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Leaf Injury Induced by Temperature Drop Shock in Gesneriaceae and Acanthaceae Plants

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ABSTRACT Leaf spots in *Saintpaulia* leaves are caused by temperature drop shock (TDS). This TDS-mediated leaf injury has not been reported in other plants besides *Saintpaulia*. To investigate how many and what kinds of plants are susceptible to temperature drop shock, Gesneriaceae and Acanthaceae plants were treated with TDS (from 30℃ to 15℃ or 5℃). Yellow or brown spots were found in 26 species or cultivars of 10 genuses of Gesneriaceae plants and in 8 species or cultivars of 7 genuses of Acanthaceae plants. Morphologically and anatomically no similarity was observed among the plants susceptible to TDS. Some plants have very thin and hard leaves, whereas other plants have thick and soft leaves. In spite of this non-similarity, the injury was restricted only to palisade cells as those of *Saintpaulia* leaves. Also the rapid and irreversible reduction of chlorophyll fluorescence was observed soon after TDS treatment in those plants. These results indicate that leaf injury induced by TDS is a more widespread leaf injury than has previously been thought.

Additional key words: *Saintpaulia*, leaf spot, overhead irrigation, TDS

Introduction

Saintpaulia (African violet), a genus of Gesneriacea family, often has yellow or brown spots on their leaves. These leaf spots have been called 'leaf spot' or 'ring spot' and considered as a kind of leaf injuries. Leaf spot was known to be caused by overhead irrigation with cold water under strong light condition, and considered as a kind of cold injury (Maekawa et al., 1987).

Research on leaf spot has shown that leaf spot has some specific characteristics. Anatomically, the injury of leaf spot is confined to the palisade cells in the leaves (Elliot, 1946). A direct factor causing leaf spot is temperature drop shock (TDS) which is induced by water of lower temperatures rather than leaf temperature (Yun et al., 1998). Because of this, leaf spot could be caused by 20℃ water if a water temperature was 15℃ lower than a leaf temperature. The other characteristic of leaf spot was an abrupt reduction in chlorophyll fluorescence to an irreversible level within a very short time (20 sec) after *Saintpaulia* leaves were exposed to TDS (Yun et al., 1998). This irreversible changes of chlorophyll fluorescence was confined only to palisade cells of the treated leaves. Also, soon after TDS treatment, an abrupt degradation of photosynthetic system in palisade cells was observed physiologically and anatomically (Yun, et al., 1996b, 1997a). Anatomically, not only chloroplast but also other organelles including nucleus and microbodies showed drastic morphological changes soon after TDS treatment (Yun, et al., 1996b). From these results, it was considered that leaf spot is not a cold or chilling injury but a specific leaf injury observed in limited chilling sensitive plants such as *Saintpaulia*.

To date, although some plants of Gesneriacea families such as *Achimenes*, *Gloxinia*, and *Columnea* have been known to be susceptible to leaf spots caused by an overhead irrigation with cold water under a strong light condition, there has been no such report in plants other than *Saintpaulia*. Screening how many and what kinds of plants are susceptible to TDS probably can offer very important information and plant materials for investigation of the mechanism of leaf spot occurrence.

In this study, many plants in Gesneriaceae and in its very near family Acanthaceae were examined to clarify whether they were susceptible to TDS or not. Additionally, morphological and anatomical examination were also conducted.

Materials and Methods

Plant materials

Plant materials have been collected at Kyoto Botanical

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Garden in Kyoto, Japan. Only mature leaves were detached, placed in transparent plastic bags with a wet paper towel, and transported to the laboratory for examination. Plant materials which already had brown or yellow spots on their leaves looking like TDS-mediated leaf spot, were also collected. These leaves were fixed at FAA (Formaldehyde+Acetic acid+50% Ethanol) solution for anatomical examination through a photomicroscopy.

Temperature drop shock (TDS) treatment

The leaves were immersed in 35℃ water for 3 min., and then the half parts of them were dipped in 15 or 5℃ water for 3 sec. Treated leaves were placed in an incubator under 25℃ and continuous irradiation of 80μmol $m^{-2}s^{-1}$ PPFD during a day, with their petiols immersed in a distilled water. After 2−3 weeks, leaves were examined visually whether leaf spots (brown or yellow spots) have appeared or not. Leaves having yellow or brown spots were considered as susceptible to TDS.

Preparing thin section for anatomical examination

The injured leaves were divided into small segments and fixed in FAA solution. The leaf segments were dehydrated in an ethanol series, embedded in the resin (Technovit 7100, Germany), and sectioned at 5μm with a microtome. Thin sections were stained for 1 min. with 0.5% toluidine blue solution, and examined under a photomicroscope.

Chlorophyll fluorescence

An important characteristic of the leaf injury induced by TDS is a rapid declination of chlorophyll fluorescence immediately after TDS treatment. To confirm whether the leaf injuries observed were caused by TDS or not, leaves right after TDS treatment were transferred for a fluorescence microscope (excitation filter BP490nm, dichroic mirror DM500 nm, long pass filter AFC+O515 nm), and changes in chlorophyll fluorescence were observed.

Results and Discussion

1. Classification of plants according to the leaf spot sensitivity

The examined plants were classified into 3 groups according to their sensitivity to TDS. Plants, whose leaf spots were caused by the temperature drop from 35 to 15℃, were considered as high sensitive plants. The plants, whose leaf spots were caused by the temperature drop from 35 to 5℃, were classified as low sensitive plants. Plants with no symptom of leaf spot were classified as insensitive plants.

In Gesneriaceae plants

By the TDS treatments described above, the symptoms of leaf spot (yellow or brown spots) were observed in 26 species or cultivars of 10 genuses of Gesneriaceae plants examined (Table 1). Even within the same genus, the degree of sensitivity to TDS varied greatly depending upon species or cultivars.

The highly sensitive plants include *Achimenes longiflora* cv. Alba, *Episcia* sp. cv. Moss Brocade, *E. linacina* cv. Mrs. Fanny Hage, *Nematanthus fissuss*, *N.* sp. cv. Apres, *N.* sp. cv. Rio, *Saintpaulia confusa*, *S. ionantha*, *S. magungensis*, *S. nitida*, *S. pendula*, *S.* sp. cv. Royal, S. sp. cv. Violet Trail, *Sinningia cunescence*, *S. claybergiana*, *Streptocarpus* sp. cv. Boysenbery Delight, *Tricantha* sp. cv. Tiger Paws, and *T. calotricha*. The low sensitive plants includes *Columnea* sp. cv. Euphora, *C. proctorii*, *C. sanguinea*, *Dalbergaria aureonitens*, *Gloxinia nematanthodes*, *G. sylvatica*, *Sinningia aggregata*, *Streptocarpus saxorum*. However, other plants such as *Achimenes erecta*, *Kohleria digitaliflora*, and *Sinningia aggregata* showed no symptom of leaf spot.

On the other hand, among the insensitive plants, some plants already had brown or yellow spots on their leaves when samples were collected in a plastic film house. These plants include *Achimenes pedunculata*, *Alsobia* sp. cv. San Miguel, *Chirita lavandulacea*, *Episcia* sp. cv. Frosty, *Gloxinia perennis*, *Gloxinia* sp. cv. Arion, *Kohleria maculata*, *Nematanthus strigil-*

Table 1. Classification of Gesneriacea plants according to their sensitivity to temperature drop shock (TDS).

Highly sensitive plants[z]	*Achimenes longiflora* cv. Alba, *Episcia* sp. cv. Moss Brocade, *E. linacina* cv. Mrs. Fanny Hage, *Nematanthus fissuss*, *N. sp.* cv. Apres, *N. sp.* cv. Rio, *Saintpaulia confusa*, *S. ionantha*, *S. magungensis*, *S. nitida*, *S. pendula*, *S. sp.* cv. Royal, *S. sp.* cv. Violet Trail, *Sinningia cunescence*, *S. claybergiana*, *Streptocarpus sp.* cv. Boysenbery Delight, *Tricantha* sp. cv. Tiger Paws, *T.* calotricha.
Low sensitive plants[y]	*Columnea sp.* cv. Euphora, *C. proctorii*, *C. sanguinea*, *Dalbergaria aureonitens*, *Gloxinia nematanthodes*, *G. sylvatica*, *Sinningia aggregata*, *Streptocarpus saxorum*.
Insensitive plants[x]	*Achimenes erecta*, *A. pedunculata*[w], *A. sp.* cv. Harvey, *Aeschynanthus radicans*, *A. longiflorus*, *Alsobia sp.* cv. San Miguel[w], *Chirita lavandulacea*[w], *Codonanthe elegans*, *C. fawcettii* cv. Mitzi, *Episcia sp.* cv. Frosty, *Gloxinia perennis*, *G. sp.* cv. *Arion*[w], *Kohleia digitaliflora*, *K. maculata*[w], *Nematanthus gregarius*, *N. strigillosus*[w], *Ramonda myconi*, *Sinningia aggregata*, *S. eumorpha*[w], *S. speciosa*.

[z]Leaf spot was caused by a TDS from 35 to 15℃.

[y]Leaf spot was caused by a TDS from 35 to 5℃.

[x]Leaf spot was not caused by a TDS in this experiment.

[w]Plants having leaf spots similar to TDS-mediated leaf spot under the natural condition.

Table 2. Classification of Acanthaceae plants according to their sensitivity to temperature drop shock (TDS).

Highly sensitive plants[z]	*Schaueria calycotricha* *Strobilanthes japonica*
Low sensitive plants[y]	*Barleria repens, Crossandra infundibuliformis* *Eranthemum pulchellum, Hemigraphis colorata* *Ruellia graecizans, Strobilanthes maculatus.*
Insensitive plants[x]	*Barleria lupulina, Dicliptera suberecta* *Peristrophe hyssopifolia* *Pseuderanthemum atropurpureum, P. reticulatum, P. laxiflorum* *Ruellia colorata*[W], *R. macrantha*[w] *Strobelanthes anisophyllus* *Thunbergia erecta, T. fragrans, T. vogeliana*

[z,y,x] and [w]See Table 1.

losus, and *Sinningia eumorpha*. However, these plants did not show leaf spot by TDS treatment in this experiment.

In Acanthaceae plants

Leaf spots of yellow or brown color were observed in 8 species or cultivars of 7 genuses (Table 2). Among them, *Schaueria calycotricha* and *Strobilanthes japonica* appeared to be very sensitive to TDS. *Barleria repense*, *Crossandra infundibuliformis*, *Eranthemum pulchellum*, *Hemigraphis colorata*, *Peristrophe hyssopifolia* cv. Bremak, *Ruellia graecizans*, and *Strobilanthes maculatus* appeared to be susceptible to leaf spot, although their sensitivity to TDS was relatively low. Twelve other plants, including *Strobilanthes anisophyllus*, *Ruellia colorata*, and *R. macrantha*, did not show any symptom of leaf spot. Among the insensitive plants, plants which already had yellow or brown spots when samples were collected include *Ruellia colorata*, *Ruellia macrantha*, and so on.

It was very interesting to know that leaf spot was also found in *Strobilanthes japonica* which is a temperate, not a tropical plant. Furthermore, *Strobilanthes japonica* was very sensitive to TDS. This result suggests that leaf spot occurs not only in tropical plants which are sensitive to chilling temperatures, but may also occur in more diverse plants.

2. Morphological and anatomical observation

Morphologically, no similarity was observed among plants susceptible to TDS. For example, *Nematanthus* sp. cv. Rio of Gesneriaceae, a highly sensitive plant to TDS, had very thick leaves with multiple epidermal cells and very hard texture (Fig. 1A, B). However, leaves of *Strobilanthes japonica* of Acanthaceae, a highly sensitive plant, were very thin with very soft texture (Fig. 2A, B). Leaves of these plants were also different anatomically from those of *Saintpaulia* having one layer of

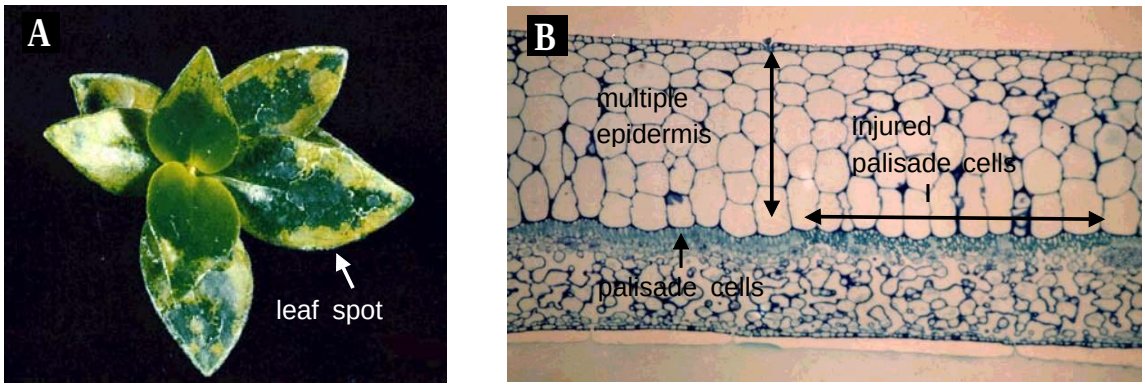

Fig. 1. Plant (A) and leaf structure (B) of *Nematanthus* sp. cv. Rio.

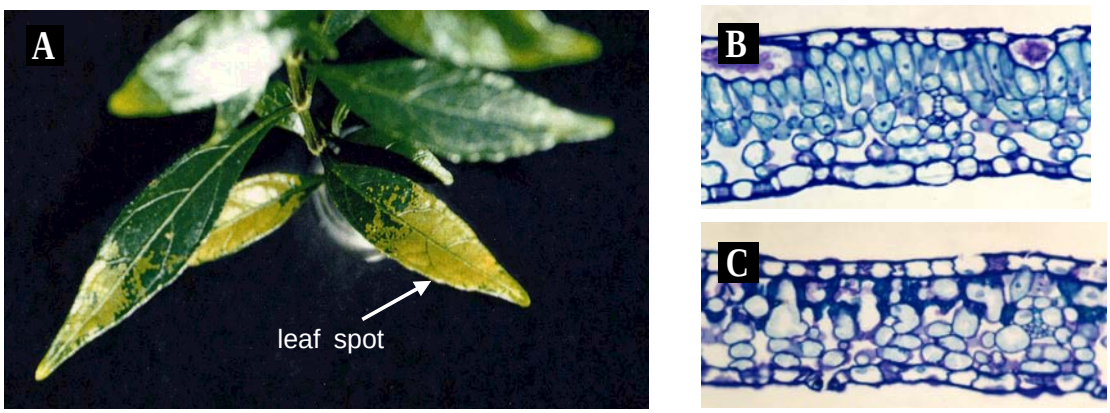

Fig. 2. Plant (A) and leaf strucures of intact (B) and injured (C) of *Strobilanthes japonica*.

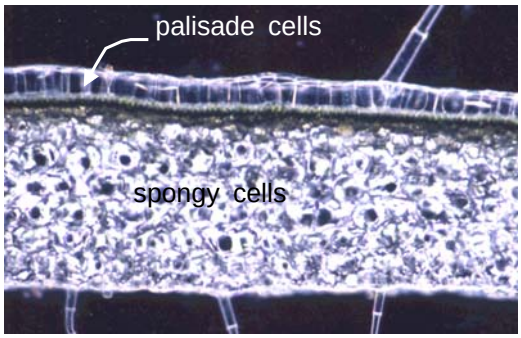

Fig. 3. Leaf section of *Saintpaulia ionantha*.

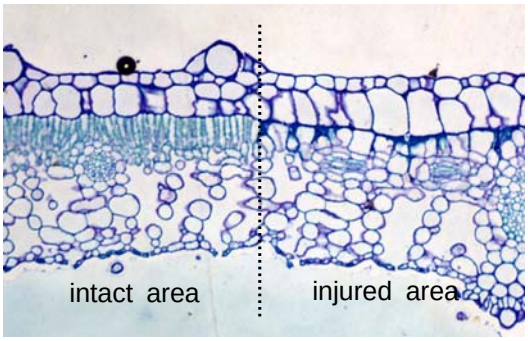

Fig. 4. Leaf structure of *Gloxinia sylvatica*.

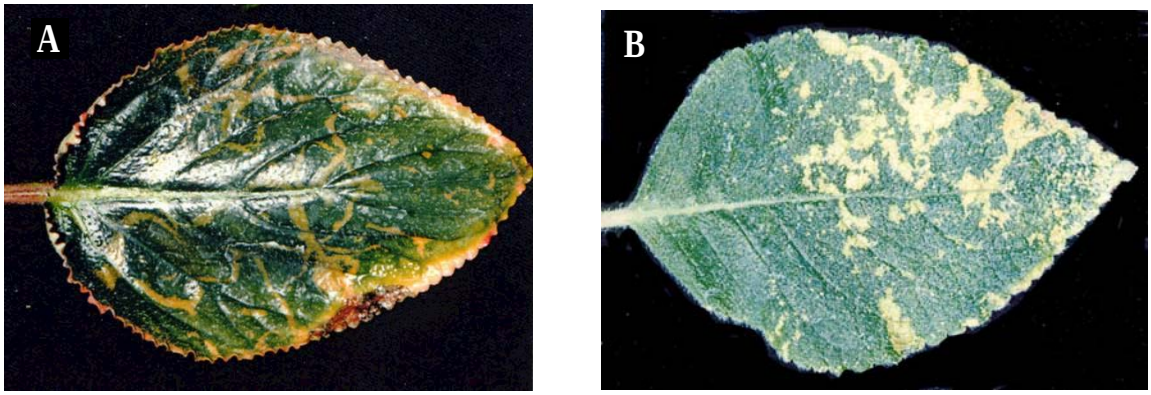

Fig. 5. Variegations of leaf spot in *Gloxinia perrenis* (A) and *Kohleria maculata* (B).

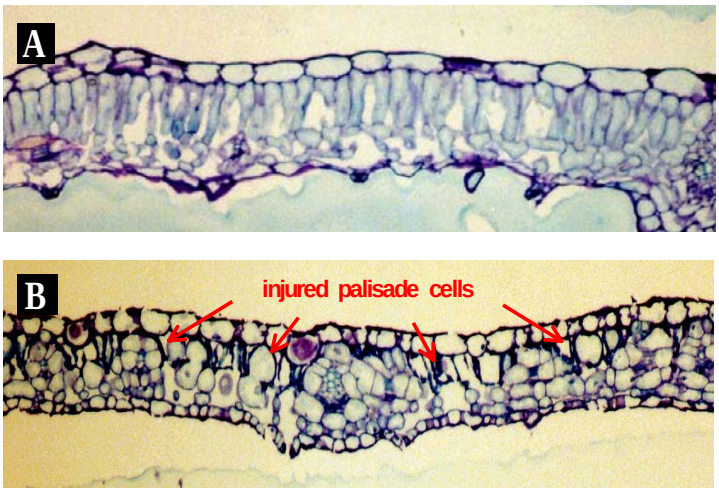

Fig. 6. Leaf section of intact (A) and injured (B) in *Kohleria maculata*.

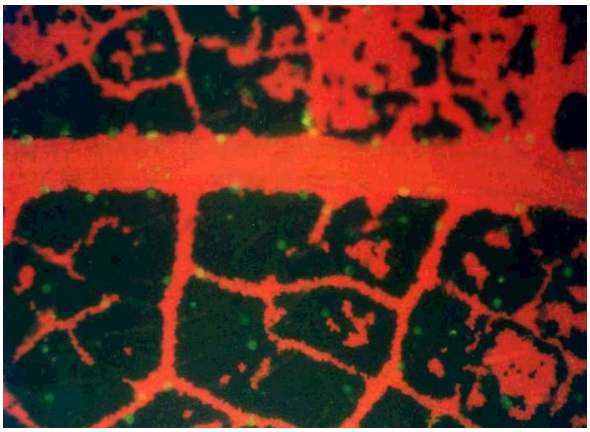

Fig. 7. Fluorescence microscope photograph soon after TDS treatment of *Strobilanthes japonica*. Red: non-injured; Dark: injured area.

epidermis, 1−2 layers of palisade cells, and multiple layers of spongy cells (Fig. 3A). Despite these non-similarities in morphological and anatomical characteristics, leaves of those plants were susceptible to TDS and the leaf injury was restricted to the palisade cells in all plants. Other tissues such as upper and lower epidermal and spongy cells were not damaged at all (Fig. 1B, 2C, 4).

These results support the fact that leaf spot caused in this experiment is the same injury as that of *Saintpaulia* in which restriction of damaged tissue to only palisade cells is a specific characteristic of leaf spot in *Saintpaulia* (Elliot, 1946). At the same time, the results indicated that leaf spot occurrence is independent of morphological or anatomical characteristics of the leaves.

Leaves of plants which did not show sensitivity to TDS in this experiment but already had yellow or brown spots on their

leaves when collected were investigated through light microscopy. The variegations of leaf injury observed in those plants were very similar to those of TDS-mediated leaf spot (Fig. 5). Also injury of those leaves was restricted to palisade cells in the same manner as TDS-mediated leaf spot (Fig. 6). At present, we have no idea what factor causes leaf spots in those plants. However, considering the facts that 1) the variegations of leaf spots in those plants are very similar to those of *Saintpaulia* (Yun et al., 1996a), 2) the injury of the leaf spots is restricted to only palisade cells (Elliot, 1946, Yun et al., 1998), and 3) the sensitivity of *Saintpaulia* leaves to TDS largely dependent upon environmental factors, such as light, temperature and humidity (Yun et al., 1997b), the leaf spots observed in those nonsensitive plants are likely to be the same or very similar injury to those of *Saintpaulia*.

3. Change of chlorophyll fluorescence (CF)

Under a fluorescence microscope, abrupt reducton in intensity of chlorophyll fluorescence (CF) was observed in the injured areas of leaves soon after TDS treatment, and the injured areas were distinguished from uninjured areas (Fig. 7). Although there are some differences among the species or cultivars, CF was reduced to the lowest level within 1−2 min after TDS treatment in most of plants. In some plants, CF reduction was observed in interveinal areas. Transverse sections of TDS-treated leaves showed that the reduction in CF was restricted to palisade cells. Because the leaf spot in *Saintpaulia* was characterized by an abrupt and irreversible reduction in CF of palisade cells (Yun et al., 1998), it is reasonable to consider the injury in this experiment is the same injury as that of *Saintpaulia*.

There have been many reports on the leaf spot. In many cases, leaf spot were reported to be caused by pathogens such as bacteria, fungus (Latterel and Rossie, 1983; Ward et al., 1994, 1997) and virus (Grieco et al., 1989; Johnston and Rochon, 1995; Miller et al., 1997, Rochon and Tremaine, 1988). Leaf spot caused by TDS has been reported only in *Saintpaulia*. The present study clarified that TDS-mediated leaf spot occurred not only in *Saintpaulia* but also in many other Gesneriaceae, and some Acanthaceae plants. Although the plants susceptible to TDS have no similarity in morphology and anatomical structures of their leaves, their injuries are restricted only to palisade cells in the same way as those of *Saintpaulia*.

Gesneriaceae와 Acnathaceae과 식물에서 급격한 엽온저하에 의해 발생하는 엽상해

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초 록

세인트폴리아(*Saintpaulia ionanth*)의 잎에는 노랑 또는 갈색의 반점이 많이 생긴다. 이러한 반점은 leaf spot라고 불리며 관수시 엽온이 급격히 떨어지는 쇼크(Temperature Drop Shock: TDS)에 기인하는 것으로 알려져 있다. TDS에 의해 발생하는 leaf spot에 대해서는 세인트폴리아 외에는 지금까지 발표된 바 없다. 본 연구에서는 이러한 엽상해가 어떤 종류의 식물에서 발생하는지 알아보기 위해, 세인트폴리아가 속해 있는 Gesneriaceae과 식물을 중심으로 조사하였다. 그 결과, Gesneriaceae과 식물에서는 *Gloxinia* 등 10속, 26종의 식물에서 leaf spot가 발생하였으며, Acanthaceae과 식물에서는 7속, 8종의 식물에서 발생하였다. Leaf spot이 발생하는 식물들의 잎에서는 어떠한 형태적, 해부학적 유사성이 발견되지 않았다. 어떤 식물의 잎은 두껍고 단단한 반면, 어떤 식물의 잎은 얇고 부드러웠다. 그러나 leaf spot이 발생한 모든 식물들에 있어 TDS에 의한 상해는 책상조직세포에만 발생하였고, 엽록소형광도 TDS 처리 직후 급격히 감소하여 회복되지 않는 등, 세인트폴리아에서의 leaf spot과 동일한 현상을 보였다. 이러한 결과들로부터 TDS에 의해 발생하는 엽상해는 세인트폴리아만이 아니라 좀더 넓은 범위의 식물에서 발생된다는 사실이 명확해졌다.

추가 주요어 : *Saintpaulia*, leaf spot, overhead irrigation, TDS

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