The spatial patterns for temperature distribution on crape myrtle leaves infested with sooty mold were investigated using a digital infrared thermal imaging camera. The mean temperatures of the control and sooty regions were 26.98°C and 28.44°C, respectively. In the thermal images, the sooty regions appeared as distinct spots, indicating that the temperatures in these areas were higher than those in the control regions on the same leaves. This suggests that the sooty regions became warmer than their control regions on the adaxial leaf surface. Neither epidermal penetration nor cell wall dissolution by the fungus was observed on the adaxial leaf surface. It is likely that the high temperature of black leaves have an increased cooling load. To our knowledge, this is the first report on elevated temperatures in sooty regions, and the results show spatial heterogeneity in temperature distribution across the leaf surface.

Keywords: Lagerstroemia indica, sooty mold, thermal imaging

Digital Infrared Thermal Imaging of Crape Myrtle Leaves Infested with Sooty Mold

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Plant leaf temperature represents the interactions between the external and internal factors involved in leaf growth and metabolism (Oerke et al., 2006). It is usually positively correlated with light intensity and negatively correlated with transpiration, which is the evaporation of liquid water in the form of water vapor (Pallas et al., 1967). As water converts from a liquid to a gas through the leaf stomatal aperture, the heat energy required to break the hydrogen bonds between water molecules is taken from the leaf. The leaf temperature changes when the absorbed heat energy interacts with the energy influx from its surroundings and from metabolic heat generation (Hellebrand et al., 2006). The measurement of leaf temperature is primarily used to identify the relationship between plant water relations and stomatal conductance because a major determinant of leaf temperature is the rate of transpiration from the leaf surface (Jones et al., 2009).

Non-destructive imaging methods could potentially produce early and intuitive information on plant responses to a variety of stresses (Chaerle et al., 2004). Thermal imaging visualizes infrared (IR) radiation, which can be used to highlight an object as the temperature changes across the object’s surface (Li et al., 2014). It is well established that the higher an object’s temperature, the more IR radiation is emitted and detected by IR thermal imaging cameras. Localized plant responses to stresses can be clearly contrasted against a background of unaffected plant tissue (Chaerle et al., 2004; Jones, 1999). Digital IR thermography has been used to find correlations between temperature and transpiration in various plants infected with fungal pathogens (Baranowski et al., 2015; Chaerle et al., 2004). It has been also used to examine the response of morning glory leaves to ozone exposure (Hur and Lee, 1996). Spatial patterns for temperature distribution can be created based on one of the images (e.g., the false-color processed images with red, green, and blue colors) and then superimposed on the others (Li et al., 2014).

Crape myrtle (Lagerstroemia indica Pers.) belonging to the family Lythraceae is a deciduous shrub with pink to red flowers in summer and is distributed across the temperate regions of the northern hemisphere. The shrub is usually heavily infested with sooty mold which includes...
E. lagerstroemiae

Sangju (36

between black regions and white regions. By comparing thermal responses to fungal colonization, mildew-colonized leaves of the shrub were also analyzed. Black body radiation-based working hypothesis, powdery mildew fungi such as Erysiphe australiana and E. lagerstroemiae infect the shrub in China, Japan, Australia, and the southern USA (Shi and Mmbaga, 2006).

Sooty mold diseases on landscape trees and shrubs commonly cause blackened plant parts through the proliferation of epiphytic fungal complexes (Kim, 2016). The black fungal covering means that less sunlight reaches the leaf surface, which reduces photosynthesis compared to green, healthy leaves (Insausti et al., 2015). It has been suggested that sooty regions might be warmer than neighboring healthy ones, as black areas absorb all wavelengths of incident electromagnetic radiation (energy) and reflect none of the incident radiation, which is known as black-body radiation in physics. However, there have been few studies on elevated temperatures in sooty regions in sooty mold-plant interactions. This study uses IR thermal imaging to show that local warming occurs in sooty regions, compared to green, healthy regions. To corroborate the black body radiation-based working hypothesis, powdery mildew-colonized leaves of the shrub were also analyzed by comparing thermal responses to fungal colonization between black regions and white regions.

Approximately one-m-tall crape myrtles were grown in Sangju (36°42′ N and 128°16′ E), Korea. Sooty mold and powdery mildew diseases on the shrubs were assessed in mid-August 2015. The attached symptomatic leaves were imaged using a digital camera, which produced real photographs. Thermographs of the same leaves were acquired using a digital IR thermal imaging camera (T620; FLIR Systems, Wilsonville, OR, USA) with 0.04°C thermal sensitivity during the day. The daily mean temperature in thermal imaging was 27.63°C. The distance between the thermal camera and the leaves was approximately 30 cm. The thermal camera was positioned nearly perpendicular to the plant surface (Baranowski et al., 2015). The real photographs were compared with their corresponding IR thermographs to reveal the spatial patterns for temperature distribution on the leaf surface. The attached asymptomatic leaves were also imaged in the same way and acted as a control. Previously, the validity and reliability of the IR thermal imaging camera performance had been ascertained by checking thermographs of human hands and light-emitting bulbs that had been acquired using the same IR thermal imaging camera (data not shown). The mean leaf temperatures of the fungi-colonized regions (n = 35 for sooty mold and n = 9 for powdery mildew) were compared to the control regions (n = 44) by Duncan’s multiple range test using SAS PROC GLM (SAS Institute, Cary, NC, USA).

Leaves that showed signs of either sooty mold infestation or powdery mildew infection were detached, kept in paper envelopes, and air-dried at room temperature (20–24°C) for two months for comparison with the epidermal coverage by fungi. To minimize the loss of fungal hyphae and spores during conventional specimen preparation for scanning electron microscopy, leaf fragments (5 × 10 mm²) were excised from the dried leaves, mounted on a metal stub with double-sided copper tape, and coated with platinum using a sputter-coater (SCD 005; BAL-TEC, Balzers, Liechtenstein) (Paudyal and Hyun, 2015). The specimens were observed with a field emission scanning electron microscope (Supra 55VP; Carl Zeiss, Oberkochen, Germany) at 2 kV.

Unshaded asymptomatic leaves that had been exposed to sunlight had no sooty regions or any detectable disease symptoms (Fig. 1A). The thermograph showed that the temperature was above 25°C throughout the adaxial leaf surface (Fig. 1B). The spatial patterns for temperature distribution were shown by a false-color scale. There was a subtle difference in leaf temperature (0.5°C) between the highest and the lowest temperatures on the same leaf surface. Some asymptomatic leaves were shaded from the sunlight either by the flowers or the flower stalks at the nodes (Fig. 1C). Even shading resulted in temperature variations (1.0°C) throughout the leaf surface (Fig. 1D). Lower temperatures were detected in shaded regions on the leaf surface, compared to unshaded leaf regions.

Sooty regions were found on the adaxial leaf surface (Fig. 2A), but no sooty regions were observed on the abaxial leaf surface (data not shown). There were distinct color variations in the thermograph, which showed temperature variations (4.5°C) between the highest temperature and the lowest temperature on the same leaf surface (Fig. 2B). The sooty regions corresponded to the location of the highest temperature in the thermograph. Furthermore, several leaves on the same twig had sooty regions on the leaf surface (Fig. 2C). Elevated temperatures were recorded in the sooty regions on each leaf (Fig. 2D). In the thermal image, the sooty regions on each leaf were a distinct yellow color, compared to the violet color in non-sooty (control) regions on the same leaf.

Powdery mildew produced white mycelia and numerous conidia as powdery mildew signs on the adaxial leaf surface (Fig. 3A, C). However, the spatial patterns for...
Thermal Imaging of Sooty Mold-Colonized Leaves

Temperature distribution were not correlated with the regions of powdery mildew signs, showing higher and lower temperatures, compared with the regions with no powdery mildew signs (Fig. 3B, D).

Different mean leaf temperatures were recorded in the control regions and fungi-colonized regions (Fig. 4). The mean leaf temperature of the control regions was 26.98°C. However, the mean temperatures recorded in the sooty mold-colonized regions and the powdery mildew-colonized regions were 28.44°C and 28.30°C, respectively. Six instances out of nine observations were noted where the powdery mildew-colonized regions were warmer than the control regions. There were also significant differences in the mean leaf temperatures between the control regions and fungi-colonized regions ($P = 0.01$).

Field emission scanning electron microscopy revealed that the adaxial leaf surface of the shrub was covered with sooty mold (Fig. 5A). Stomata were present only on the abaxial leaf surface of the shrub (data not shown). Neither epidermal penetration nor cell wall dissolution by the fungi was observed on the leaf surface. The hyphae grew on the leaf surface and had tapering distal ends. Spores had both transverse and longitudinal septa (data not shown). Such mycological characteristics were strikingly similar to those found in *Metacapnodium* species (Rikkinen et al., 2003). In addition, the powdery mildew-colonized leaves also showed hyphal proliferation on the leaf surface (Fig. 5B). The hyphae were branched and formed erect and cylindrical conidiophores, which are typical of the *Erysiphe* species anamorph. There were no significant differences in the epidermal coverage by the sooty mold or powdery mildew.

This study demonstrated that the application of digital IR thermal imaging of the sooty mold disease improves our understanding of sooty mold infestation from the thermodynamic perspective. Sooty mold is reported to
aggravate sunburn injuries because black surfaces absorb more heat from the sun and become warmer than normal plant surfaces (Sibbett et al., 1971). Based on the false-color scale, the digital IR thermal imaging provided simple, rapid, and intuitive information on local warming in the sooty mold-colonized regions of crape myrtle leaves, which proved the long-standing hypothesis of sooty mold infestation. To our knowledge, this is the first use of digital IR thermal imaging to investigate plant responses to sooty mold infestation.

The elevated temperature in sooty regions appears to be derived from the black coating to the leaf surface caused by the sooty mold. Through black-body radiation, the black melanin pigment in the sooty mold may absorb all incident solar radiation, which leads to an increase in local temperature in a particular region. This phenomenon is similar to the higher accumulation of heat in a house with a black roof compared to one with a white or green roof (Sproul et al., 2014). Such a black roof effect is considered to be an important factor in sooty mold infestation: warmer, black leaves due to the absorbed sunlight are assumed to increase the cooling load on plant metabolism. The effects of local warming by sooty mold on the physiological aspects of trees such as photosynthesis, (photo) respiration, and transpiration await further studies.

Our current understanding of the superficial growth of sooty mold on the adaxial leaf surface without stomata suggests that the effects of host cell degeneration and abnormal stomatal opening on the leaf temperature changes are negligible. Melanin is also known to protect fungal cells from a variety of stress factors, such as desiccation, ultraviolet rays, and extreme temperatures, all of which are against fungal epiphytic growth (Kim, 2016).

In contrary to the hypothesis that powdery mildew-

**Fig. 2.** Comparison between photographs and thermographs of attached sooty leaves from *Lagerstroemia indica*. (A) Photograph of sooty leaves. Arrows indicate sooty regions on the leaves. (B) Thermograph of the sooty leaves shown in Fig. 2A. (C) Photograph of sooty leaves on a twig. There were several sooty regions (arrows) on the leaves. (D) Thermograph of the sooty leaves on the twig shown in Fig. 2C. The color bars (B, D) show the temperature gradient.
Thermal Imaging of Sooty Mold-Colonized Leaves

Colonized regions are colder than sooty regions, powdery mildew-colonized white leaf regions showed an overall increase in temperature, which was similar to sooty regions. This result was inconsistent with the wheat-powdery mildew *Blumeria graminis* (synonym *Erysiphe graminis*) pathosystem (Bayoumi and Abdullah, 2016). The obligate parasite *Erysiphe* species rarely destroys host plants. It obtains its nutrition and water from host cells through haustoria. It is possible that the loss of host cell membrane integrity may result in larger amounts of apoplastic water in infected tissues, leading to a reduction in natural cooling capacity, as suggested in downy mildew-infected leaves (Lindenthal et al., 2005). The temperature fluctuations in white regions might be attributed to the infection stage of powdery mildew and this may occur before the development of symptoms. The types of fungal growth, such as epiphytic saprotrophy and

Fig. 3. Comparison between photographs and thermographs of attached powdery mildew-infected leaves from *Lagerstroemia indica*. (A, C) Photographs of powdery mildew-infected leaves. (B, D) Thermographs of the powdery mildew-infected leaves shown in Fig. 3A and C, respectively. The color bars (B, D) show the temperature gradient.

Fig. 4. Comparison of mean leaf temperatures recorded in the control regions and the fungi-colonized regions. Bars indicate the standard deviations. The same letters denote no significant difference at $P = 0.01$ according to the Duncan’s multiple range test.
in planta biotrophy, are probably more associated with the leaf temperature change than the surface color of each fungal colonizer.

In conclusion, digital IR thermography can be used to evaluate the effects of sooty mold infestation on leaf temperature changes. The temperature distribution across crape myrtle leaves was spatially heterogeneous and was dependent on the epidermal coverage of sooty mold. The hot spots that appeared in the sooty regions were possibly due to the black fungal mycelial mats. Further studies are needed to ascertain whether the sooty mold-colonized shrub has the ability to acclimate to shading and warming without significant changes in plant growth.

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Fig. 5. Field emission scanning electron micrographs of the adaxial leaf surface of Lagerstromia indica. (A) Sooty mold-infested leaf. Hyphae had tapering distal ends (arrows). (B) Powdery mildew-infected leaf. Erect conidiophores (arrows) were present on the leaf surface. Scale bars = 20 μm.
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