Alternaria Leaf Spot on Three Species of *Pelargonium* Caused by *Alternaria alternata* in Japan

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ABSTRACT

A new disease of pelargonium (Pelargonium domesticum Bailey), ivy geranium (P. peltatum (L.) L'Her. ex Ait.) and scented geranium (P. graveolens L'Her.), primarily causing brown spots on leaves, was found in Kawasaki-shi in Kanagawa Prefecture and Tachikawa-shi in Tokyo. An Alternaria sp. was consistently isolated from these diseased leaves, and the isolates were pathogenic to their host leaves. Based on morphological characteristics, the causal fungus in all three cases was identified as Alternaria alternata (Fr.) Keissler. Because Alternaria leaf spot of geranium by A. alternata has already been reported, the pathogenicity of isolates from four groups of genus Pelargonium was investigated. The isolates from scented geranium were pathogenic only to their original host, but the isolates from pelargonium, ivy geranium and geranium were pathogenic to all groups of pelargonium. This is the first report of this disease on pelargonium, ivy geranium and scented geranium caused by A. alternata in Japan. We propose the names for these diseases as Alternaria leaf spot of pelargonium (kappan-byo), Alternaria leaf spot of ivy geranium (kappan-byo) and Alternaria leaf spot of scented geranium (kappan-byo).

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Key words: ivy geranium, pelargonium, scented geranium, Alternaria alternata.

INTRODUCTION

Plants belonging to the genus Pelargonium are one of the most popular and widely cultured ornamental plants in the world. The genus Pelargonium is horticulturally divided into four groups⁷⁾. The zonal group, commonly called geranium, has a characteristic dark-colored circular band bordering their leaves. The regal group, usually called pelargonium, has a trumpet-shaped flower. Ivy geranium has thick leaves and grows in the manner of ivy. Scented geranium has small flowers and is cultured to produce perfumes or mosquito-repelling incense. Alternaria leaf spot of geranium by Alternaria alternata has already been reported in Japan^{10,11)}, Italy²⁾, Greece⁵⁾ and the United States⁹⁾, but not on the other groups of Pelargonium in Japan¹²⁾. This paper describes Alternaria leaf spot of pelargonium, ivy geranium and scented geranium by A. alternata in Japan. Preliminary results were reported elsewhere4).

MATERIALS AND METHODS

Isolation of the causal fungi Fungi were isolated

from the spots on leaves of pelargonium in Tachikawashi, Tokyo in 1998, of ivy geranium in Kawasaki-shi, Kanagawa Prefecture in 1999 and of scented geranium in Tachikawa-shi, Tokyo in 1999. Spots from the leaves of each plant were cut and surface-sterilized with 80% ethanol for 10 sec, washed with sterilized water and placed on water agar. The plates were incubated for a few days at room temperature, and small pieces of mycelia were cut and placed on PDA. After 7 days' incubation in the dark at room temperature, they were placed under continuous BLB irradiation for about 7 days. Monoconidium was isolated for each and used for further experiments.

Cultural and morphological characteristics The isolates were identified by comparing cultural characteristics and conidial morphology^{3,6,10,13)}. The size of conidia from isolates on leaf spots of geranium caused by *Alternaria* was also measured for detailed comparison.

Inoculation tests Each of the nine isolates was cultured on V8 medium at 25° C in the dark for 1 week, then under continuous BLB light irradiation for 1 week. The conidia were collected and suspended in distilled water. The concentration of conidia was adjusted to approximately 1×10^{5} /ml. Small, healthy plants of pelar-

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gonium were obtained from Tochigi Agricultural Experiment Station, those of ivy geranium were obtained from a local market and those of scented geranium were propagated from cuttings. All were potted and grown in a glasshouse. Suspensions of conidia were sprayed on two or three intact healthy plants, which were covered with plastic bags after inoculation, then placed in shade outdoors for 48 hr. After removal of the bags, the plants were placed in a glasshouse. Leaves with spots were counted 1 month after inoculation. Sterilized water was used for a negative control.

RESULTS

Symptoms

We found severe leaf spots on leaves of potted plants of pelargonium in Tachikawa-shi, Tokyo in 1998 (Plate I-A). On the leaves, brown irregular spots developed, then entire leaves turned yellow and fell. The spots were also found on flower petals when the disease was severe (Plate I-B). The same symptoms were found on plants in Kita-ku in Tokyo and in Tochigi Prefecture. Many field-grown plants were also diseased in Tochigi Prefecture.

Similar spots were also observed on potted plants of ivy geranium in Kawasaki-shi, Kanagawa Prefecture in 1999 and scented geranium in Tachikawa-shi in 1999 (Plate I-C, D). In the case of ivy geranium and scented geranium, brown spots formed only on leaves.

Isolation and identification of the causal fungi

An Alternaria sp. was most frequently isolated from the lesions of leaves (Plate I-E). On PDA, the fungal

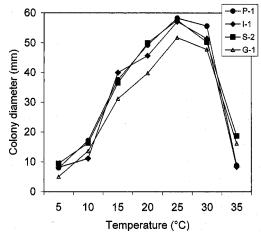


Fig. 1. Growth of mycelial colonies of Alternaria isolates on PDA at different temperatures. Colony diameters of 7-day-old cultures in the darkness were measured. P-1 is the isolate from pelargonium. I-1 is from ivy geranium. S-2 is from scented pelargonium. G-1 is from geranium.

Table 1. Comparison of morphology of conidia produced on PDA by the isolates of Alternaria

| Reference | | | | | | | | | | Takano et al, | $1993^{5)10)}$ | Takano et al., | $1993^{\circ)10)}$ | Inoue and Nasu, | 20006) | |
|----------------------------|-----------------------|-----------------|---------------|-----------------------------|-------------------|----------------------------|------------------|-------------------------------------|-----------------------|-------------------------------|----------------------|----------------|---------------------------|-----------------|--------------------|---|
| Septa | Longitudinal/Obilique | Mean±S.E. | | 0.8 ± 0.1 | | 0.8 ± 0.1 | | $1\!\pm\!0.1$ | | 2 ± 0.1 | T | | T | | - | |
| | Longitue | Range | | 0-2 | | 0-2 | | 0-3 | | 0-3 | ١ | | 1 | | 0-4 | |
| | Transverse | Range Mean±S.E. | | 3 ± 0.1 | * 4 | 3 ± 0.1 | | 4 ± 0.1 | s* . | $4\!\pm\!0.2$ | 1 | | 1. | | 1 | |
| | Tra | Range | | 1-4 | | 1-4 | | 2-7 | | 2-7 | 1 | | I | | 0-10 | |
| Rooka) | Deak | Kate (%) | | 75.2 | | 91.3 | ` | 77.4 | | 86.7 | 1 | | 1 | | 1 | |
| rio in | liam. | Range Mean±S.E. | | $5\!\pm\!0.2$ | | 5 ± 0.1 | | 7 ± 0.3 | | $4\!\pm\!0.2$ | ľ | , | 1 | | I | |
| ۳ | ١ | Kange | | 3-9 | | 3-7 | | 3-13 | | 3-5 | g | | . [| | 1 | |
| Size (lenoth × width) (.m) | widelly (will) | Mean⊤5.E. | | $31\pm1.0\!	imes\!14\pm0.4$ | | $32\pm0.9{	imes}16\pm0.6$ | | $28\!\pm\!1.0\!	imes\!12\!\pm\!0.4$ | | $27{\pm}1.0{	imes}13{\pm}0.3$ | $49{	imes}16$ | | $26.5{\times}11.4$ | | $32{	imes}13$ | |
| | ।∨गावधाना) चटाट | Kange | | $22.552.5\times\ 1017.5$ | | $22.5-47.5 \times 10-27.5$ | | $15-45 \times 10-17.5$ | nium | $13.8-40 \times 8.8-18.8$ | 29–87 $	imes 10$ –23 | | $16.3-60.6\times7.5-17.3$ | | $9-71 \times 6-18$ | |
| | Isolate | | from Geranium | G-1 | from Ivy geranium | 1.1 | from Pelargonium | P-1 | from Scented geranium | S-2 | A. alternata | | A. alternata | | A. alternata | a |

d) Not reported. c) Conidia formed on PSA. b) Conidia formed on diseased leaves. Percentage of conidia with beaks.

isolates from leaf spots grew most rapidly at 25°C (Fig. 1). At first, the colonies were white, then turned pale gray with age. The appearance of all isolates was the same. After BLB irradiation, cultures conidiated to some extent on PDA and extensively on V8. Based on cultural and morphological characteristics such as size of conidia, existence of a short beak and chain number, the causal fungus was identified as *Alternaria alternata* (Fries) Keissler (Table 1).

Optimal growth temperature of the *Alternaria* isolates

As shown by the colony diameters of 7-day-old cultures of the strains on PDA in 9-cm petri dishes at temperatures ranging from 5°C to 35°C (Fig. 1), these strains grew well between 15°C and 30°C. The optimal temperature was all 25°C.

Pathogenicity

All the isolates were pathogenic to their hosts, though the incidence of infected leaves was not high and varied among samples (Table 2). Yellowish spots began to form on intact leaves about a week after inoculation. One month after inoculation, the spots turned brown, as did the naturally occurring spots. The spots on ivy geranium became dented within 1 month after inoculation.

A. alternata was isolated from the spots and was pathogenic to their hosts.

Table 2. Pathogenicity of the isolates of Alternaria on Pelargonium

| returgontum | | | | | | | | | | | | |
|-----------------------|---|-----------------|------------------------------------|------------------------|--|--|--|--|--|--|--|--|
| | Percentage of infected leaves ^{a)} | | | | | | | | | | | |
| Isolate | Geranium | Ivy geranium | Pelargo- nium | Scented geranium | | | | | | | | |
| from Geranium | | | | | | | | | | | | |
| G-1 | $36\pm~7$ | 28 ± 7 | $20\pm~3$ | 10 ± 1 | | | | | | | | |
| G-2 | 36 ± 10 | $17\!\pm\!1$ | 17 ± 1 | $14\pm~2$ | | | | | | | | |
| G-3 | $24\pm~3$ | $29\!\pm\!4$ | 22 ± 7 | $20\pm~2$ | | | | | | | | |
| from Ivy geranium | | | | | | | | | | | | |
| I-1 | $49\pm~6$ | $25\!\pm\!3$ | $13\pm~3$ | 24 ± 1 | | | | | | | | |
| from Pelargonium | | | | | | | | | | | | |
| P-1 | $26\!\pm\!10$ | 33 ± 1 | $40\pm 10^{\rm b}$ | 11± 1 | | | | | | | | |
| P-2 | $6\pm~1$ | 18 ± 5 | $27\pm~3^{\scriptscriptstyle (b)}$ | $11\pm~3$ | | | | | | | | |
| P-3m | $25\pm~8$ | 23 ± 3 | $19\pm~8^{\mathrm{b}}$ | 10± 5 | | | | | | | | |
| from Scented geranium | | | | | | | | | | | | |
| S-2 | O _{p)} | 0 | 0 | 23± 3 ^{b)} | | | | | | | | |
| S-3 | $O_{\rm p)}$ | O _{p)} | 0 | $26 \pm 10^{\text{b}}$ | | | | | | | | |
| $Control^{c)}$ | $0_{p)}$ | O _{p)} | $O_{\rm p)}$ | 0 _p) | | | | | | | | |

- a) Percentage of infected leaves=(No. of infected leaves/No. of total leaves) \times 100. Two plants were used for each experiment unless indicated otherwise. Values are average \pm S.E.
- b) Three plants were used for each experiment.
- c) Water was sprayed as control.

Cross inoculation

The isolates from pelargonium, geranium and ivy geranium were pathogenic to all four groups of *Pelargonium* (Table 2). Pathogenicity to the original host plants and to plants of the other groups was not significantly different for each isolate except for the isolates from scented geranium, which were pathogenic only to their original host.

DISCUSSION

Leaf spot diseases found on pelargonium, ivy geranium and scented geranium were examined. The causal fungus on all three groups was A. alternata (Fr.) Keissler, the same agent that causes Alternaria leaf spot of geranium¹⁰⁾.

In artificial inoculation tests with the causal A. alternata, the disease severity on each host plant was not significantly different among the four groups. This tendency does not fit with the natural occurrence of the disease on the four groups. Alternaria leaf spot of geranium occurs on plants grown in many places under ordinary culture conditions. The severity is, however, light, and serious symptoms are scarce. Similarly, no serious damage was found on ivy geranium and scented geranium. On the contrary, the disease could be severe on pelargonium both in yards and fields under specific conditions such as high humidity.

The host range of the isolates from pelargonium, ivy geranium and geranium was wide; they were pathogenic to all four groups of *Pelargonium*. Plants of these four groups are widely cultivated in commercial fields and home gardens. Once one species becomes diseased, plants of the other groups may also be infected. On the contrary, the isolates from scented geranium were pathogenic only to the original host.

Because some kinds of pathogenic A. alternata produce host-specific toxins, which determine host range, such as AK-, AM-, and AAL-toxin[®], we examined whether each isolate produces diffusible toxin(s) by the standard method using detached leaves (unpublished results). However, none of the germination media with or without conidia caused a toxic reaction in any leaves of the four groups of Pelargonium, suggesting that these isolates do not produce host-specific toxins.

Though Takano had named spot disease of geranium by A. alternata "leaf spot", we named the leaf spot diseases caused by A. alternata "Alternaria leaf spot" This is because spot disease of geranium caused by Fusarium had already been named "leaf spot" and the name "Alternaria leaf spot" has been used in some countries^{2,9}. We named each of this disease on the four groups of the genus Pelargonium independently: Alternaria leaf spot of

pelargonium (Pelargonium kappan-byo), Alternaria leaf spot of ivy geranium (Ivy geranium kappan-byo) and Alternaria leaf spot of scented geranium (Nioi geranium kappan-byo). We did not adopt the general name Alternaria leaf spot of *Pelargonium* because these four groups are treated separately in the commercial flower market and in books for the home gardener¹⁾.

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Plate I

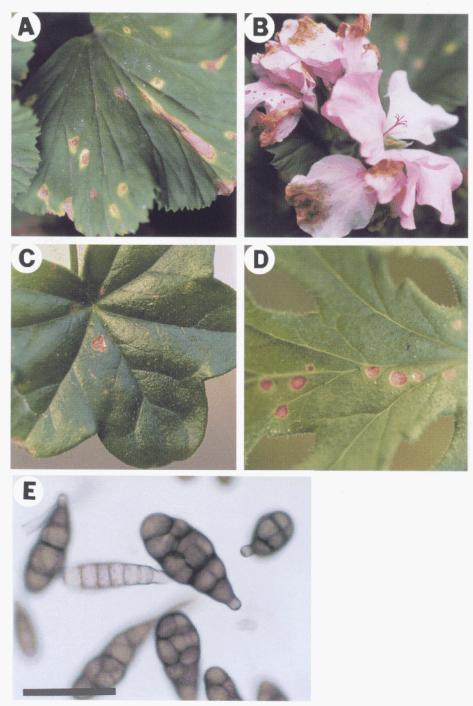


Plate I

- A. Spots on naturally infected leaves of pelargonium.
- B. Spots on naturally infected flowers of pelargonium.
- C. Spots on naturally infected leaves of ivy geranium.
- D. Spots on naturally infected leaves of scented geranium.
- E. Conidia of Alternaria alternata P-1 formed on PDA (Bar is $30 \mu m$).