) implemented using Newton-Raphson with ridging as the optimization technique. Relevant pairwise comparisons were conducted using either the Tukey-Kramer or Bonferroni adjustment, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons.

## **Results and Discussion**

### ***UVB Validation***

Light treatments were validated using UVB measurements collected with a spectroradiometer. Throughout both experimental runs, Treatment UVB had significantly ( $P < 0.0001$ ) higher levels of UVB radiation than any of the other three treatments (Fig. 2.1).

### ***Cultivar Differences***

Across both experimental runs of the study, cultivars were found to be significantly different ( $P < 0.0001$ ) in their incidence of intumescent leaves. More specifically, the 'Sidekick Black' cultivar showed almost no intumescence development (estimated probability of intumescence-affected leaf  $\pm$  SE =  $0.06 \pm 0.02$  %), while 'Ace of Spades' was significantly more likely to develop intumescences (estimated probability of intumescence-affected leaf  $\pm$  SE =  $11.82 \pm 0.85$  %). Differences in cultivar susceptibility to this disorder have been noted for other species, including potato (*Solanum tuberosum* L.; Petite and Ormrod, 1986; Seabrooke and Douglass, 1998), eggplant (*Solanum melongena* L.; Eisa and Dobrenz, 1971), *Cuphea* spp. (Jaworksi et al., 1988), tomato (Metwally et al., 1970b), and geranium (Balge et al., 1969). Based

on these cultivar differences, Eisa and Dobrenz (1971) suggested that genetic composition may play a partial role in the development of intumescences, though it is still uncertain as to what specific attributes or genetic factors may be affecting this resistance and susceptibility response.

### *Intumescent Leaves*

Due to the observed low susceptibility of the ‘Sidekick Black’ cultivar to intumescence development, it was not possible to evaluate the effect of light treatment on this cultivar. So treatment effects on the “Sidekick Black” cultivar are not discussed further.

For the ‘Ace of Spades’ cultivar, Treatment UVB significantly decreased the probability of intumescence development compared to the other three light treatments ( $P < 0.0001$ ; Fig. 2.2). More specifically, the estimated probabilities of affected leaves for Treatments Normal and UVB-Blocked exceeded that of Treatment UVB by more than 10 times, and were not significantly different from each other ( $P = 0.1872$ ). For Treatment Full-Spectrum, the probability of intumescence development was roughly half that of Treatments Normal ( $P = 0.0016$ ) and UVB-Blocked ( $P = 0.0002$ ), but was still greater than that of Treatment UVB ( $P < 0.0001$ ).

Our results are consistent with the findings of Lang and Tibbits (1983), Morrow and Tibbits (1987), and Rud (2009), by which UVB light effectively prevented intumescence development on tomato plants. Our results further indicate that UVB radiation can be used as a means to effectively minimize the disorder on ornamental sweet potato.

The reduced occurrence of intumescences on plants in Treatment Full-Spectrum (Fig. 2.2) was initially believed to be related to expected higher levels of UVB radiation in this treatment compared to Treatments Normal and UVB-Blocked. However, levels of UVB radiation emitted under Treatment Full-Spectrum were not consistently greater than Treatment Normal. In particular, UVB radiation under Treatment Full-Spectrum was significantly greater than under Treatment Normal only on days 6 and 9 for experimental run 1 and days 0, 6 and 9 for experimental run 2. Moreover, estimated differences in levels of UVB radiation emitted between these treatments never exceed  $0.017 \text{ W} \cdot \text{m}^{-2}$ . Thus, it seems unlikely that the low levels of UVB radiation emitted from Treatment Full-Spectrum had a major role in preventing intumescence development. However, we expect that the presence of other wavelengths of light in Treatment Full-Spectrum, such as red and far-red radiation, may have played a contributing role in the moderate prevention of intumescence development observed. This idea is supported

by the findings of Morrow and Tibbits (1988) who reported that red light induced intumescence development on tomato, while far-red light seemed to diminish these effects. These authors further implicated the involvement of phytochrome in this disorder.

The plants in Treatment UVB were not completely void of intumescence development. Occasional intumescences formed on the leaves under Treatment UVB, but the severity of the lesions and number of leaves affected was significantly less than for the other treatments. However, with intumescences not typically observed in the natural environment, one would expect that the amount of UVB light supplied to the plants under Treatment UVB to be sufficient for the prevention of the disorder, as UVB levels surpassed the natural levels observed outdoors. A similar observation was made by Kirkham and Keeney (1974), who came to the conclusion that UV light may act to alleviate enation development, but will not completely prevent it. Rud (2009) also discussed this phenomenon concerning intumescences forming on tomato leaves regardless of UVB exposure. In that study, Rud suggested that the occurrence of intumescences under these conditions may be due to either shading from the upper canopy leaves reducing UVB exposure or to naturally cloudy days, which could have reduced the overall UV levels in the treatments.

While the differences in UVB exposure due to natural light conditions in the greenhouse seems marginal for our study, leaves developing intumescences in Treatment UVB were often shaded by the upper canopy. We expect the upper canopy likely reduced the levels of UVB light reaching the lower canopy leaves, thus contributing to their development of intumescences. To further assess this possibility, we collected pilot data to conduct a preliminary evaluation of a potential shading effect by the upper canopy leaves on UVB radiation. We measured UVB radiation by placing the spectroradiometer probe directly above and below the upper leaf canopy on six 'Ace of Spades' plants under UVB lighting. These measurements were collected after completion of the study presented herein and are only intended to support future work evaluating the proposed shading mechanism. These pilot data were analyzed using a general linear mixed model that fitted a randomized complete block design with heterogeneous residual variances, following procedures described in the Statistical Analysis section. As expected, levels of UVB radiation were significantly lower ( $P < 0.0001$ ) under the upper leaf canopy ( $0.014 \pm 0.003 \text{ W} \cdot \text{m}^{-2}$ ) compared to above it ( $0.945 \pm 0.013 \text{ W} \cdot \text{m}^{-2}$ ). These pilot data support the shading mechanism suggested by Rud (2009), and display that the upper canopy may cause a substantial decrease in

the UVB radiation reaching the lower canopy leaves. This reduction in UVB may lead to potential intumescence development within the lower canopy. Future research should further investigate this phenomenon.

To further support the possible involvement of shading, Rud (2009) hypothesized that a threshold mechanism may be involved. Specifically, that intumescence development on susceptible plants may be prevented if a specific duration or intensity of UV light is made available. This idea should be further evaluated by exposing plants to UVB of various time intervals or intensities.

The specific mechanism by which UVB radiation prevents intumescence development is still uncertain. One theory states that the plant hormone auxin may act in the development of these intumescent growths. Morrow and Tibbitts (1988) cited that auxin may be involved in intumescence development due to its role in cell expansion. They suggest that the inhibitory action of UV light on intumescence development may be due to the ability of these wavelengths to degrade auxins. This theory of auxin inactivation or degradation by UV light is further supported in a review published by Springer (1978). However, why auxin levels would become more prevalent within a controlled environment in the first place is still uncertain. One can hypothesize that the lack of UVB radiation may be causing the increase in auxin levels, but the inability of Treatment UVB to completely eradicate these levels, due to the occurrence of few intumescences, leaves one questioning this theory. One possible explanation is the shading effect by upper canopy leaves discussed previously. Under these conditions, auxin in the lower canopy leaves may not be effectively degraded due to the observable reduction in UVB radiation. Additional plant hormones such as cytokinin (Morrow and Tibbitts, 1988) and ethylene (Wallace, 1928; Kirkham and Keeney, 1974) have also been theorized to play a role in intumescence development.

### ***Plant Growth***

No evidence for treatment differences on fresh and dry weight nor start and harvest width were apparent throughout the experiment ( $P>0.05$ ; Table 2.2). Thus, we concluded that no significant reduction in plant growth occurred from UV light. These findings differ from those of Frantz et al. (2012), whereby UV light was found to be a potential means of regulating plant growth in plugs. This contrast is likely due to differences in plant age, as plugs may be more

vulnerable to growth inhibition from UV light than the plants in our study or that dose differences between studies impacted the results. Additionally, it is possible that plant species react differently to UV light. This may have led to less noticeable changes in growth on ornamental sweet potato than in many of the species used by Frantz et al (2012).

However, plant growth differences were found between cultivars regardless of treatment (Table 2.2). In particular, during experimental run 2, plants from the ‘Sidekick Black’ cultivar had significantly greater fresh ( $P=0.0003$ ) and dry ( $P=0.0008$ ) weights compared to plants from the ‘Ace of Spades’ cultivar. Additionally, ‘Ace of Spades’ plants were wider than ‘Sidekick Black’ plants at both the beginning and end of each experimental run ( $P<0.0001$ ). The ‘Sidekick Black’ cultivar consisted of more compact and dense plants, while the ‘Ace of Spades’ cultivar showed a more trailing habit.

### ***Plant Ratings***

While plant growth was not significantly affected by the UVB radiation in this study, negative effects to plant aesthetics were observed in Treatment UVB. Both leaf deformities and discoloration were observed during plant salability ratings of our study (Fig. 2.3), similar to those reported by Frantz et al. (2012). Nevertheless, no significant treatment effects were apparent in the overall plant salability ratings ( $P=0.2058$ ). As stated previously, the rating scale for plant salability took into consideration damage accrued to the plant both by intumescence development and by UVB radiation. We speculate that by accounting for both forms of damage, one due to the disorder and the other to the treatment itself, the rating scale yielded no significant differences between treatments (Fig. 2.4). In other words, while Treatment UVB did effectively reduce the occurrence of intumescence development on the plants, the negative effects of the UVB radiation on plant aesthetics seemed to nullify this benefit. It may be possible to minimize these side effects with a better understanding of the critical minimum levels of UVB intensity and dosage necessary to prevent intumescence development.

Plant rating differences were apparent between the two cultivars regardless of treatment ( $P<0.0001$ , Fig. 2.5). In particular, ‘Sidekick Black’ received an overall lower cumulative probability of high (undesirable) ratings compared to ‘Ace of Spades.’ This was to be expected following from our observations that plants of the ‘Sidekick Black’ cultivar were more resistant to intumescence development than those of the ‘Ace of Spades’ cultivar.

### ***Temperature Associated With Light Treatments***

Average daily temperature fluctuated over the duration of each experimental run (Fig. 2.6). During experimental run 1 and most of experimental run 2, no differences were apparent between treatments in average daily temperature ( $P>0.05$ ). However, during days 1, 3, 4, 5, 9, 12, 13, and 15 of experimental run 2, average daily temperature under Treatment UVB was significantly increased relative to Treatment UVB-Blocked ( $P<0.05$ ). Additionally, on day 5 of experimental run 2, Treatment UVB had a greater average daily temperature than Treatment Full-Spectrum ( $P=0.0107$ ). The estimated differences in average daily temperature never exceeded 3°C (Fig. 2.6). No evidence for differences in average daily temperature was apparent between other treatments ( $P>0.05$ ).

Even with the slightly higher temperatures recorded in Treatment UVB during experimental run 2, intumescence development was still suppressed. In regards to temperature, Balge (1969) and Eisa and Dobrenz (1971) found that high temperatures were necessary for oedema and intumescence development on zonal geranium and eggplant respectively. However, Lang and Tibbits (1983) found that intumescence development on tomatoes was more severe at cooler temperatures. While it is possible that temperature may act synergistically with other factors to increase severity or occurrence intumescence development, it appears that the quality of light the plants are subjected to may override those conditions to prevent intumescence development on ornamental sweet potato.

### ***Relative Humidity Associated With Light Treatments***

For all light treatments, levels of relative humidity fluctuated greatly over the course of the study (Fig. 2.7). However, relative humidity was lowest under the conditions of Treatment UVB compared to any of the other treatments regardless of the experimental run ( $P<0.001$ ; Fig. 2.7). Eisa and Dobrenz (1971) reported relative humidity as being a potential causative factor for intumescence development on eggplant; in contrast, Lang and Tibbits (1983) stated that this factor did not contribute to intumescence development on tomato. Given the results of this study, we speculate that a mechanism similar to that proposed for temperature may be at work. Namely, while relative humidity may act synergistically with other factors to increase the occurrence or severity of intumescence development, it appears that light quality may override these conditions and act to prevent their development on ornamental sweet potato.



## Conclusions

The results from this study show that UVB light plays a significant role in the prevention of intumescence development on ornamental sweet potato. The striking cultivar differences in susceptibility to intumescence development observed are also of interest, as it suggests the role of a genetic component. At this point, the best recommendation for intumescence control would seem to be cultivar or variety selection (Jaworski et al., 1988). Future research on genetic control of susceptibility to intumescence development in ornamental sweet potato is warranted. Additionally, the fact that Treatment UVB still had the occasional leaf develop intumescences suggests that a more complex interaction, likely involving other factors such as temperature, humidity, hormones, etc., may be at work. This insight will assist in an enhanced understanding of intumescence development and the mechanism by which this disorder develops.

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## Figures and Tables

**Table 2.1 Plant salability rating scale used to quantify damage on plants as a result of intumescence development and UVB damage.**

<b>Rating Scale</b>	<b>Intumescence</b>	<b>UVB</b>
<b>1</b>	None; smooth leaves	None; normally developed leaves
<b>2</b>	Few (1-2) leaves with solitary growths	Few leaves with minor discoloration
<b>3</b>	Multiple (3-4) leaves with mass groupings appearing along the veins of the plant	Multiple leaves with severe discoloration and deformity (such as leaf curling)
<b>4</b>	Many (5-6) leaves with mass groupings forming sporadically across the leaf surface	Many leaves displaying severe discoloration and deformity
<b>5</b>	Majority of leaves senescing due to severe development	Majority of leaves with severe discoloration; deformity resulting in leaf senescence

Figure 2.1 Estimated least squares mean UVB intensity ( $\text{Watts}\cdot\text{m}^{-2}$ ) ( $\pm$  SE) for each of the 4 light treatments in experimental run 1 (A) and 2 (B) as determined using a portable UV-VIS spectroradiometer (BLACK-Comet, StellarNet; Tampa, FL). Measurements were taken every 3 days, with a total of 5 measurements during the 2-week observation period of each experimental run. Measurements were collected on a spectrum ranging from 280-315 nm.

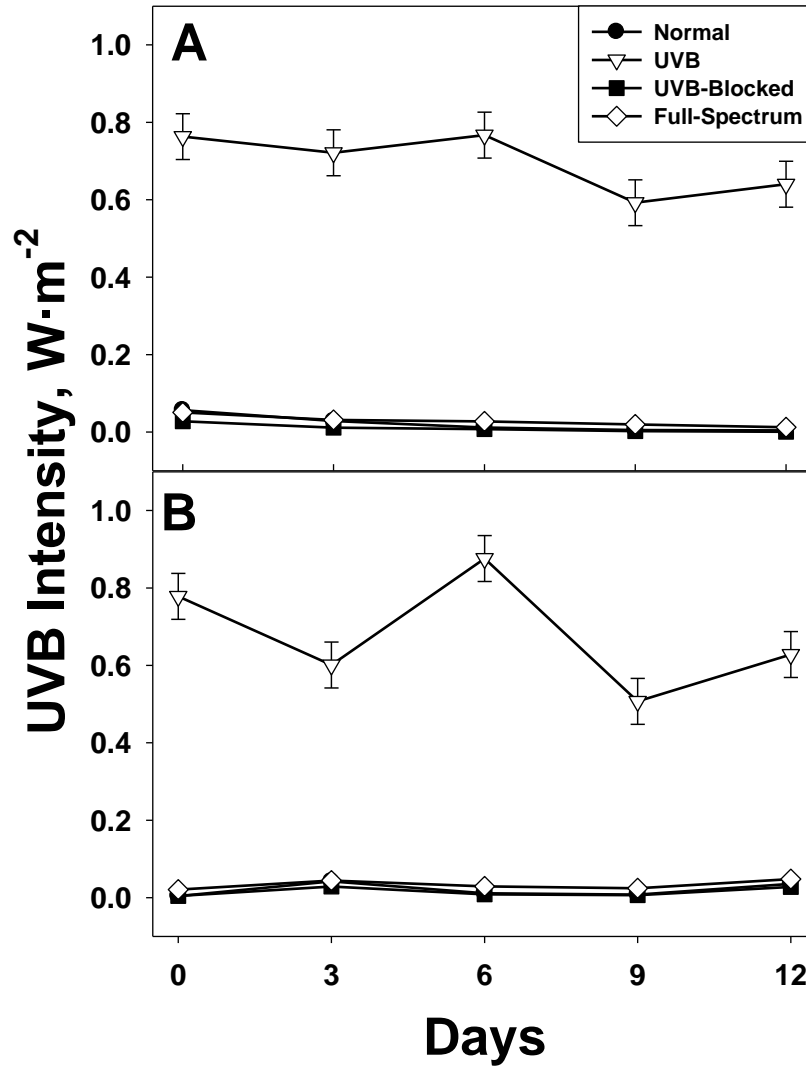
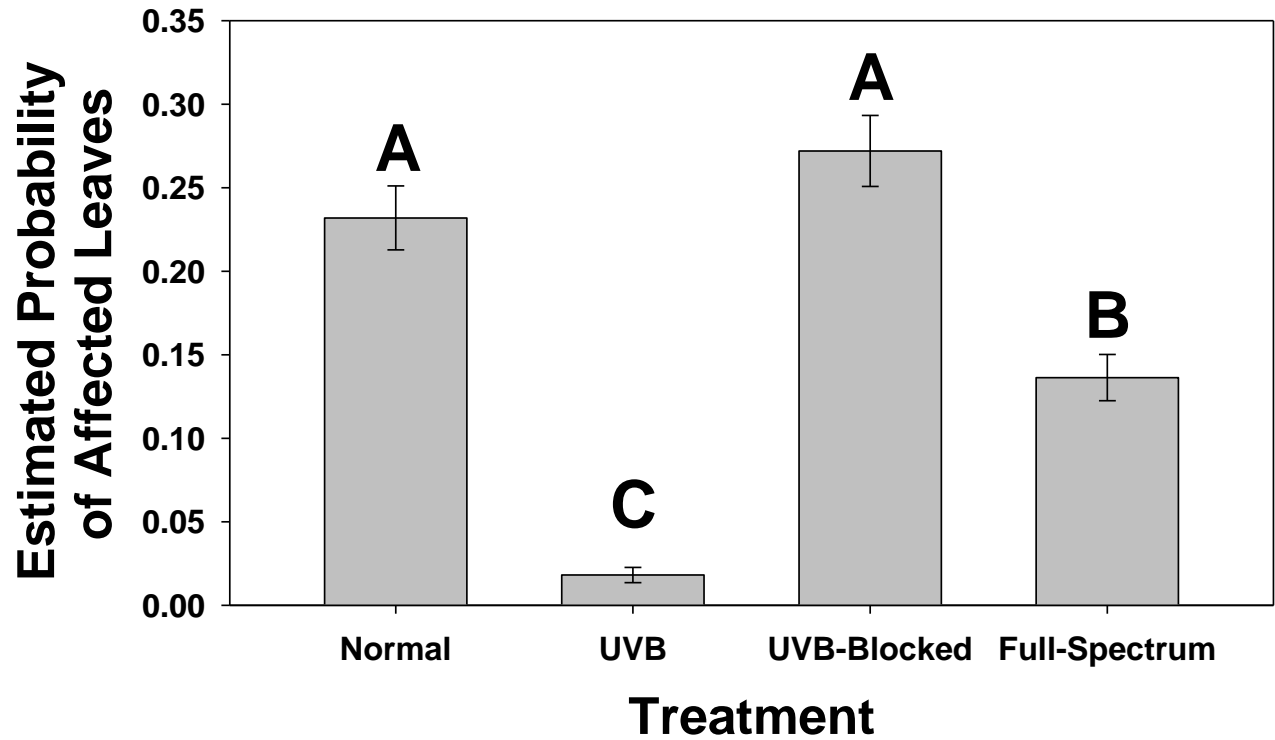


Figure 2.2 Least square mean estimated probability of intumescence-affected leaves ( $\pm$  SE) on the 'Ace of Spades' cultivar for each of the 4 light treatments. (A, B, C) Different letters indicate significant differences between treatments ( $P < 0.05$ ).



**Table 2.2 Least square mean estimates ( $\pm$  SE) for start and harvest plant width and for fresh and dry weight of ornamental sweet potato plants cultivars ‘Ace of Spades’ and ‘Sidekick Black’ grown under light treatments. Regardless of cultivar, no evidence for treatment differences was apparent for any of the response variables ( $P>0.05$ ).**

Cultivar	Run	Treatment	Fresh Weight (g)	Dry Weight (g)	Start Width (cm)	Harvest Width (cm)
‘Ace of Spades’	1	Normal	49.7 $\pm$ 2.8	5.1 $\pm$ 0.3	33.3 $\pm$ 1.2	43.0 $\pm$ 1.2
		UVB	43.1 $\pm$ 2.8	4.5 $\pm$ 0.3	34.0 $\pm$ 1.2	42.3 $\pm$ 1.2
		UVB-Blocked	46.0 $\pm$ 2.8	4.6 $\pm$ 0.3	31.9 $\pm$ 1.2	41.3 $\pm$ 1.2
		Full-Spectrum	49.6 $\pm$ 2.8	5.0 $\pm$ 0.3	33.4 $\pm$ 1.2	44.7 $\pm$ 1.2
	2	Normal	93.9 $\pm$ 8.1	10.0 $\pm$ 0.9	43.4 $\pm$ 1.2	56.3 $\pm$ 1.2
		UVB	101.2 $\pm$ 8.1	10.2 $\pm$ 0.9	42.2 $\pm$ 1.2	57.4 $\pm$ 1.2
		UVB-Blocked	95.6 $\pm$ 8.1	9.9 $\pm$ 0.9	43.8 $\pm$ 1.2	56.4 $\pm$ 1.2
		Full-Spectrum	101.6 $\pm$ 8.1	10.5 $\pm$ 0.9	44.0 $\pm$ 1.2	58.8 $\pm$ 1.2
‘Sidekick Black’	1	Normal	50.2 $\pm$ 2.8	5.4 $\pm$ 0.3	30.5 $\pm$ 1.2	40.9 $\pm$ 1.2
		UVB	40.7 $\pm$ 2.8	4.2 $\pm$ 0.3	30.3 $\pm$ 1.2	36.0 $\pm$ 1.2
		UVB-Blocked	45.3 $\pm$ 2.8	4.6 $\pm$ 0.3	29.3 $\pm$ 1.2	40.2 $\pm$ 1.2
		Full-Spectrum	48.8 $\pm$ 2.8	5.2 $\pm$ 0.3	28.9 $\pm$ 1.2	39.4 $\pm$ 1.2
	2	Normal	121.1 $\pm$ 2.8	12.7 $\pm$ 0.3	39.5 $\pm$ 1.2	49.2 $\pm$ 1.2
		UVB	113.9 $\pm$ 2.8	11.0 $\pm$ 0.3	38.7 $\pm$ 1.2	49.9 $\pm$ 1.2
		UVB-Blocked	126.4 $\pm$ 2.8	13.0 $\pm$ 0.3	41.4 $\pm$ 1.2	52.1 $\pm$ 1.2
		Full-Spectrum	128.5 $\pm$ 2.8	13.2 $\pm$ 0.3	39.9 $\pm$ 1.2	50.9 $\pm$ 1.2

**Figure 2.3** Illustration of aesthetic damage due to the high intensity of UVB radiation emitted from Treatment UVB. A) UVB damage on a plant of the ‘Ace of Spades’ cultivar showing deformity (arrow) of affected leaves (Plant Rating = 3.0). B) UVB damage on a plant of the ‘Sidekick Black’ cultivar displaying discoloration (arrow) of affected leaves (Plant Rating = 4.0).

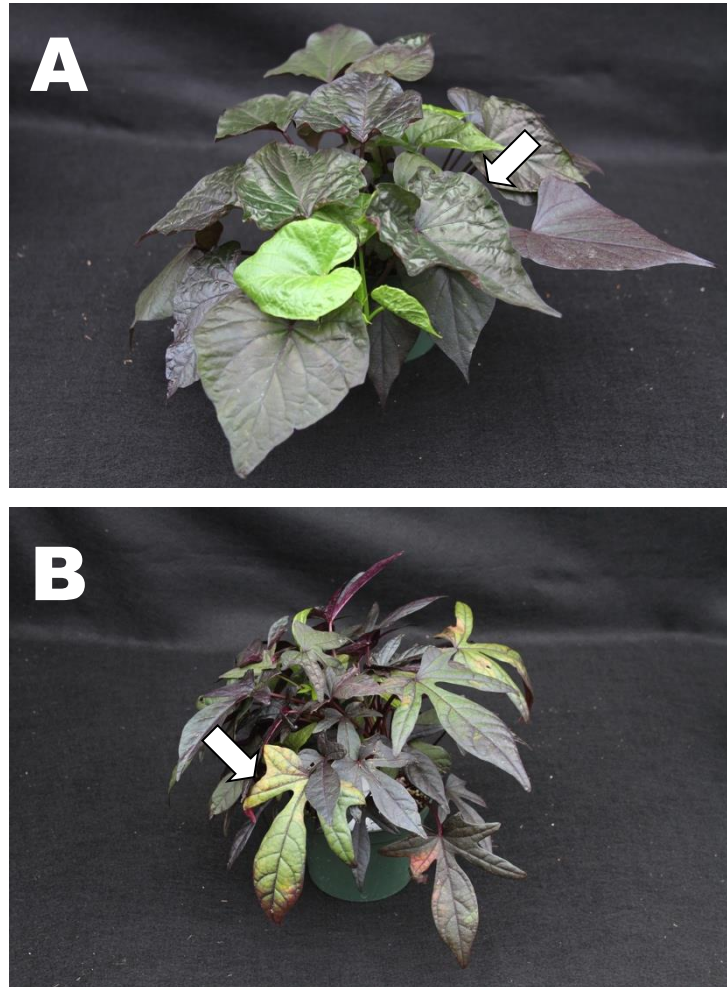




Figure 2.4 Estimated cumulative probability of a plant displaying damage equivalent to or greater than the rating administered to plants of the ‘Ace of Spades’ (A) and ‘Sidekick Black’ (B) cultivars under light treatments. The rating system was based on a 1 to 5 scale considering the number of leaves displaying intumescence and UVB damage, as well as the severity of damage, where 1) None; 2) Few (1-2); 3) Multiple (3-4); 4) Many (5-6); 5) Majority. A rating of 5 is not included in this table due to damage severity in this study never exceeding a rating of 4. No evidence for main treatment effects or interactions involving treatment was apparent based on  $\alpha=0.05$ .

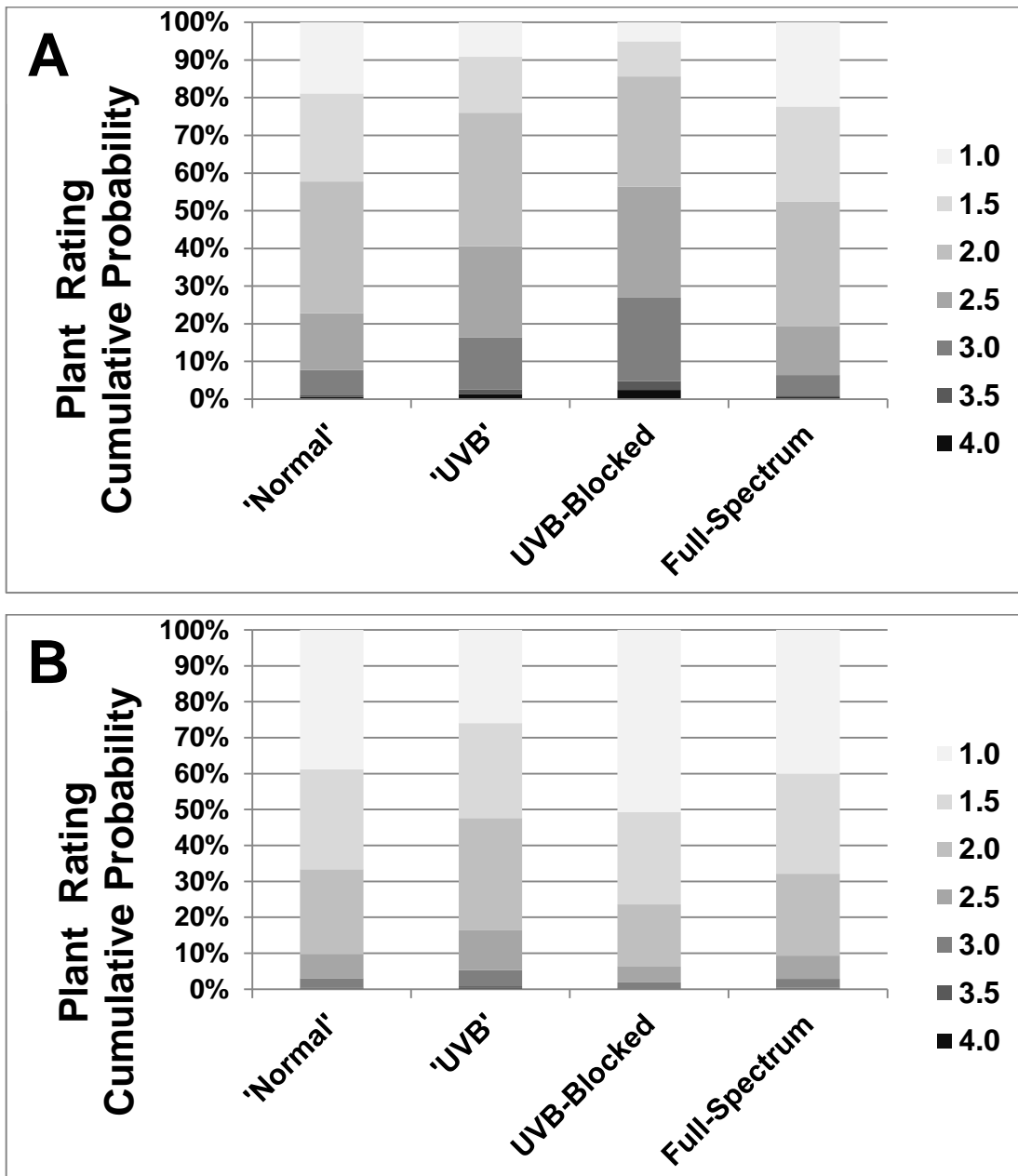


Figure 2.5 Estimated cumulative probability of a plant displaying damage equivalent to or greater than the rating administered to plants of the ‘Ace of Spades’ and ‘Sidekick Black’ cultivars, adjusted for treatments. The rating system was based on a 1-5 scale considering the number of leaves displaying intumescence and UVB damage, as well as the severity of damage, where 1) None; 2) Few (1-2); 3) Multiple (3-4); 4) Many (5-6); 5) Majority. A rating of 5 is not included in this histogram due to damage severity in this study never exceeding a rating of 4.

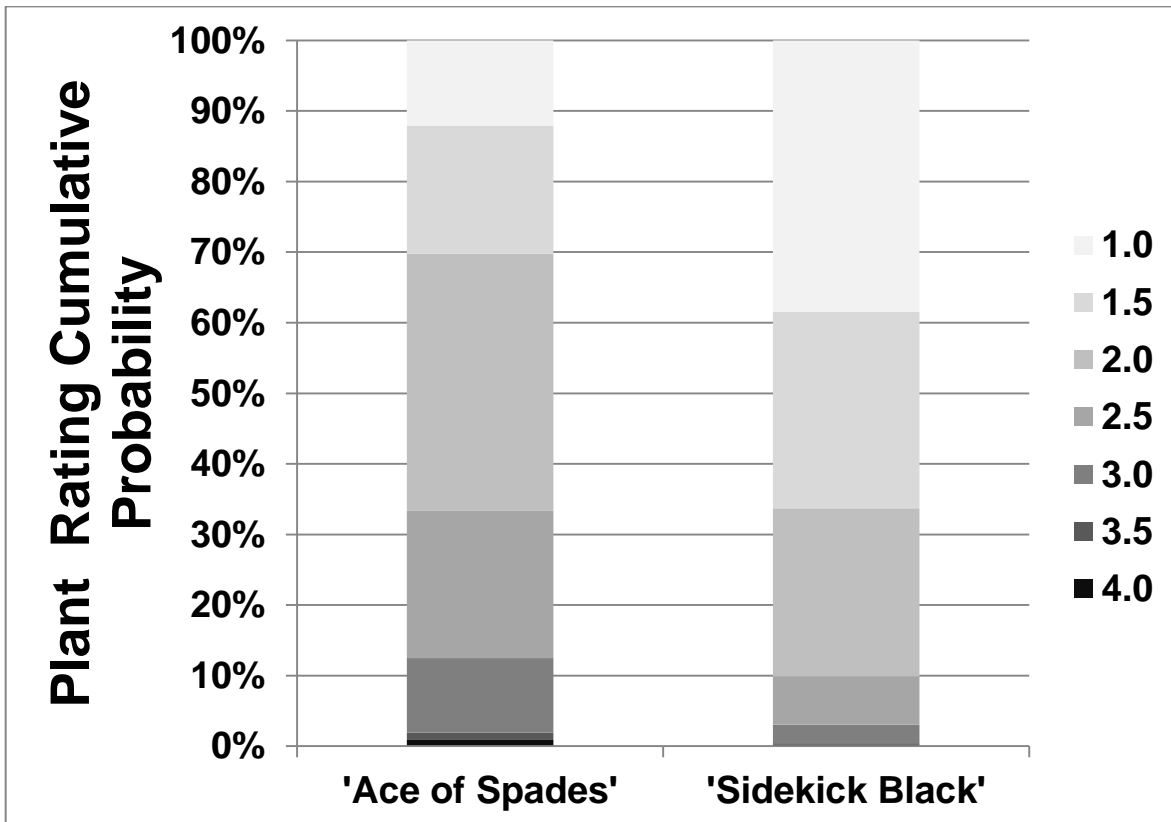


Figure 2.6 Least square mean estimates of average daily temperatures ( $^{\circ}\text{C} \pm \text{SE}$ ) under light treatments during experimental run 1 (A) and 2 (B). During days 1, 3, 4, 5, 9, 12, 13, and 14 of experimental run 2, average daily temperatures under Treatment UVB were significantly higher than under Treatment UVB-Blocked ( $P < 0.05$ ). Also on day 5 of experimental run 2, the average daily temperature of Treatment UVB was significantly higher than Treatment Full-Spectrum ( $P < 0.05$ ). Days displaying significant differences are noted on the graph with a “\*” above the corresponding day.

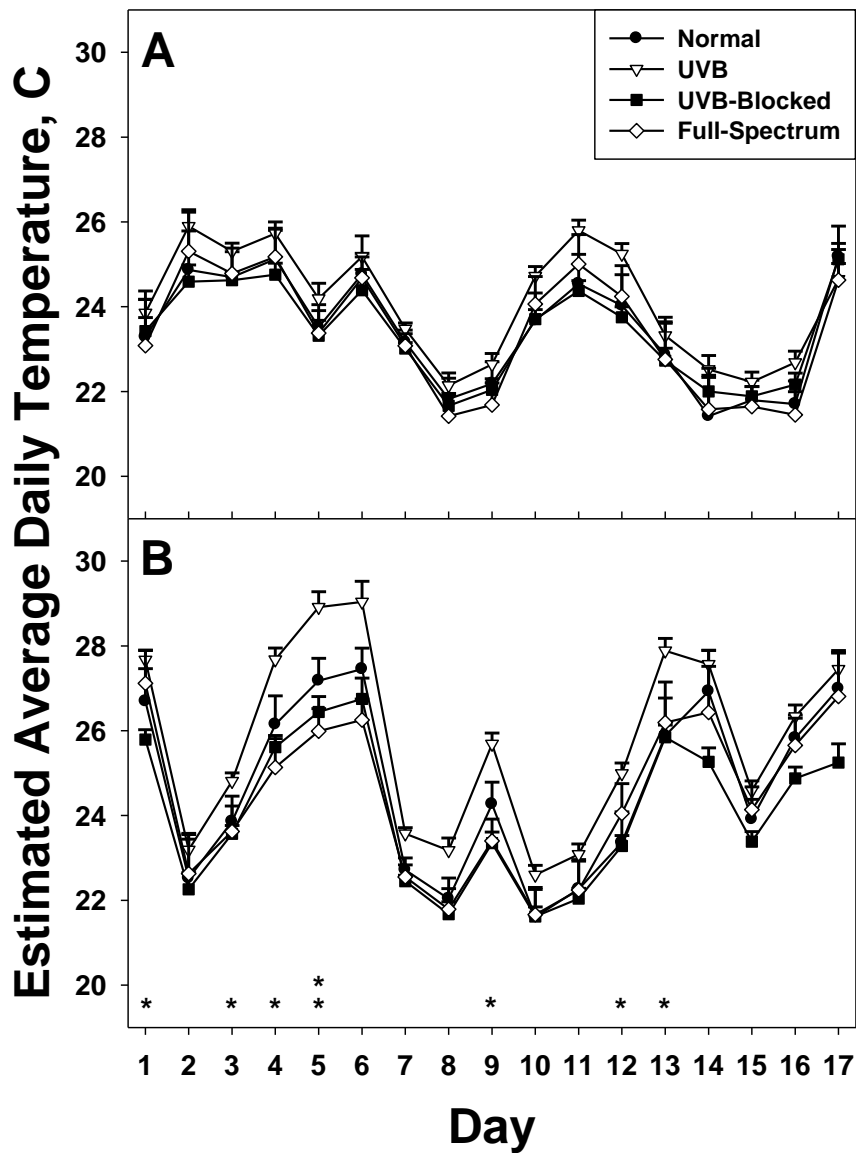
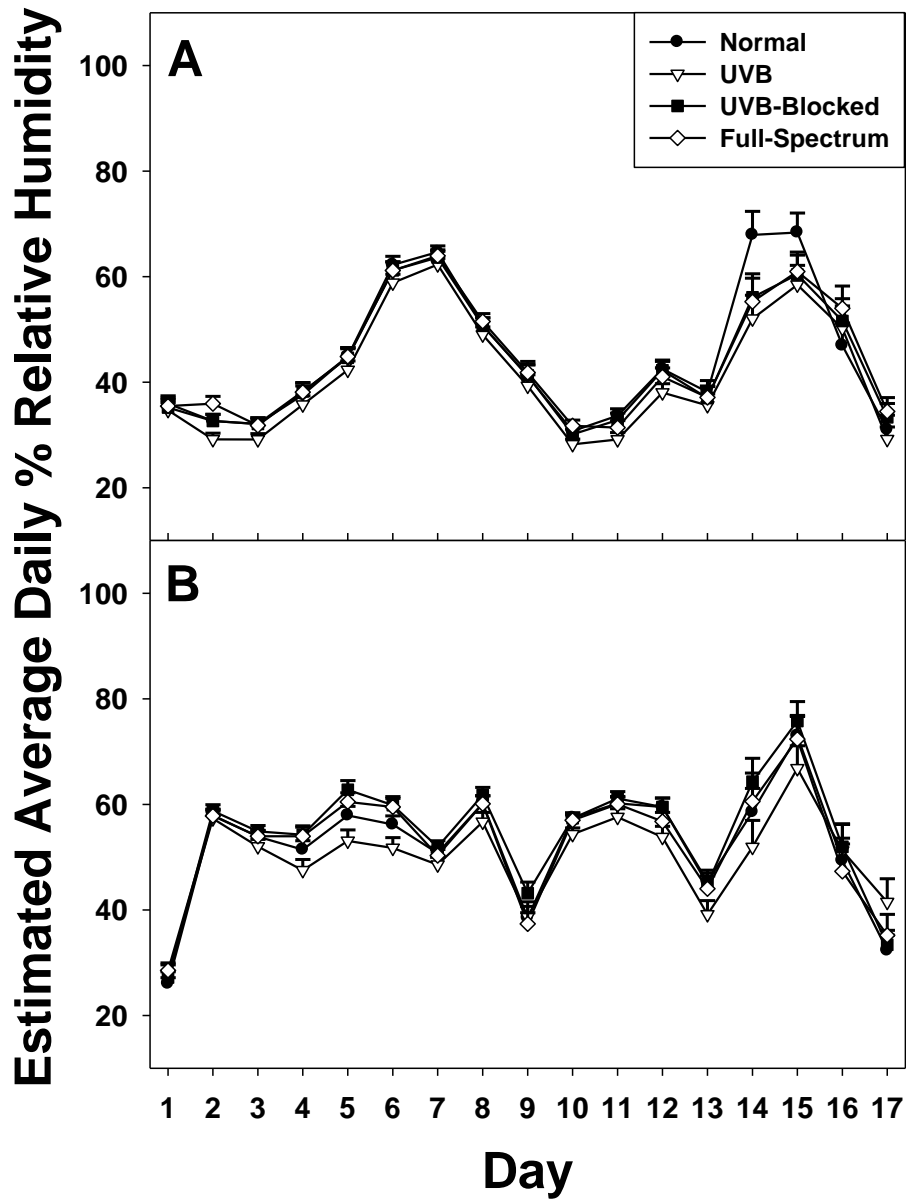


Figure 2.7 Least square mean estimates of average daily relative humidity (%  $\pm$  SE) under light treatments during experimental run 1 (A) and 2 (B). Treatment UVB had a significantly lower relative humidity than Treatments Normal ( $P=0.0049$ ), UVB-Blocked ( $P=0.0009$ ), and Full-Spectrum ( $P=0.0084$ ) regardless of run or bench based on multiple comparisons at  $\alpha=0.05$ .



# **Chapter 3 - Characterization and Comparison of Lesions on Ornamental Sweet Potato (*Ipomoea batatas*), Tomato (*Solanum lycopersicum* ‘Maxifort’), and Interspecific Geranium (*Pelargonium* ×‘Caliente Coral’)**

## **Introduction**

Intumescence is a physiological disorder that develops sporadically on the leaf tissue of many plant species, including tomato (*Solanum lycopersicum* L. ‘Maxifort’ [an interspecific tomato cultivar bred as a grafting rootstock that includes var. *hirsutum* in its parentage]; Rud, 2009), sweet potato (*Ipomoea batatas* (L.) Lam.; Wetzstein and Frett, 1984), and *Cuphea* P. Br. spp. (Jaworski et al., 1988). This disorder is often described as abnormal, translucent outgrowths on the leaf surface with a gall or wart-like appearance (Morrow and Tibbitts, 1988; Wetzstein and Frett, 1984). While the term intumescence is commonly used to describe this disorder, other common and interchangeably used nomenclature in published literature includes excrescences, neoplasms, galls, genetic tumours, leaf lesions, enations and oedemata (Pinkard et al., 2006).

Interchangeably using these terms causes confusion as to whether these names refer to the same or different disorders. In 1951, White reviewed neoplastic growths on plant tissue and concluded that there was a wide variety of such growths that existed, and that these growths may have originated by many different means. In other words, much of the confusion concerning the nomenclature of intumescence development may actually be due to multiple disorders being observed. Two of the most commonly used names when referring to these growths are intumescence, as described above, and oedema. Oedema is most commonly found on geranium (*Pelargonium* spp.), while the term intumescence is more often associated with growths on sweet potato (*Ipomoea batatas*) and tomato (*Solanum* spp.).

In 1905, Dale suggested that these disorders were equivalent by stating that these growths were first called “intumescences”, but the term “oedemata” was later adopted by American authors. For decades, including today, many plant scientists have adopted (or accepted) that the two names were synonymous. However, Lang and Tibbitts (1983) suggested that the names oedema and intumescence refer to completely different disorders. According to these authors, oedema can best be defined as “a ‘watery swelling of plant organs or parts,’ resulting from water

congestion in plant tissue”. On the other hand, they suggested the disorder observed on tomatoes in their research should be termed intumescence, as the plants exhibited symptoms when relative humidity levels were low and water status within the tissue was normal (Lang and Tibbitts, 1983). Morrow and Tibbitts (1988) added to this differentiation by stating that “edema” have traditionally been found to develop under conditions where excess water and high humidity prevent sufficient transpiration by the plant.

Rangarajan and Tibbitts (1994) further described and suggested differences between oedema and intumescence. Based on the results obtained in their research involving the failure of far-red light to inhibit oedema injury on ivy geranium (*Pelargonium peltatum* (L.) L'Hér. ex Aiton), they suggested that the causative factors and physiological systems that regulate oedema on geranium and intumescence on solanaceous species were different. They supported this statement through their observation that UV radiation aids in the prevention of intumescence on solanaceous species (Morrow and Tibbitts 1988), but has little to no effect regarding the development of oedema on geraniums (Rangarajan and Tibbitts, 1994).

There are multiple instances in which microscopy methods have been utilized to assist in better defining and understanding development of these disorders. Light microscopy has been used to evaluate intumescences on tomato (*S. lycopersicum* L.; Lang et al., 1983), sweet potato (*Ipomoea batatas*; Wetzstein and Frett, 1984), and eucalyptus (*Eucalyptus nitens* Labill. and *E. globulus* (Deane and Maiden); Pinkard et al., 2006); and oedema on geranium (*Pelargonium* (L.) L'Hér. ex Aiton *×hortorum*; Balge et al., 1969; Metwally et al., 1970) and eggplant (*Solanum melongena* L.; Eisa and Dobrenz, 1971). Additionally, electron microscopy has been used to further analyze intumescences on sweet potato (Wetzstein and Frett, 1984) and tomato (Rud, 2009) leaves.

While lesions have been previously observed using microscopy on a single species, no extensive comparison of these lesions among different species has been conducted. To remove bias related to accurately characterizing this physiological disorder on each species, the term “lesion” will be used when referring to these abnormal growths. The objective of this study was to further characterize lesions and their development on three plant species: ornamental sweet potato (*Ipomoea batatas* ‘Blackie’), tomato (*Solanum lycopersicum* ‘Maxifort’), and interspecific geranium (*Pelargonium* (L.) L'Hér. ex Aiton *×* ‘Caliente Coral’). Specific goals were to 1) use digital imaging, including field emission scanning electron microscopy (FESEM) and light

microscopy, to evaluate stages of development for the disorders on each species; and 2) characterize and determine cellular layers involved in lesion development in order to evaluate differences and similarities in abnormal cellular growth among the species.

## **Materials and Methods**

### ***Plant Materials***

Rooted cuttings of ornamental sweet potato (*Ipomea batatas* 'Blackie'), were obtained from a commercial supplier (Four Star Greenhouses; Carlton, MI) and were potted on 20 Jan. 2014 in 11.4 cm diameter (465 ml volume) pots. Tomato (*Solanum lycopersicum* 'Maxifort') seeds were sown on 20 Nov. 2013 and were kept under mist until transplanting into 15.2 cm diameter (940 ml volume) pots on 9 Dec. 2013. Plants were transplanted a second time on 5 Feb. 2014 into 11 L volume pots. Interspecific geranium (*Pelargonium* × 'Caliente Coral') tip cuttings were taken from stock plants maintained in greenhouses at Kansas State University's Throckmorton Plant Sciences Center (Manhattan, KS) and stuck in Oasis<sup>®</sup> Wedge<sup>®</sup> Growing Medium (Smithers-Oasis North America; Kent, OH) on 17 Dec. 2013 and placed under mist; cuttings were transplanted on 2 Jan. 2014 into 12.7 cm diameter (625 ml volume) pots. A peat-based root medium (Fafard #2; Conrad Fafard Inc.; Agawam, MA) was used at each instance of transplant. Plants were grown at a greenhouse temperature set-point of 22° C day and 20° C night. Data loggers (Onset Computer Corporation; Bourne, MA) were placed in the greenhouse to monitor temperature and relative humidity. Plants were fertigated with a 200 mg·L<sup>-1</sup> N constant liquid feed using 20N-4.4P-16.6K (Peters Professional Peat-Lite Special; Everris NA Inc.; Dublin, OH) when greater than half of the plants reached ~ 45% container capacity due to water loss.

### ***Light Microscopy Measurements***

Lesions on each species were characterized by measuring the height, width and area of three lesions on six separate plants per species at the same stage of lesion development. On 20 Feb. 2014, six ornamental sweet potato plants displaying signs of lesion development were randomly sampled. A single leaf from each plant that was near full expansion but had not fully developed the typical purple pigmentation was selected. The lesions targeted for sampling were translucent protrusions on the adaxial surface that had not yet begun to senesce. On 21 Feb.

2014, six tomato plants displaying signs of lesion development were randomly sampled. A single leaf from each plant, five to seven nodes from the apical meristem, was selected and on that leaf, the terminal three leaflets were used for sampling. The lesions sampled were green protrusions or bumps on the abaxial surface that had not yet begun to senesce. On 26 Feb. 2014, six geranium plants displaying signs of lesion development were randomly sampled. From each plant, two to three leaves (excluding the youngest leaves approximately two nodes from the apical meristem) were selected for sampling. The sampled lesions were green protrusions or bumps on the abaxial surface that had not yet begun to senesce.

Leaf sections with developing lesions of about 1 cm diameter were excised for each species. These were further sectioned to 200  $\mu\text{m}$  thick cross-sections using a tissue chopper. Sections were carefully selected so that the optimal center of each lesion was achieved. Three sections of different representative lesions from each of the six plants for each species were mounted with deionized water and observed using a Nikon Eclipse E600 light microscope (Nikon Inc., Melville, NY). Lesion measurements were obtained using ImageJ (Image Processing and Analysis in Java) software. Lesion height ( $\mu\text{m}$ ) was measured from the apex of the lesion to the leaf lamina surface (Fig. 3.1A). Lesion width ( $\mu\text{m}$ ) was measured along the epidermis, with start and end measurement points where the lesion began to protrude (Fig. 3.1A). Area ( $\text{mm}^2$ ) of the lesion was measured above the leaf lamina surface (Fig. 3.1B). Homogeneity of variance for each response variable was evaluated using the Browne-Forsythe test ( $\alpha=0.05$ ). For the height response variable, a log transformation was necessary to achieve homogeneous variance and meet model assumptions. Additionally, for the area response variable, a reciprocal square root transformation was used. These data were analyzed using a general linear mixed model that fitted a completely randomized design. Data were analyzed using the GLIMMIX procedure of SAS ver. 9.2 (SAS Institute, Cary, NC). Pairwise comparisons were made using the transformed data for both height and area measurements. Estimated means are reported prior to transformation.

### ***Light Microscopy of Samples Stained with Toluidine Blue O***

The same sampling criteria described for the light microscopy measurements was used for Toluidine Blue O (TBO) staining. Leaf sections,  $\sim 0.5$  cm in diameter, were excised from plants of each species displaying lesion development. The leaf sections were fixed in a 10%



formalin solution for two hours, removed from the fixative and subjected to an ethanol series before being mounted in paraffin. Cross-sections (10  $\mu\text{m}$  thick) were obtained using a microtome, then mounted and stained with 0.5% TBO. Images were obtained using the same Nikon Eclipse E600 light microscope listed in the previous section.

### ***Digital Photography and FESEM***

Lesions on each species were photographed at three separate stages of development (Canon 7D; Canon U.S.A, Melville, NY). These stages included 1) initial lesion development and early expansion; 2) full expansion of the lesions and beginning stages of senescence; 3) complete senescence of the lesion and surrounding tissue. These stages of development were also imaged using FESEM. For FESEM imaging, tissue displaying the specific stage of each disorder was excised into ~0.5 cm sections in diameter and fixed using a 2% paraformaldehyde/2% glutaraldehyde solution in a 0.2 M phosphate buffered saline (PBS) solution, pH 7.2. The tissue was fixed for two hours and then transferred to a 0.2 M PBS solution, pH 7.2 until imaging was conducted. Tissue sections were then mounted on carbon tape and imaged using a Nova NanoSEM 430 (FEI Company; Hillsboro, OR) with an X-Max Large Area Analytical EDS silicon drift detector (Oxford Instruments; Abingdon, Oxfordshire). Water vapor was pumped into the system in an attempt to maintain tissue turgidity.

## **Results and Discussion**

### ***Ornamental Sweet Potato***

#### ***Morphology***

Lesions on ornamental sweet potato initiated predominately on the adaxial surface of leaves approaching full expansion, similar to results reported by Wetzstein and Frett (1984). In the earliest stages of development, lesions appeared as small green bumps, which formed along leaf veins as well as interveinally (Fig. 3.2A and B). Both individual and small groupings of lesions were observed. Lesion formation appeared to initiate around the stomata because guard cells displayed the first signs of cellular hypertrophy (Fig. 3.3A). After the initiation of guard cell hypertrophy, the surrounding epidermal cells also appeared to hypertrophy (Fig. 3.3B).

Wetzstein and Frett (1983) reported similar observations as they observed extensive hypertrophy that included the enlargement of stomata.

In the intermediate stages of development, the lesions became more elongated and translucent (Fig. 3.2C and D). The more translucent coloration observed was due to extensive cell hypertrophy. As the cells continued to expand, anthocyanin and chlorophyll pigments were not observed. Our observations are similar to the findings of Wallace (1928), who described the fate of chloroplasts in hypertrophied cells of *Pyrus malus* var. *transparent*, reporting that the chlorophyll granules gradually lost their green color, eventually disappearing completely. Moreover, La Rue (1932) reported that hypertrophic poplar (*Populus tremuloides* Michx. and *P. grandidentata* Michx.) leaf cells did not display a change in the number of chloroplasts contained within the cell; rather, the chloroplasts were spaced more widely apart and the cells appeared translucent rather than green.

Lesion senescence was observed in the latter stages of development (Fig. 3.2E). These stages occurred mostly on mature foliage, as the leaf continued to age after the initial onset of lesion development. The senescence began as a blackening of the lesion apex, and ultimately led to the senescence of the entire lesion (Fig. 3.2F). These senesced lesions remained on the leaf surface or abscised over time.

### ***Anatomy***

Ornamental sweet potato leaves had a single-layered upper and lower epidermis, a single-layered palisade parenchyma, and multiple layers of loosely packed spongy parenchyma (Fig. 3.4A). Lesions were mostly groups of hypertrophic cells that elongated above the leaf lamina surface (Fig. 3.4B). Lesions on ornamental sweet potato displayed a much greater height than width (Table 3.1). These data support the tendency of these cells to elongate vertically as hypertrophy continued. The extensive amount of elongation present in these cells led to a high overall area (Table 3.1) of lesion growth above the leaf lamina.

Based on the light microscopy images with TBO staining, the cell layer that was most directly involved in lesion development was the palisade parenchyma (Fig. 3.4B). Hypertrophy of these cells resulted in the significant elongation of the lesion, which was also apparent in the height measurements observed. Additionally, it appeared that these hypertrophic palisade parenchyma cells pushed the upper epidermis to the side as continued elongation occurred (Fig. 3.4B). In contrast, however, findings from the FESEM images suggested the participation of the

upper epidermis in the onset of these hypertrophic lesions due to the incorporation of stomata and the surrounding epidermal cells in lesion expansion (Fig. 3.3C). The involvement of the upper epidermis is further suggested by the occurrence of trichomes near the lesion apex (Fig. 3.3D). However, due to the significant desiccation of the tissue while observing latter stages of lesion development under FESEM, the potential for artifacts, such as the trichome incorporation, is plausible. Thus, it is proposed that while epidermal cells may be incorporated in the initiation and early stages of lesion development, these cells are soon pushed aside to make way for continued palisade parenchyma hypertrophy. This idea is in contrast to the findings of Wetzstein and Frett (1983), who state that intumescences developing on sweet potato leaves displayed epidermal continuity and involved both the epidermis and palisade parenchyma. While some of the FESEM images of early stages of lesion development observed in this study support their statement (Fig. 3.3B and C), the cross-sections stained with TBO suggest that lesion development was predominately due to palisade parenchyma hypertrophy (Fig. 3.4B).

While hypertrophy was the most evident cellular response, hyperplasia may have also occurred. The palisade parenchyma cells underwent hypertrophic elongation and expansion predominately, but hyperplasia was also evidenced due to what appeared to be an increase in the number of cellular layers within the palisade parenchyma (Fig. 3.4B). Wetzstein and Frett (1983) reported that intumescences on sweet potato formed due to the occurrence of both hypertrophy and hyperplasia. On the other hand, Trotter (1904) indicated that intumescence development on sweet potato was solely due to cellular hypertrophy. In our study, we observed that while hyperplasia may have been present, the most evident response was the extensive hypertrophy of these cells.

## *Tomato*

### *Morphology*

Lesion development on tomato occurred predominately on the abaxial surface of the leaf, typically beginning five to seven leaves below the apical meristem. The early stages of development appeared as large white-green bumps (Fig. 3.5A and B). Lesions initiated as groups of single hypertrophic cells scattered across the abaxial surface (Fig. 3.6A), which ultimately spread horizontally to encompass many of the surrounding cells to form a mounded shape.

Lesion development was not concentrated in a specific area of the leaf, such as near the veins, but occurred sporadically.

The intermediate stages of lesion development exhibited senescence as the surface of the lesions turned brown and began to collapse (Fig. 3.5C and D). Large senescent areas on the leaf surface where the lesions once formed characterized the latter stages of lesion development (Fig. 3.5E). While the lesions initially developed in a solitary fashion, upon collapse the affected areas coalesced to form large senesced regions (Fig. 3.5F). This cellular collapse (Fig. 3.6B) occurred through all leaf tissue layers, causing these senescent regions to be observed on both the abaxial and adaxial surface of the leaf (Fig. 3.7). This finding shows that the extent of the damage is not solely restricted to the cell layers immediately affected by the abnormal cell growth. In severe occurrences of lesion development, the entire leaflet would begin to senesce upon lesion collapse (Fig. 3.7). Rud (2009) stated that the cells involved with these lesions would ultimately rupture due to extensive hypertrophy. Regardless of whether rupture occurred, the end result was always cellular collapse.

### ***Anatomy***

Tomato leaf tissue had a single-layered upper and lower epidermis, a single-layered palisade parenchyma, and a loosely arranged spongy parenchyma (Fig. 3.8A). These anatomical observations regarding tomato leaf tissue are similar to those of Lang et al. (1983). However, our study differed in the location of lesion development. Lang et al. (1983) reported that lesions formed primarily on the adaxial surface of the leaf, while we found lesions predominately on the abaxial surface.

The lesions appeared to expand both horizontally and vertically (Fig. 3.8B). Both the spongy parenchyma and lower epidermis were involved in lesion development, with the potential for rupture of hypertrophied epidermal cells also apparent due to cells swelling many times greater than their normal size (Fig. 3.8B). These findings are similar to those of Lang et al. (1983) in which they observed hypertrophy of the palisade parenchyma and upper epidermis that led to the occasional rupture of epidermal cells. As such, extensive hypertrophy was the most evident response, with hyperplasia in the spongy parenchyma also proposed due to an increase in spongy parenchyma cell layers (Fig. 3.8B). Additionally, lesions on tomato were greater in width than height (Table 3.1). This may be due to these cells undergoing more horizontal expansion than vertical. The horizontal expansion, resulting in greater lesion width, is logical because of the

loosely packed spongy parenchyma layer, which would have allowed for more expansion within this tissue before cells were forced above the leaf lamina surface. It is likely that this expansion occurring within the confines of the mesophyll led to the lower area of lesion growth above the epidermis when compared to lesions on ornamental sweet potato (Table 3.1). While our study suggests that hyperplasia may play a role in lesion development on tomato, this finding was not observed by Lang et al. (1983).

## *Geranium*

### *Morphology*

Lesion development on geranium occurred solely on the abaxial leaf surface. For the early stages of development, small green bumps formed sporadically on the leaf surface (Fig. 3.9A and B). Leaves that were nearing full expansion or mature were most susceptible to this disorder. Younger, underdeveloped leaves showed no signs of lesion development, which is in accordance with previously reported literature (Balge et al., 1969; Metwally et al., 1970). Similar to lesion development on ornamental sweet potato, lesions on geranium often formed along veins and near the petiole, although development was not limited to these areas of the leaf. The lesions formed as both solitary growths and groupings, and were noticeably smooth and rounded (Fig. 3.9B). This smoothness was likely due to the epidermal layer not specifically being affected by cellular hypertrophy. Rather, pressure appears to have been placed on the lower epidermis from the expansion of underlying cells, which ultimately caused a reduction in the definition between epidermal cells (Fig. 3.10A). Thus, it appears that the epidermis was stretched due to the expansion of the mesophyll underneath.

In the intermediate stages of development, the lesions appear to have senesced (Fig. 3.9C), as a brown coloration formed at the lesion apex (Fig. 3.9D). We propose this browning was due to cellular senescence, initiated from the tearing of the lower epidermis due to mesophyll hypertrophy (Fig. 3.10B and C). Upon significant expansion of the underlying cell layer, the epidermis would have been torn from the increasing pressure. Continued browning and senescence on the lesion surface were observed in the latter stages of development (Fig. 3.9E and F), similar to the lesion senescence observed on tomato. However, in contrast to latter stages of tomato development, the senesced lesions did not always appear to collapse. When lesion collapse was present (Fig. 3.9F), the adaxial surface of the leaf was occasionally affected in a

response similar to that seen on tomato. Under these circumstances, a small senescent circle directly underneath the collapsed lesion would appear on the adaxial surface (Fig. 3.11). Regardless, lesions did not spread to the surrounding tissues on the abaxial leaf surface, as observed in tomato. Upon closer examination, we propose that these areas affected by lesion development may develop layers of suberized cells as a wound response to the tearing of the lower epidermis. This wound response will be discussed further in the following section.

### ***Anatomy***

Geranium leaf tissue was comprised of a single-layered upper and lower epidermis, a single-layered palisade parenchyma, and multiple layers of spongy parenchyma (Fig. 3.12A). The lesions developed solely on the abaxial surface and involved both the hypertrophy and hyperplasia of spongy parenchyma cells (Fig. 3.12B). The lower epidermal cells were not involved in the development of these lesions. Rather, the lower epidermis was subjected to tension as the spongy parenchyma cells beneath expanded and multiplied. This ultimately resulted in the tearing of the lower epidermis (Fig. 3.12B). This evidence was further supported as lesions on geranium showed a greater width than height (Table 3.1). This may have been due to increased periclinal division in the spongy parenchyma. Balge et al. (1969) reported similar observations, as they found that spongy parenchyma cells would undergo periclinal division. This further supports the finding in our study that hyperplasia was apparent within this cell layer. Additionally, until this epidermal layer was torn, the cells could only expand outward as far as the lower epidermis would allow. Thus, as the cells underwent hypertrophy and hyperplasia, the lesion was forced to grow horizontally. This restriction of lesion growth by the epidermis was also the reason for a lower total area affected when compared to lesion development on ornamental sweet potato (Table 3.1).

Our observations of spongy parenchyma cells undergoing hypertrophy are in agreement with Metwally et al. (1970) and Balge et al. (1969); however, in their studies with lesion development on geranium, they reported hypertrophy of epidermal cells as well. Metwally et al. (1970) concluded that the swelling of both parenchyma and epidermal cells resulted in pressure on the guard cells which caused them to close, and ultimately resulted in stomatal collapse. Their findings are in contrast to ours, as epidermal cells did not undergo hypertrophy. Rather, we observed that the lower epidermis might have been subjected to tearing due to excessive pressure from the hypertrophic spongy parenchyma cells. These results are similar to the findings of La

Rue (1963). While observing lesion development on poplar leaves, he found that the mesophyll cells closest to the epidermis underwent the most expansion, while the epidermis was then forced to stretch as these cells swelled outward. This outward growth was due to very limited space for lateral expansion among the palisade and spongy parenchyma layers. Additionally, Schrenk (1905) described a similar phenomenon in cauliflower (*Brassica oleracea* L.) leaves where mesophyll cells, both spongy and palisade parenchyma, would enlarge until eventually breaking through the epidermis.

Balge et al. (1969) further stated that the senesced regions in the late stages of lesion development were spongy parenchyma cells that had re-differentiated into a cork cambium layer. As these cork cambium cells elongated, the authors proposed that a raised periderm was formed. While leaves usually do not produce periderm (Fahn, 1982; Esau, 1977), they have been found to produce cork cells as a result of wounding, where living plant tissue is exposed to the ambient air (Fahn, 1982). Under these conditions, the dead plant tissue will become separated from the living by a layer of suberized cells. Thus, phellogen may develop and give rise to both phellum and a phelloderm. The layer of cork that is formed would then allow for protection against pathogens as well as prevent water loss through the newly developed wound (Fahn, 1982). However, for the lesion development on geranium observed in the present study, this explanation does not fully characterize the phenomenon. The hypertrophy of spongy parenchyma cells appeared to occur prior to the exposure of the interior tissues to the ambient air. Thus, while the development of suberized cells may act as a wound response to epidermal tearing, it does not explain why the spongy parenchyma cells would initially undergo hypertrophy and hyperplasia to cause this tearing. Additionally, even though a protective layer of suberized cells may have been formed, these lesions on geranium would still often collapse (Fig. 3.9F). We propose that this observation may have been due to the extensive hypertrophy of the spongy parenchyma cells, which, regardless of the protective layer of cells formed above, ultimately collapsed.

The wound response proposition in geranium is further supported when observing western flower thrips (*Frankliniella occidentalis* Pergande) feeding damage on the abaxial surface of the leaf. Damage by this pest on ivy geranium has been described as pale yellow to dark brown spots on the abaxial leaf surface (Chen and Williams, 2006). Thus, thrips feeding damage may appear very similar to the late stages of lesion development on geranium (Fig. 3.13). Thrips will feed on both epidermal and mesophyll cells by damaging the cell tissue and

then imbibing the cellular fluids (Cloyd, 2010). In the case of both thrips feeding damage and lesion development on geranium, the epidermal layer is compromised. Thus, while the mechanism of tissue wounding may differ, the wound response by geranium in response to this epidermal damage may be identical. As a result, the proposed idea of a wound response would assist in explaining the similarity between thrips feeding damage and the development of lesions on geranium leaves. Additionally, lesions on geranium leaves may act to attract thrips due to the swollen spongy parenchyma and taut or torn epidermis.

Pinkard et al. (2006) suggested a similar wound response theory as they observed intumescence development on the leaves of *Eucalyptus nitens* (Deane and Maiden) and *E. globulus* Labill. seedlings. The authors proposed that these intumescences were actually environmentally induced lenticel-like structures (ERLS). They believed that these ERLS formed on leaves under the conditions of high relative humidity as a means to facilitate increased aeration of the interior tissues. Lenticels will often develop underneath stomata (Esau, 1977; Fahn, 1982). The underlying mesophyll cells beneath these stomata undergo a series of divisions until a phellogen is formed. The phellogen will then push the overlying cells outward until the epidermis ruptures. We propose that a response similar to the function of lenticel development may have been observed in the lesion development on geranium in the present study. Lesion development on geranium commonly occurred underneath stomata (Fig. 3.10A) until the eventual rupture of the epidermis (Fig. 3.10B). Thus, upon tearing, it is plausible that the exposed epidermal cells would senesce while a layer of suberized cells was re-differentiated below (Fahn, 1982) (Fig. 3.14). While lesions on geranium are not anatomically similar to lenticels, it is possible that this physiological response may serve a similar function in facilitating increased gas exchange to the underlying tissues.

### ***Light Microscopy Measurement Comparisons***

Lesion development on ornamental sweet potato was nearly three times greater in estimated mean height compared to those on geranium and tomato (Table 3.1). Also, tomato lesions were marginally greater in height than those on geranium ( $P=0.052$ ). The difference in cellular expansion between species was further observed by the average width measurements, as geranium and tomato lesions were nearly two and three times wider, respectively, than ornamental sweet potato lesions (Table 3.1). We propose that the greater average height for the



growths on ornamental sweet potato was due to the cells involved predominately being the palisade parenchyma. With this cell layer being more tightly packed together than the spongy parenchyma, the hypertrophic cells tended to elongate upwards due to this constraint. On the contrary, the naturally occurring air spaces within the spongy parenchyma of tomato leaves allowed for greater horizontal cell expansion and possibly division, resulting in the reduced height and more horizontal lesion development. Additionally, the greater lesion width on geranium might be explained either by an increase in periclinal divisions of the spongy parenchyma; or by the restriction of outward growth from the lower epidermis, causing cellular expansion to spread horizontally until the epidermis was torn.

The average lesion area for ornamental sweet potato was more than double those on both geranium and tomato (Table 3.1). This was also due to the tendency of lesions on ornamental sweet potato to display greater vertical elongation of hypertrophic cells, resulting in a greater lesion area. Intuitively, with much of the cellular growth occurring horizontally within the mesophyll in geranium and tomato, the total lesion area above the epidermis for those two species would be significantly lower.

## Conclusions

An understanding of the causative factors related to lesion development on each species further assists in providing a comprehensive characterization and comparison. Light quality, specifically ultra-violet B (UVB) radiation, was found to be directly related to preventing or reducing lesion development on ornamental sweet potato (Craver et al., *in preparation*) and tomato (Lang and Tibbitts, 1983; Morrow and Tibbitts, 1987; Morrow and Tibbitts, 1988; Rud, 2009). On the contrary, UVB was found to have no effect regarding lesion development on geranium (Rangarajan and Tibbitts, 1994). Rather, lesion development on geranium is thought to be affected by water relations; specifically, that high humidity, warm soils, and poor ventilation are conducive to development (Balge et al., 1969; Lang and Tibbitts, 1983; Metwally et al., 1970; Rangarajan and Tibbitts, 1994). This finding led Rangarajan and Tibbitts (1994) to propose that “oedema injury” on geranium may have different causative factors and be regulated by different physiological systems than on solanaceous species.

With lesions on tomato and ornamental sweet potato, the epidermis was often subjected to the same hypertrophy apparent in the underlying cells. However, in geranium the epidermis

resisted the expansion of the underlying cells, resulting in the eventual tearing of the tissue layer. Because lesions on geranium are thought to be caused by water congestion and high humidity, a plant response that results in a function similar to that provided by lenticels, as proposed by Pinkard et al. (2006), is an appropriate hypothesis. As leaves were unable to transpire at rates equivalent to the amount of water uptake, the cells underneath the stomata may have undergone a response to facilitate increased aeration. Thus, swelling and division of spongy parenchyma cells would have continued until the eventual tearing of the lower epidermis. This response involving facilitated gas exchange does not seem to be related to lesion development on tomato, as epidermal cells underwent significant hypertrophy, and this hypertrophy did not seem to instigate around stomata. Additionally, lesion development on tomato resulted in the ultimate senescence of leaf tissue, which does not support the idea of a wound response or response to facilitate gas exchange for this species. However, lesions on ornamental sweet potato appeared to initiate around the stomata as guard cells underwent hypertrophy. The occurrence of the lesions around the stomata is similar to the development on geranium, and may point toward the involvement of air quality, hormones, or solutes in the initiation of lesion development. While tearing of the epidermis was not apparent in lesions on ornamental sweet potato, it is probable that the epidermis was compromised around the stomata as the underlying palisade parenchyma elongated outward. This also may account for the multiple observations of stomata at the apex of lesion development on ornamental sweet potato. However, the development of lenticel-like structures on ornamental sweet potato seems unlikely as palisade parenchyma cells underwent significant hypertrophy above the epidermis soon after the initiation of lesion development, with no apparent signs of cellular suberization.

UVB has been found to reduce stomatal density and opening on rice (*Oryza sativa* L.; Dai et al., 1995), which would seemingly result in lower levels of transpiration and increased hypertrophic lesions if water congestion were the causative factor for lesion development on tomato and ornamental sweet potato. Rather, UVB radiation has been found to be a preventative measure for these two species. Thus, it appears that lesion development on these species is not directly related to the need for increased gas exchange, but may involve a different physiologically mechanism from geranium lesion development altogether.

In conclusion, these results point toward a differentiation in lesion nomenclature. Specifically, that lesion development on geranium would be referred to as “oedema”, while

lesion development on tomato and ornamental sweet potato would be referred to as “intumescence”. Lesion development on geranium has previously been found to be closely related to water status within the plant and appeared to result in a wound response or provide a means of facilitated gas exchange. On the contrary, development of lesions on ornamental sweet potato and tomato were found to involve light quality. Lesions on these two species resulted in cellular abnormalities, which often included the epidermis, that ultimately caused cell and tissue senescence. Additionally, it is important to consider that plants possess a limited means by which to respond to stress. In other words, while the occurrence of hypertrophy was apparent in lesion development on all three species, the cause and mechanism by which these abnormal growths occur on each species may differ. Thus, it is important to consider the proposed causative factors alongside morphological and anatomical aspects of lesion development to most accurately determine the appropriate terminology for these physiological disorders.

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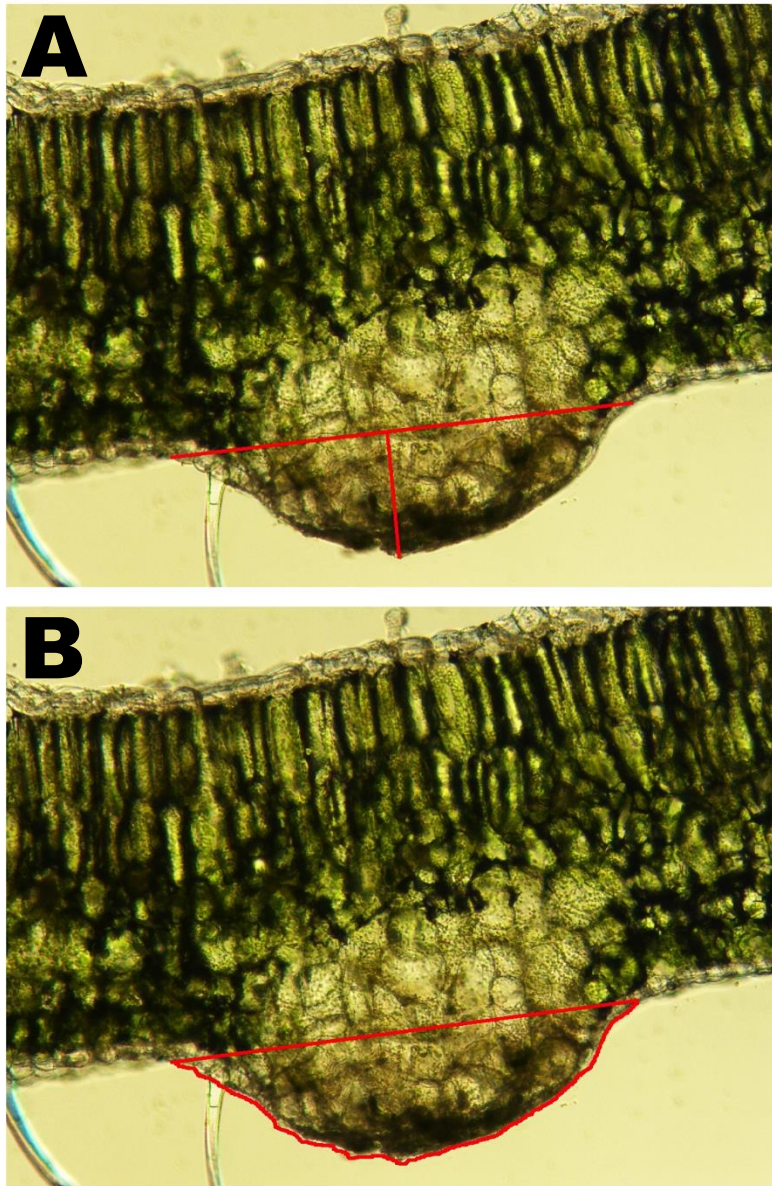
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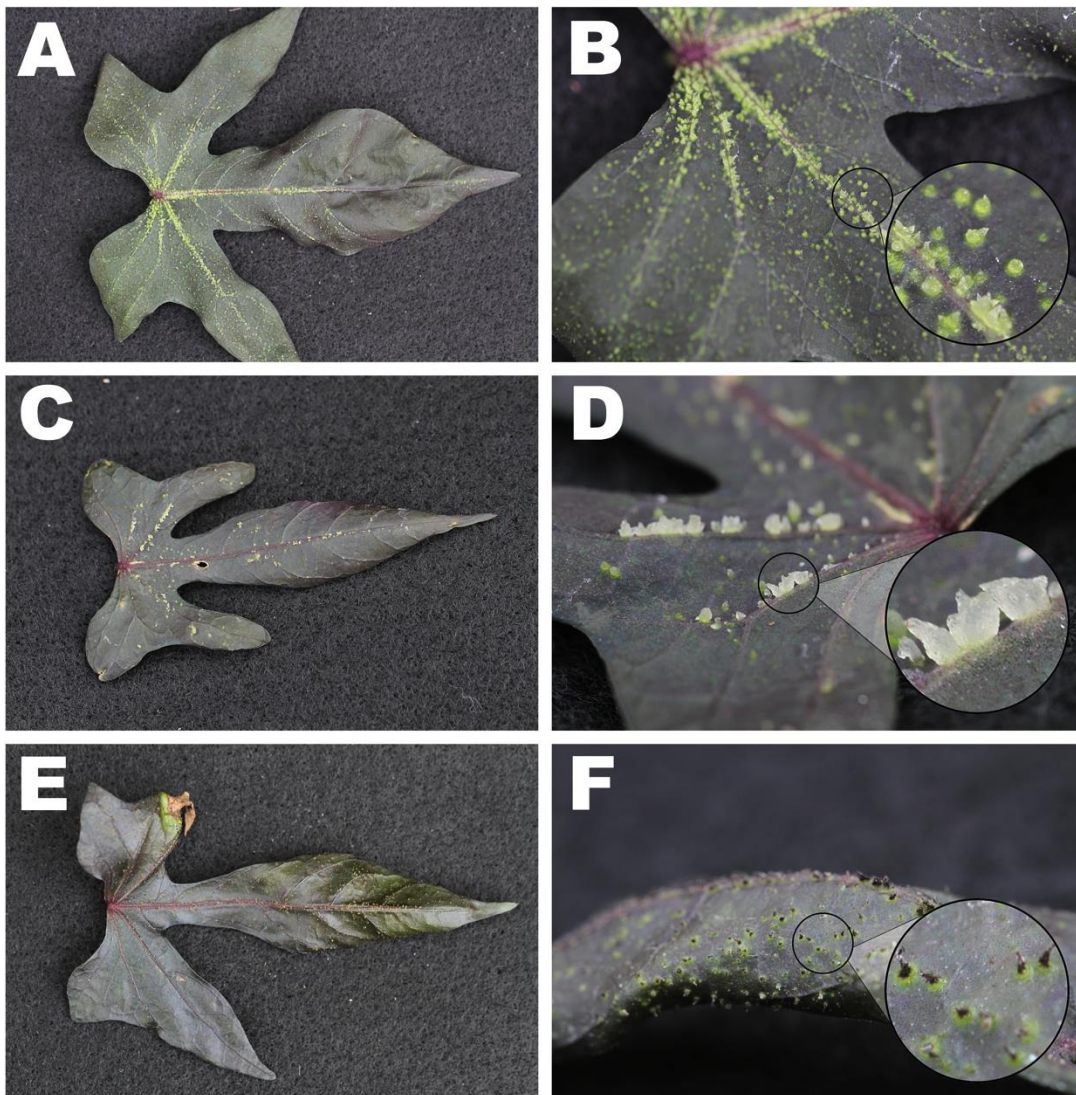
## Figures and Tables

**Figure 3.1** Method of measuring lesion height, width, and area on geranium (*Pelargonium* ×‘Caliente Coral’) using ImageJ Processing and Analysis in Java. A) Height ( $\mu\text{m}$ ) was measured from the apex of the lesion to the leaf lamina surface, and width ( $\mu\text{m}$ ) along the epidermis. B) Area ( $\text{mm}^2$ ) of the lesion was measured above the leaf lamina surface.

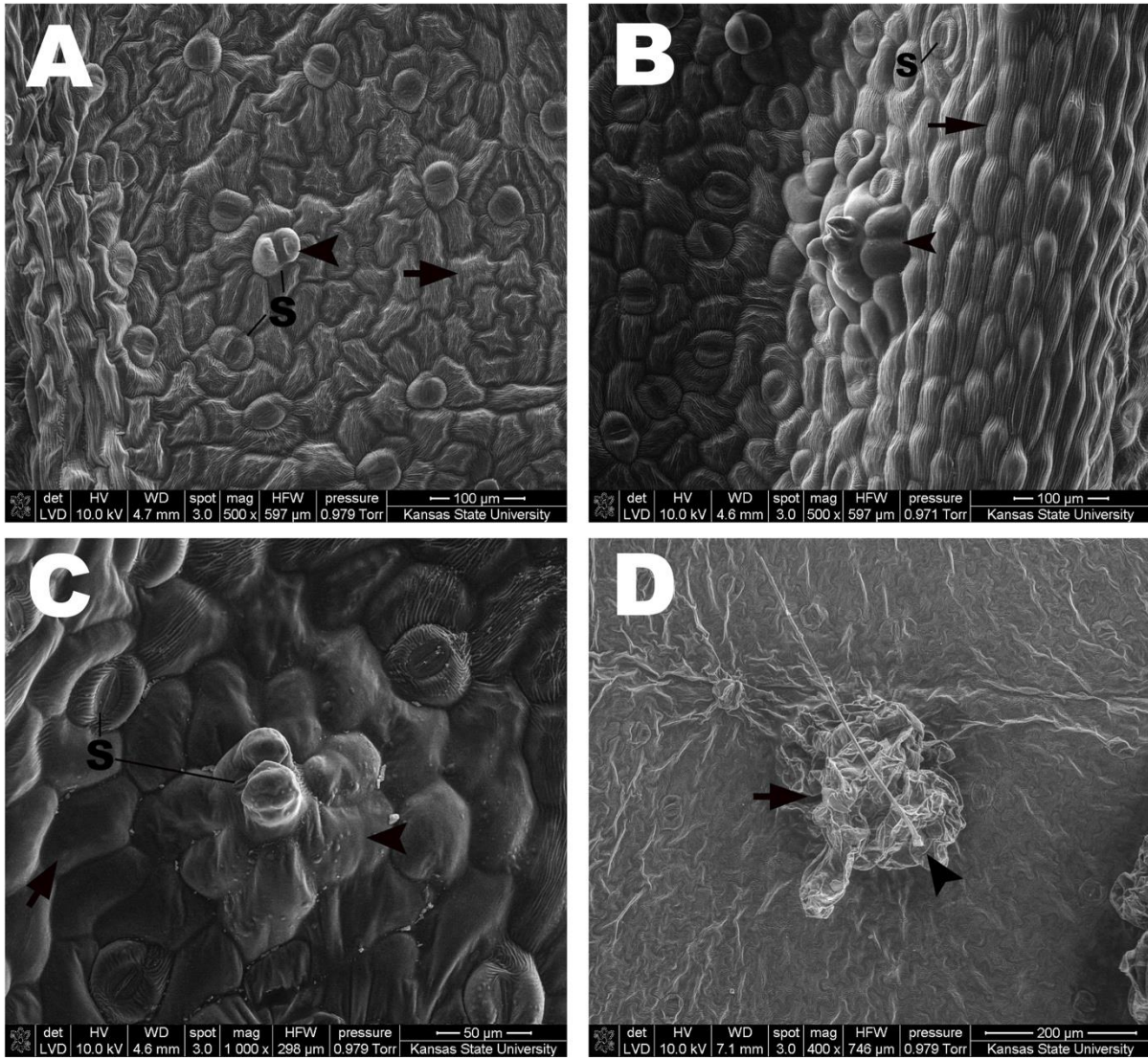




**Figure 3.2** Digital images of three stages of lesion development on the adaxial leaf surface of ornamental sweet potato (*Ipomoea batatas* ‘Blackie’). **A)** Leaf displaying the early stages of lesion development as small green bumps began to form along the leaf veins as well as interveinally. **B)** Leaf area close-up characterizing the early stages of lesion development as rounded green lesions formed and began to elongate. **C)** Leaf displaying further elongated and increasingly translucent lesions which characterize the intermediate stages of development. **D)** Leaf area close-up characterizing the significant hypertrophy of cells which gave rise to the increasingly translucent appearance of the lesions. **E)** Leaf displaying the latter stages of lesion development, typical on more mature leaves, with the senescence of lesions apparent. **F)** Leaf area close-up characterizing the senescence and blackening of the lesion apex.

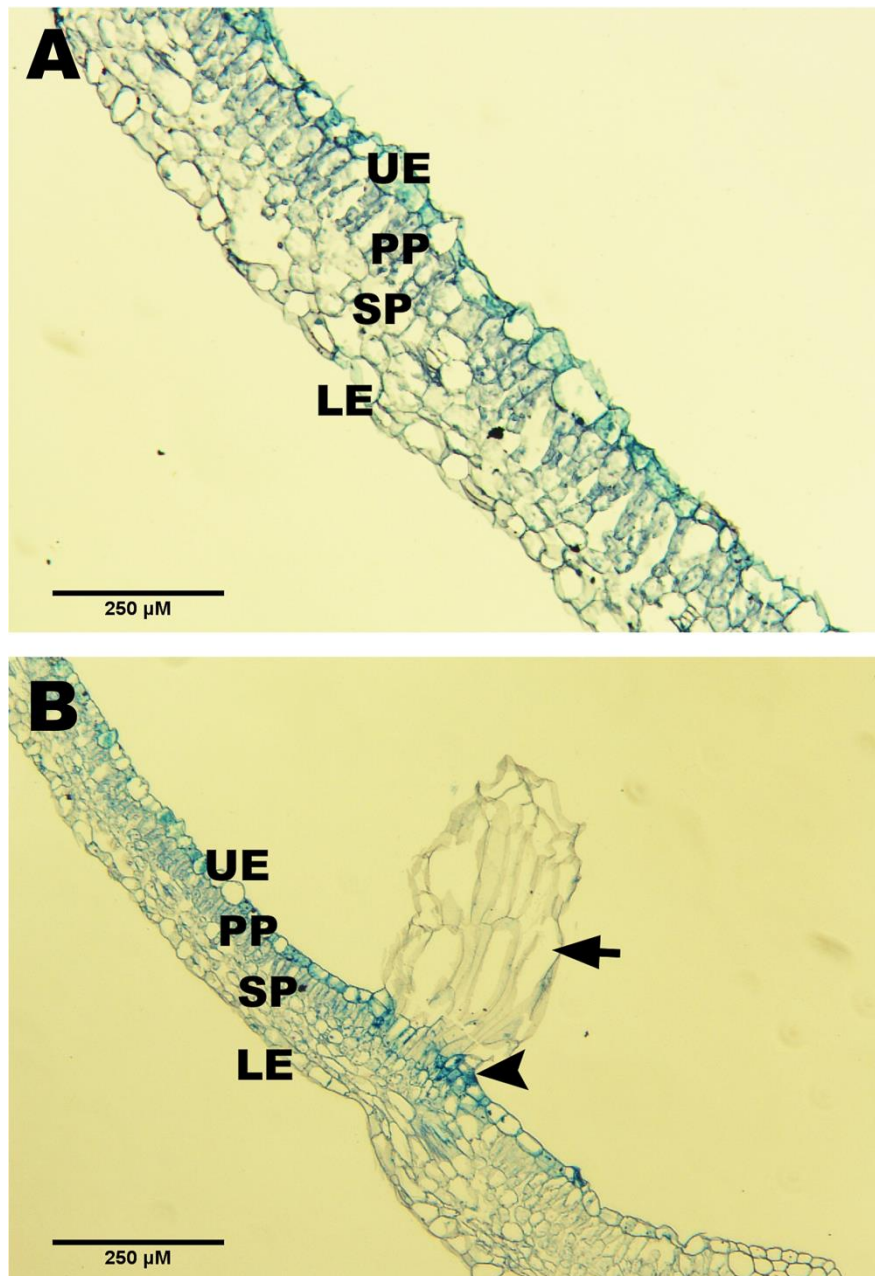


**Figure 3.3** Field emission scanning electron microscopy (FESEM) of the adaxial leaf surface of ornamental sweet potato (*Ipomoea batatas* ‘Blackie’). **A)** Hypertrophy of guard cells (point) occurred during the initial stages of lesion development; hypertrophy did not appear to affect normal epidermal cells (arrow) during these initial stages. **B)** Hypertrophy of multiple cells (point) during the early stages of lesion development that occurred on the side of a leaf vein (arrow). **C)** Lesion elongation involved the surrounding epidermal cells (point) with stomata often located at the lesion apex. Normal cells (arrow) around the lesion are unaffected by hypertrophy. **D)** A lesion in the intermediate stages of development (arrow) with a trichome (point) near the lesion apex. The potential for artifacts was possible due to the significant desiccation of the tissue in vacuum. Abbreviations: stoma (S).





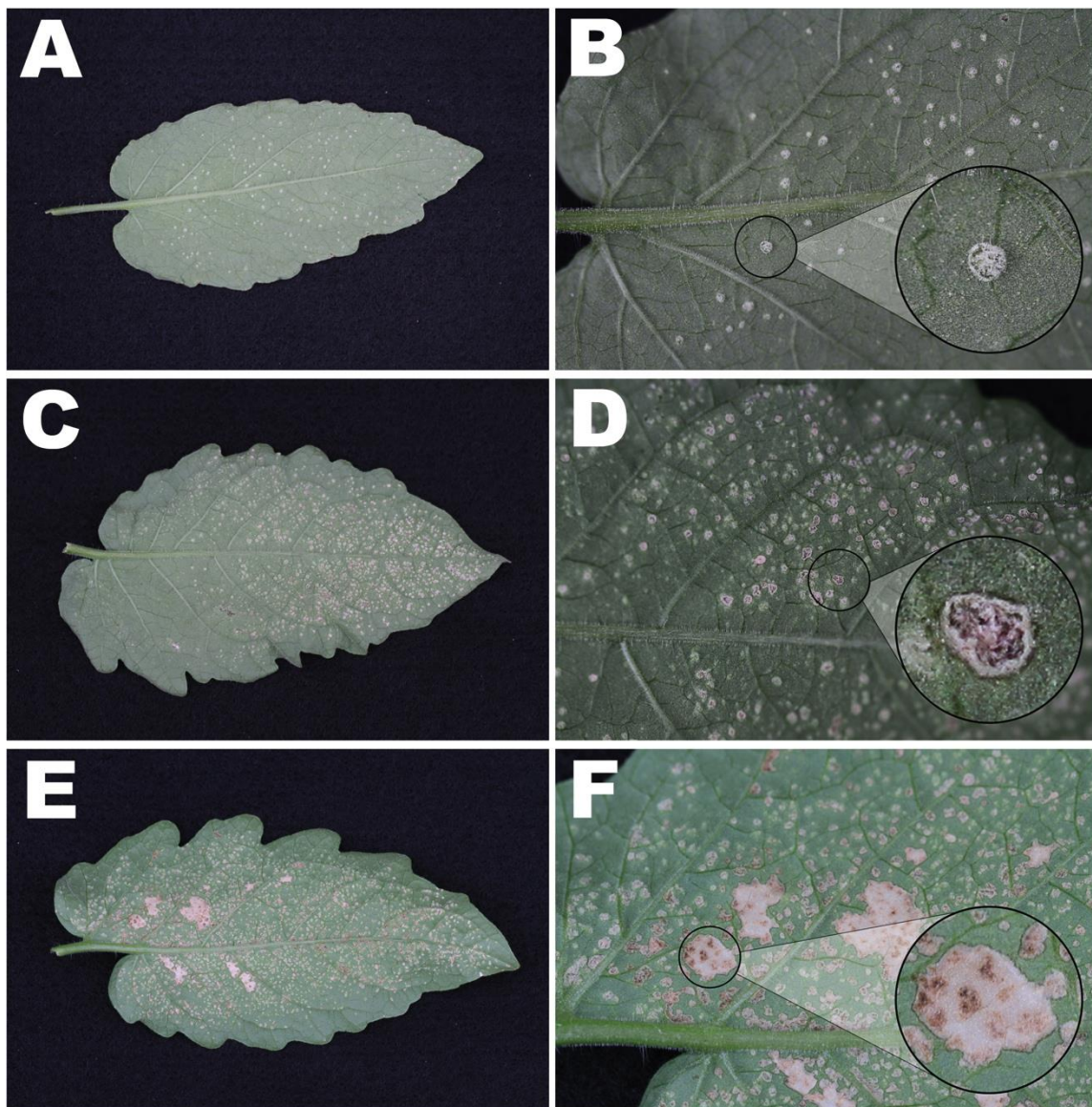
**Figure 3.4** Light microscopy cross-sections stained with Toluidine Blue O of lesions on the adaxial leaf surface of ornamental sweet potato (*Ipomoea batatas* 'Blackie'). **A)** Asymptomatic leaf; tissue with no lesion development. **B)** Lesion displaying significant hypertrophy of palisade parenchyma cells above the leaf lamina surface (arrow). Epidermal cells (point) are pushed aside as palisade parenchyma cells expand. Abbreviations: lower epidermis (LE); palisade parenchyma (PP); spongy parenchyma (SP); and upper epidermis (UE).



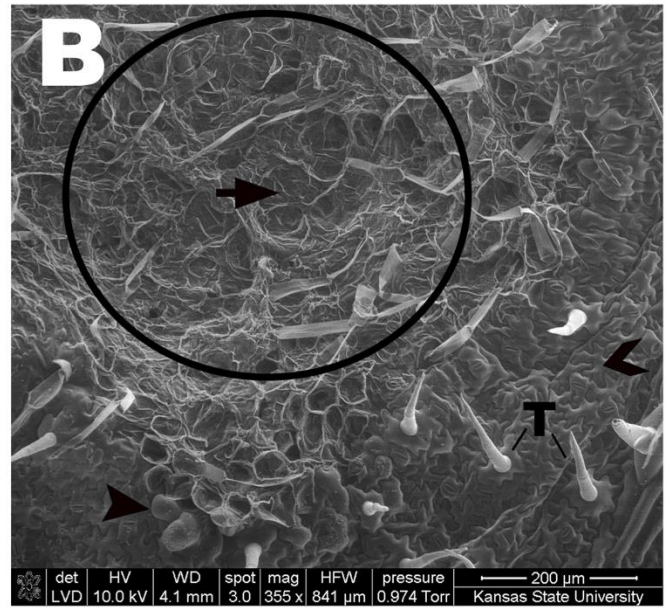
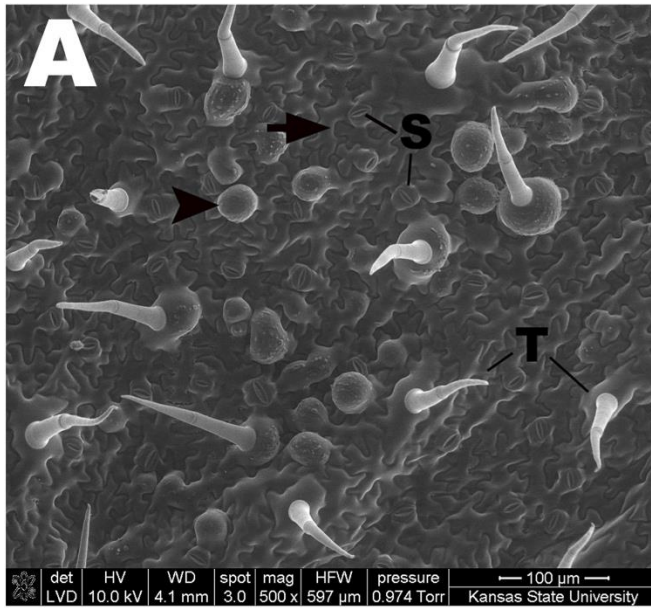
**Table 3.1 Estimated mean lesion height, width and area on interspecific geranium (*Pelargonium* ×‘Caliente Coral’), ornamental sweet potato (*Ipomoea batatas* ‘Blackie’), and tomato (*Solanum lycopersicum* ‘Maxifort’) taken on cross-sections (n=6). Estimated means prior to transformation are reported for height and area. Estimated means with a common letter within each column are not significantly different based on multiple comparisons at  $\alpha=0.05$ .**

<b>Species</b>	<b>Height (<math>\mu\text{m}</math>)</b>	<b>Width (<math>\mu\text{m}</math>)</b>	<b>Area (<math>\text{mm}^2</math>)</b>
<b>Geranium</b>	186.6b	807.5b	0.10b
<b>Sweet Potato</b>	613.2a	375.6c	0.23a
<b>Tomato</b>	155.9b	1140.6a	0.12b

**Figure 3.5** Digital images of three stages of lesion development on the abaxial leaf surface of tomato (*Solanum lycopersicum* ‘Maxifort’). **A)** Leaf displaying the early stages of lesion development as rounded bumps form sporadically on the surface. **B)** Leaf area close-up characterizing solitary lesions that appeared as white-green bumps. **C)** Leaf displaying the intermediate stages of development as lesions began to senesce and collapse. **D)** Leaf area close-up characterizing lesions that became brown due to senescence and began to collapse. **E)** Leaf displaying the latter stages of lesion development as large senescent regions began to appear where lesions previously collapsed. **F)** Leaf area close-up characterizing a large senesced region where multiple lesions previously collapsed.



**Figure 3.6** Field emission scanning electron microscopy (FESEM) of the abaxial leaf surface of tomato (*Solanum lycopersicum* ‘Maxifort’). **A)** The initiation of lesion development occurred as cells sporadically underwent hypertrophy (point) on the leaf surface. Surrounding cells were often unaffected at this stage (arrow). **B)** A large collapsed lesion (circle) that appears to be multiple cell layers deep (arrow). Hypertrophic cells (point) were still apparent around the lesion perimeter. The collapsed lesion did not result in the senescence of surrounding cells at this stage (caret). Abbreviations: stoma (S); and trichome (T).

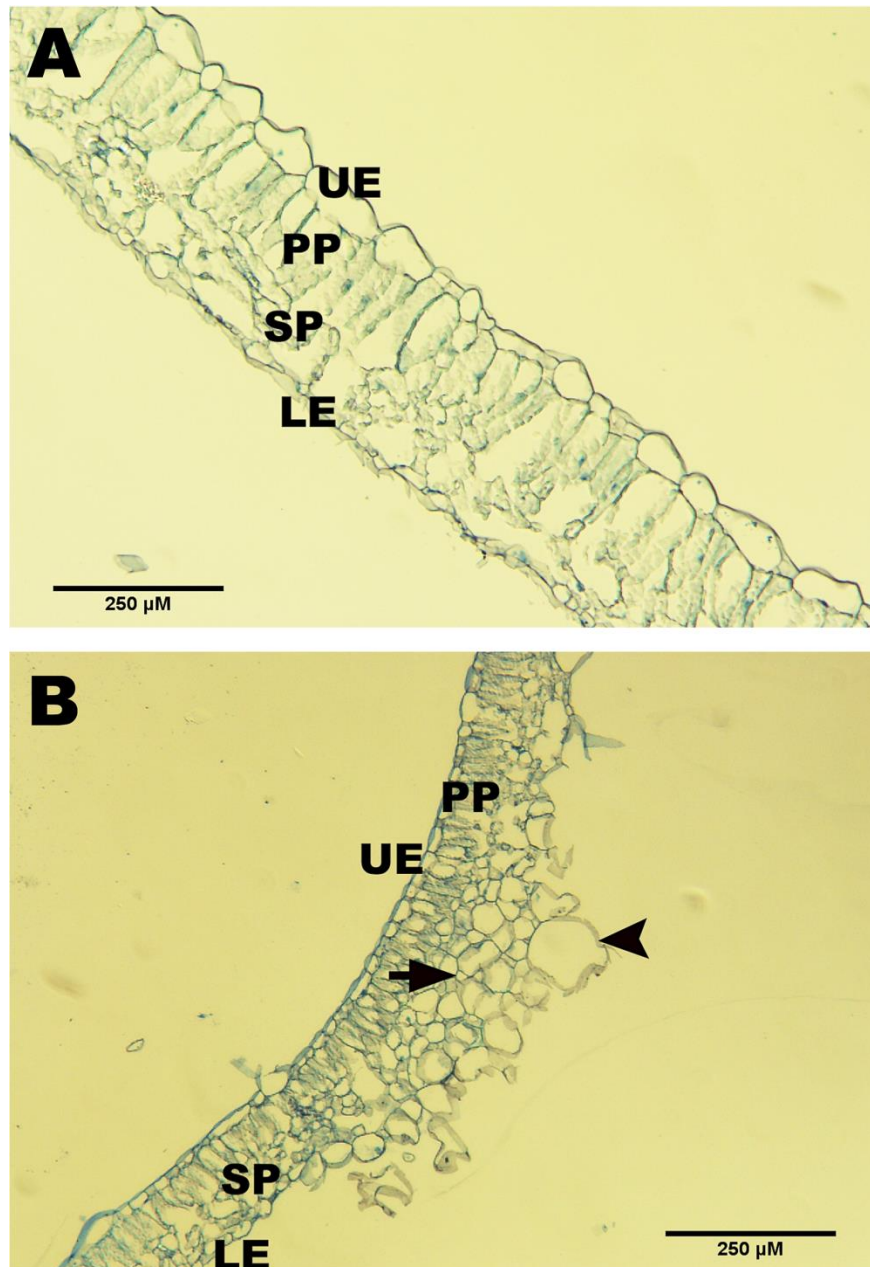




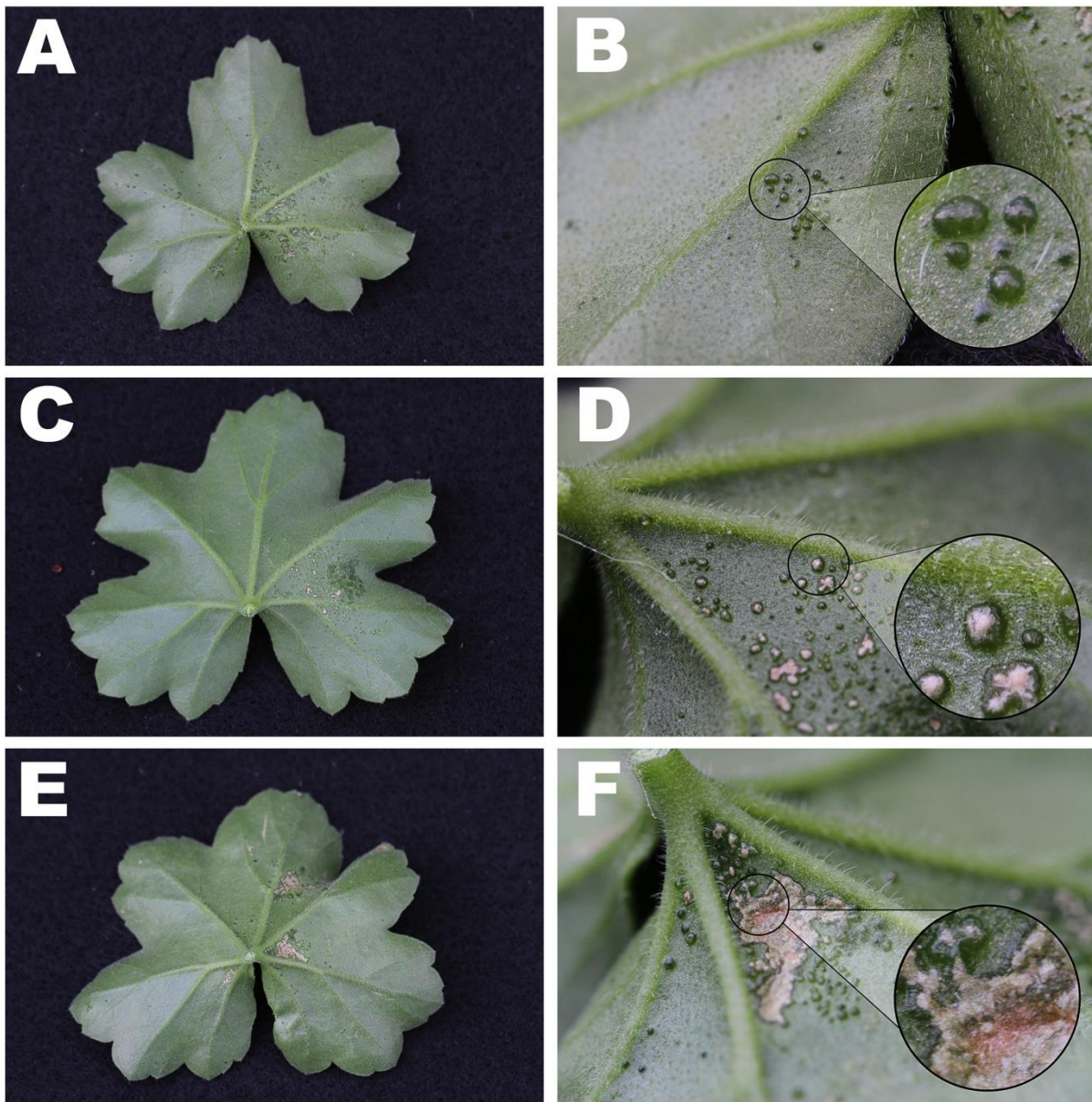
**Figure 3.7** Tomato (*Solanum lycopersicum* ‘Maxifort’) leaflet undergoing senescence and epinasty due to the collapse of lesions on the abaxial surface. Lesion collapse resulted in senescent regions on the adaxial surface as well.



**Figure 3.8** Light microscopy cross-sections stained with Toluidine Blue O of lesions on the abaxial leaf surface of tomato (*Solanum lycopersicum* 'Maxifort'). **A)** Asymptomatic leaf; tissue with no lesion development. **B)** Lesion displaying lower epidermis and spongy parenchyma cells undergoing significant hypertrophy (point) and hyperplasia (arrow) both horizontally and vertically. Abbreviations: lower epidermis (LE); palisade parenchyma (PP); spongy parenchyma (SP); and upper epidermis (UE).

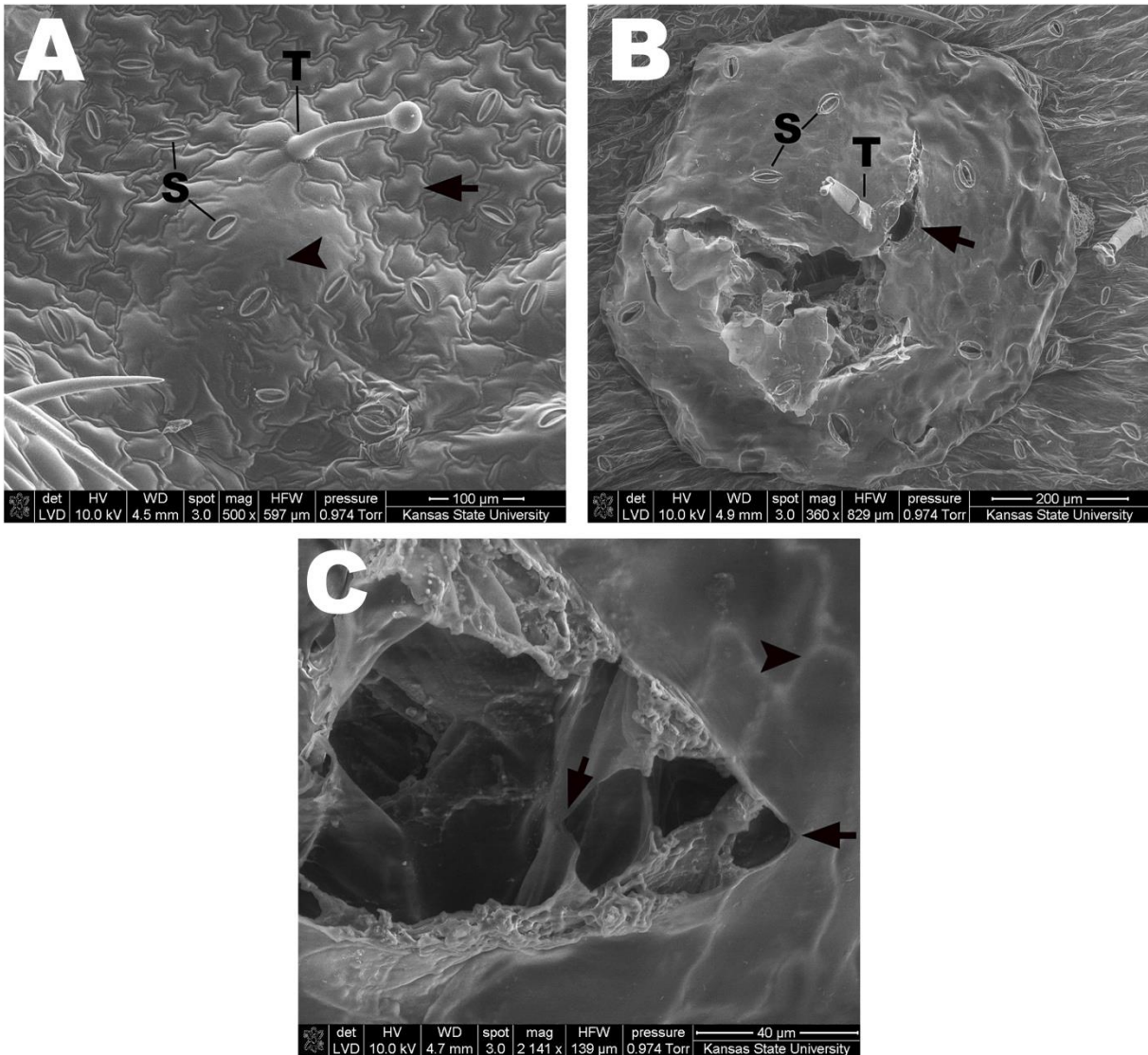


**Figure 3.9** Digital images of three stages of lesion development on the abaxial leaf surface of geranium (*Pelargonium* ×‘Caliente Coral’). **A)** Leaf displaying the early stages of lesion development as small green bumps form sporadically on the surface. **B)** Leaf area close-up characterizing the smooth and rounded appearance of lesions that often appeared in small groupings. **C)** Leaf displaying the intermediate stages of lesion development as senescence was initiated. **D)** Leaf area close-up characterizing lesion senescence which originated around the lesion apex. **E)** Leaf displaying the latter stages of development as lesions senesced and formed large regions of senescent cells. **F)** Leaf area close-up characterizing a large region of senesced cells and lesion collapse.





**Figure 3.10** Field emission scanning electron microscopy (FESEM) of the abaxial leaf surface of geranium (*Pelargonium* ×‘Caliente Coral’). **A**) Lesion development initiated as epidermal cells were stretched (point) due to the expansion of underlying cells. A loss in the definition of these stretched epidermal cells was apparent when compared to the surrounding unaffected cells (arrow). **B**) The lower epidermis was torn (arrow) across the lesion surface due to pressure from mesophyll expansion below. **C**) Evidence of epidermal tearing due to tension, where epidermal cells have lost definition (point) and torn (arrows) due to underlying cell expansion. Abbreviations: stoma (S); and trichome (T).

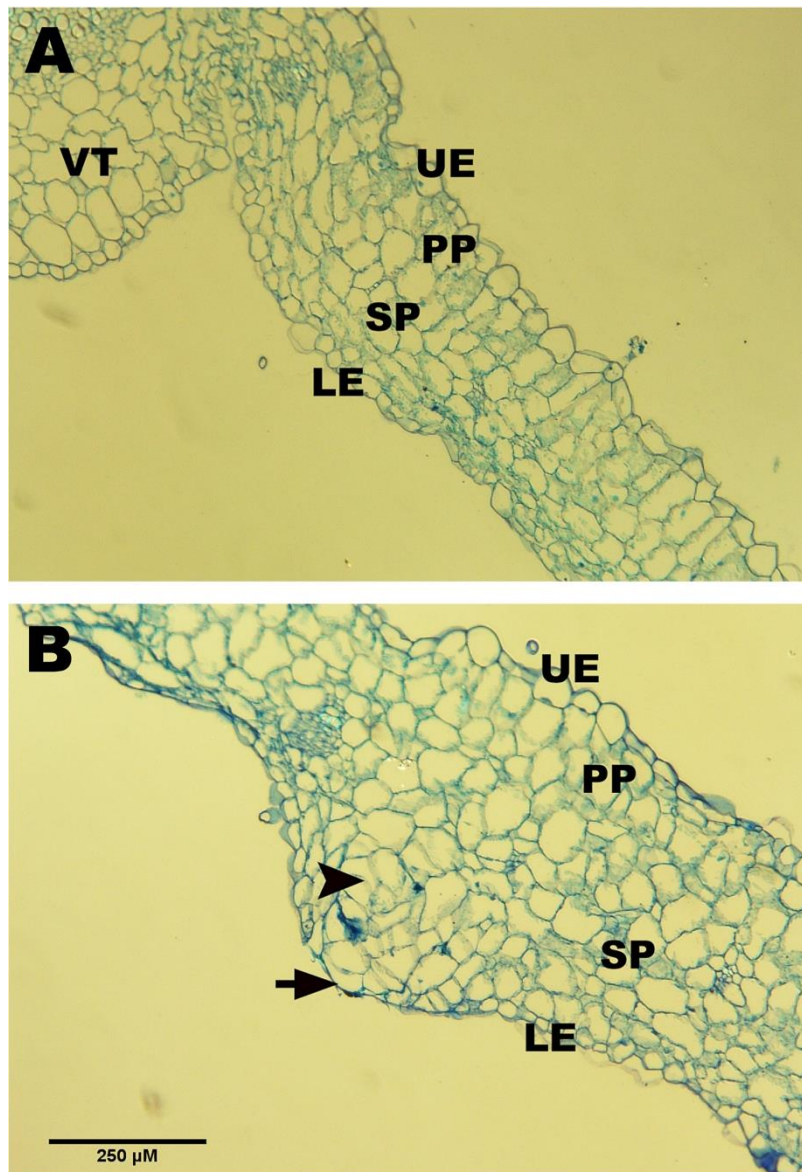




**Figure 3.11** Geranium (*Pelargonium* ×‘Caliente Coral’) leaf displaying a small circle of senesced cells (arrow) on the adaxial surface where a lesion had previously collapsed on the abaxial surface directly below.



Figure 3.12 Light microscopy cross-sections stained with Toluidine Blue O of lesions on the abaxial leaf surface of geranium (*Pelargonium* ×‘Caliente Coral’). A) Asymptomatic leaf; tissue with no lesion development. B) Hypertrophy and hyperplasia of spongy parenchyma cells (point) applied pressure to the lower epidermis. Evidence for tearing of the lower epidermis (arrow) as the epidermal cells became increasingly thin from the underlying cell expansion. Abbreviations: lower epidermis (LE); palisade parenchyma (PP); spongy parenchyma (SP); upper epidermis (UE); and vascular tissue (VT).

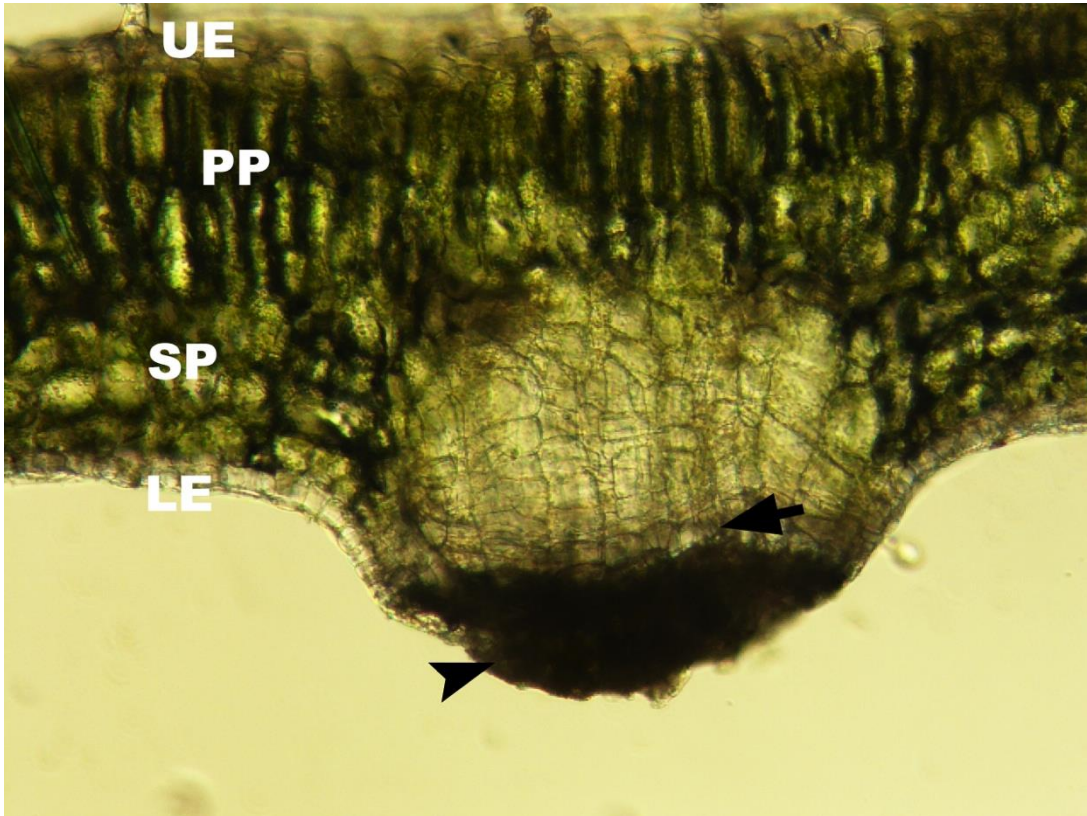


**Figure 3.13** Western Flower Thrips (*Frankliniella occidentalis* Pergande) (arrow) feeding damage on the abaxial leaf surface of interspecific geranium (*Pelargonium* × 'Caliente Coral'). Damage (point) appears similar to the latter stages of lesion development on geranium, suggesting a potential wounding response.





**Figure 3.14** Cross-section (200  $\mu\text{m}$  thick) of a lesion on the abaxial leaf surface of interspecific geranium (*Pelargonium*  $\times$  'Caliente Coral'). Senescing cells at the apex of the lesion appear black (point), while stacked cells underneath may act as a protective layer and be suberized (arrow). Abbreviations: lower epidermis (LE); palisade parenchyma (PP); spongy parenchyma (SP); and upper epidermis (UE).



## Appendix A - Confocal Microscopy

Multiple confocal microscopy staining techniques were attempted during Fall 2013 to best evaluate and quantify intumescence development on ornamental sweet potato (*Ipomoea batatas* 'Blackie') and oedema development on interspecific geranium (*Pelargonium* × 'Caliente Coral'). Ultimately, the confocal microscopy provided unsatisfactory results. Further evaluation of these staining methods is necessary.

### Sytox<sup>®</sup> Orange Dead Cell Stain

SYTOX<sup>®</sup> Orange Dead Cell Stain is a nucleic acid stain that is cell-impermeant. This stain was selected in an attempt to quantify the exact number of cells involved in lesion development on both ornamental sweet potato and geranium (Fig A.1, 2). Target areas on selected leaves were sampled as leaf discs (0.7 cm diameter). Leaf discs were then sectioned to 200 µm slices using a tissue chopper. Optimal slices that best represented the lesion center were then selected. Tissue sections were then fixed with 2% paraformaldehyde in a 200 mmol PBS (pH 7.2) for 30 minutes, rinsed twice with double deionized water (DDH<sub>2</sub>O), and stored at 2.5°C. Sections were then stained for 10 minutes using 5 µM SYTOX<sup>®</sup> Orange Dead Cell Stain (Life Technologies Corporation; Grand Island, NY) and mounted on slides using DDH<sub>2</sub>O. Slides were imaged using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal. Ultimately, staining was not uniform and provided inconsistent results between species.

### DiI

DiI (1,1'-Dioctadecyl-3,3',3'-Tetramethylindocarbocyanine Perchlorate) is a lipophilic membrane stain. Specifically, DiI is a long-chain dialkylcarbocyanine that will label neurons via lateral diffusion in the plasma membrane (Anonymous, 2011). The function of this stain is to cause the plant cell membranes to fluoresce, allowing for an effective means of quantifying cell size and specific cell layers involved in the development of lesions on ornamental sweet potato and geranium (Fig A.3, 4). Target areas on selected leaves were sampled as leaf discs (0.7 cm diameter). Leaf discs were then sectioned to 200 µm slices using a tissue chopper. Optimal slices

which best represented the lesion center were then selected and stained with 20  $\mu$ M DiI (Life Technologies Corporation; Grand Island, NY) in 50% ethanol for 10 minutes. Sections were then mounted on slides using DDH<sub>2</sub>O. Slides were evaluated using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal. Labeling of other lipids in the cells by DiI and inconsistent staining made evaluation of cell size and layers difficult. Ultimately, the use of Toluidine Blue O and light microscopy provided a simpler protocol and more desirable results.

### **CellTrace™ CFSE Cell Proliferation Kit**

A CellTrace™ CFSE Cell Proliferation Kit was used in an attempt to quantify cell proliferation. Specifically, this stain will effectively label eight or more generations of cells, allowing for the quantification of hyperplasia in lesion development. The stain is cell permeant and will bind to free amines on the cell surface as well as within the cell. A total of eight ornamental sweet potato plants were selected for staining. Leaves that were nearing full expansion were marked on these plants, and 2 to 3 small circles (~1 cm in diameter) were made on the adaxial surface. Plants were placed in a light controlled environment (dark conditions), and ~35  $\mu$ L of 20  $\mu$ M CellTrace™ CFSE Cell Proliferation Kit (Life Technologies Corporation; Grand Island, NY) in 50% ethanol was applied inside each circle. The plants were left in the light controlled environment for ~3 hours, until all the stain had passed into the leaf tissue. Seven plants were then moved back into the greenhouse environment for 5 days to develop lesions, while one plant was left in the light controlled environment to serve as a control. Target areas on the marked leaves were then sampled as leaf discs (0.7 cm diameter). Leaf discs were then sectioned to 200  $\mu$ m slices using a tissue chopper and mounted on slides using DDH<sub>2</sub>O. Slides were evaluated using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 488 nm laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and a BP 505-550 band pass filter to collect the signal. Staining from the CellTrace™ CFSE Cell Proliferation Kit was ultimately unobservable. This may have been due to the degradation of the stain due to light in the

greenhouse environment. However, plants grown entirely under light controlled conditions still did not produce a strong signal, making measurements of hyperplasia impossible to collect.

### **Glutaraldehyde Fixation**

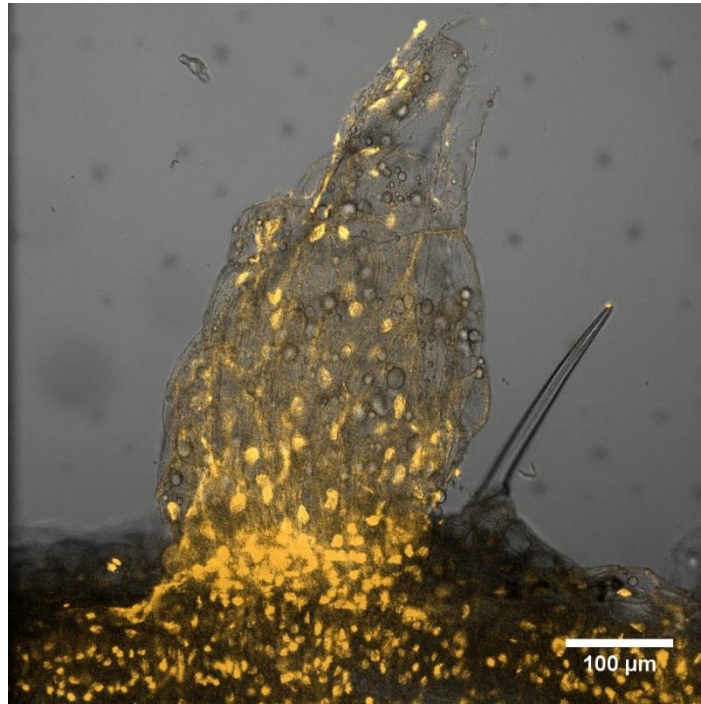
Glutaraldehyde can be used as a fixative to allow for a green auto-fluorescence in the cells. The mechanism of auto-fluorescence is achieved by the cell proteins after cross-linking with glutaraldehyde. Specifically, glutaraldehyde has two aldehyde moieties that will cross-link protein amino groups (Fester et al., 2008). This will ultimately fluoresce green and allow the cells to be more easily imaged. Target areas for lesion development on both ornamental sweet potato and geranium leaves were selected and sampled as leaf discs (0.7 cm diameter). Leaf discs were then sectioned to 200  $\mu\text{m}$  slices using a tissue chopper. Optimal slices which best represented the lesion center were then selected. Tissue sections were fixed with 2% paraformaldehyde/2% glutaraldehyde in a 200 mmol PBS (pH 7.2) for 2 hours, rinsed twice with  $\text{DDH}_2\text{O}$ , and stored at  $2.5^\circ\text{C}$ . Tissue sections were then stained with DiI using the same protocol listed above. Slides were evaluated using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope with a 20x/0.50 NA Plan-NEOFLUAR objective. A 488 nm laser was used to excite the glutaraldehyde fluorochrome, and a 543 nm HeNe laser was used to excite the DiI fluorochrome. An NFT 545 secondary dichroic and an LP 560 long pass filter were used to collect signal from the DiI stain, while a BP 505-550 band pass filter collected the signal for the glutaraldehyde autofluorescence. Staining from the glutaraldehyde was ultimately unobservable.

### **Literature Cited**

- Anonymous. 2011. Vybrant<sup>TM</sup> cell-labeling solutions. Molecular Probes Inc., Eugene, OR. 3 April 2014. <<http://tools.lifetechnologies.com>>.
- Fester, T., R.H. Berg and C.G. Taylor. 2008. An easy method using glutaraldehyde-introduced fluorescence for the microscopic analysis of plant biotrophic interactions. *J. Microsc.* 231(2):342-348.

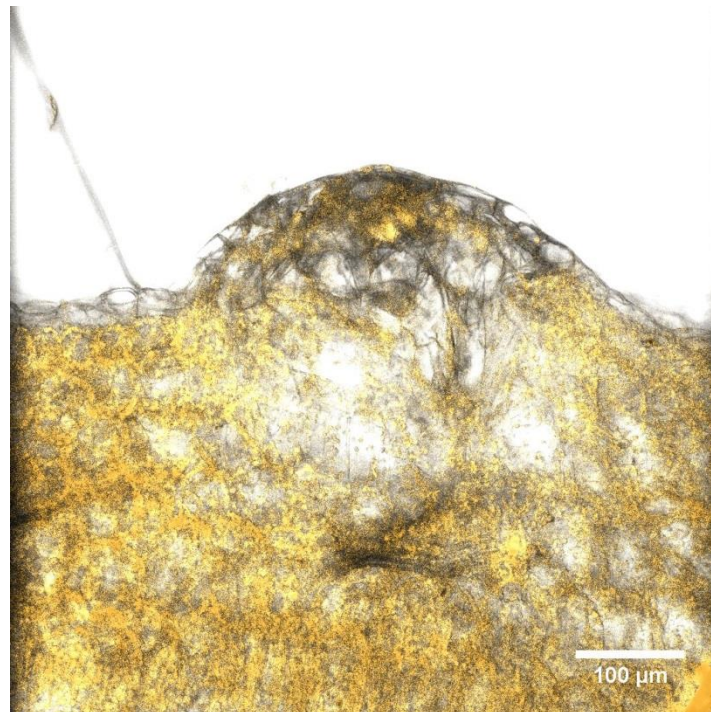
## Appendix Figures

**Figure A.1** Intumescence on the adaxial leaf surface of ornamental sweet potato (*Ipomoea batatas* ‘Blackie’) stained with SYTOX® Orange Dead Cell Stain (Life Technologies Corporation; Grand Island, NY). Image was obtained using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal.

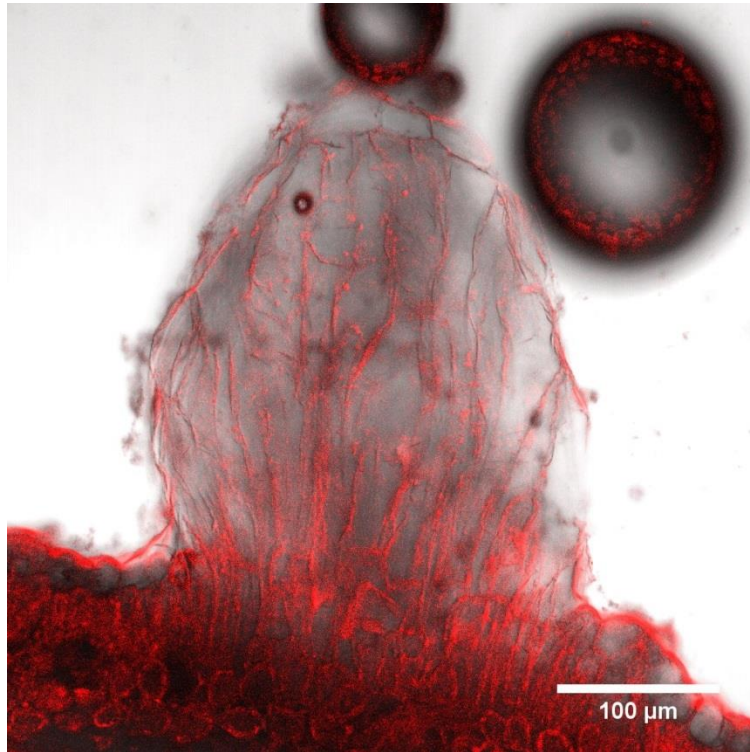




**Figure A.2 Oedemata on the abaxial leaf surface of geranium (*Pelargonium* ×‘Caliente Coral’) stained with SYTOX® Orange Dead Cell Stain (Life Technologies Corporation; Grand Island, NY). Image was obtained using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal.**



**Figure A.3** Intumescence on the adaxial leaf surface of ornamental sweet potato (*Ipomoea batatas* ‘Blackie’) stained with DiI. Image was obtained using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal.



**Figure A.4 Oedemata on the abaxial leaf surface of geranium (*Pelargonium* ×‘Caliente Coral’) stained with DiI. Image was obtained using an LSM-5 PASCAL (Carl Zeiss Microscopy; Thornwood, NY) confocal microscope and a 20x/0.50 NA Plan-NEOFLUAR objective. A 543 nm HeNe laser was used to excite the fluorochrome, with an NFT 545 secondary dichroic and an LP 560 long pass filter to collect the signal.**

