

## Puccinia benkeiによるカラシコエのさび病(新称)

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## Rust of Kalanchoe Caused by *Puccinia benkei*\*

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### Abstract

Since summer in 1991, a new rust disease has occurred on leaves of potted plants of *Kalanchoe blossfeldiana* growing in greenhouses in Konosu, Saitama Prefecture. Dark brown pustules in lesions on the leaves were mainly composed of 2-celled teliospores, with a few 1-celled ones. Leaves of potted plants of *K. blossfeldiana* were artificially inoculated with basidiospores. Whitish spots appeared on the upper surface of the leaves eight to nine days after inoculation. Sixteen to 19 days after inoculation, dark brown pustules containing teliospores appeared in the lesions. The fungus was also able to infect *Sedum kamtschaticum* and *Sedum* sp. From morphological observation of the teliospores and from results of inoculation experiments, the causal fungus of the disease was identified as *Puccinia benkei* Kusano, a microcyclic rust. We propose 'Sabi-byo' and 'Rust' as a Japanese common name and an English common name of the new disease on Kalanchoe, respectively.

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**Key words:** *Puccinia benkei*, rust fungus, *Kalanchoe blossfeldiana*, *Sedum kamtschaticum*, *Sedum* sp., identification.

### INTRODUCTION

*Kalanchoe blossfeldiana* Poelln. is a popular potted plant in Japan<sup>1)</sup>. In summer 1991, a new rust disease was found on leaves of this plant growing in greenhouses in Konosu, Saitama Prefecture. Whitish or brown spots (up to 5 mm in diam.) with dark brown pustules appeared on the leaves. Because of these dirty spots, value of the damaged plants in the market was reduced. Heavy infection caused yellowing and early defoliation of the leaves. The occurrence of the disease was particularly serious on potted plants which had been cultivated in mountainous areas in Nikko (about 1,300 m above sea level), Tochigi Prefecture or Karuizawa (about 1,000 m above sea level), Nagano Prefecture. To induce flowering, these plants were grown during summer in the cooler mountainous areas, and subjected to a short-day treatment.

In the present paper, the rust fungus causing the disease is identified based on morphological observations and results of inoculation experiments. Furthermore, possible primary inoculum sources in the field are discussed.

### MATERIALS AND METHODS

Diseased plants of *K. blossfeldiana*, collected in greenhouses in Konosu, Saitama Prefecture, were used for morphological observation of spores, a germination study, and as a source of inoculum.

For morphological observation of spores, dark brown pustules produced in lesions on the leaves

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were cut by scalpel, and mounted in a drop of water or 1% lacto-fuchsin on a glass slide. Spores were observed under a light microscope.

Pustules were also cut from the lesions and suspended in a drop of water on a glass slide. The slide was kept in a moist chamber at 20°C in the dark for 24 hr. Germination of spores was observed under a light microscope.

To examine symptom development and life cycle of the fungus, inoculation experiments were performed. Pustules were cut from lesions and placed on small pieces of moist filter paper (ca. 3 mm × 3 mm). The filter paper with pustules were then placed on the upper surface of young leaves of potted plants of *K. blossfeldiana*, which had been grown in a growth cabinet controlled at about 20°C with 16 hr light/8 hr dark. After inoculation, the plants were kept in a moist chamber at about 20°C in the dark for 2 days, and then returned to the growth cabinet. The inoculated plants were observed for 1.5 months.

Potted plants of *Sedum kamtschaticum* Fish. obtained from Tsukuba Botanical Garden, National Science Museum and *Sedum* sp. collected from the field in Nikko, were also inoculated by the method described above.

Spores and sori resulting from the inoculation experiments were also observed in water or 1% lacto-fuchsin, and by making sections of the leaves. The following dry herbarium specimens of *Puccinia benkei* collected various localities in Japan were used for morphological comparison: TSH-R1382 (a part of holotype HH-95803) on *S. telephium* L. var. *purpureum* L., TSH-R1383 (HH-95795), TSH-R1384 (HH-95801), TSH-R1385 (HH-95798) on *S. kamtschaticum*, and TSH-R1388 (HH-95794) on *S. aizoon* L.

Since the disease was particularly serious on potted plants which had been cultivated in mountainous areas during summer, we speculated that a primary inoculum source was present in these areas. Field surveys were conducted in these areas in summer 1993 to find the primary inoculum sources, or natural host plants.

## RESULTS AND DISCUSSION

### *Morphological observation of spores produced on naturally infected leaves*

The dark brown pustules produced in the lesion on the leaves of *K. blossfeldiana* collected from the greenhouse were mostly composed of 2-celled teliospores (Plate I-1). A few 1-celled teliospores (Plate I-2) were also present in the same pustules. The 2-celled teliospores were yellowish brown to chestnut-brown, ellipsoid, fusiform or sometimes irregular in shape, and 30–42 × 20–27 μm. The apex of the spores was roundish or obtuse and thickened (up to 8 μm). The pedicel was colorless, persistent, and up to 138 μm. The 1-celled spores were ellipsoid or fusiform, and 26–45 × 20–28 μm, but otherwise the same as the 2-celled spores. The morphological characteristics of the fungus was identical to the description of *Puccinia benkei* Kusano<sup>5,6,8,9</sup>, which is parasitic on *Sedum* spp. (Crassulaceae).

### *Germination experiment*

Most of the teliospores germinated without dormancy. In most cases, the spores formed a 4-celled basidium. A basidiospore was produced at the tip of the sterigma extruded from each cell (Plate I-3).

### *Inoculation experiment*

Whitish spots (Plate I-4) appeared on the upper surface of leaves of *K. blossfeldiana* eight to nine

Table 1. Results of inoculation experiments of *Kalanchoe blossfeldiana* and *Sedum* spp. with basidiospores of *Puccinia benkei*.

Plant inoculated	Date of inoculation	Days required for the first appearance of	
		Whitish lesion	Telium
<i>Kalanchoe blossfeldiana</i>	Nov. 17, 1992	8	16
	Dec. 16, 1992	9	19
<i>Sedum kamtschaticum</i>	Nov. 17, 1992	— <sup>a)</sup>	20
	Dec. 16, 1992	—	19
<i>Sedum</i> sp.	Nov. 10, 1993	9	16

a) Whitish lesions were not able to be recognized.

Table 2. Dimensions of 2-celled teliospores of *Puccinia benkei* on *Kalanchoe blossfeldiana* and on *Sedum* spp.

Specimen No.	Host plant	Locality of collection	Size ( $\mu\text{m}$ )	Wall thickness at the apex ( $\mu\text{m}$ )	Wall thickness at the side ( $\mu\text{m}$ )	Length of pedicel ( $\mu\text{m}$ )
TSH-R1403	<i>Kalanchoe blossfeldiana</i>	Konosu, Saitama Pref.	30-42 (37.3 $\pm$ 2.9) <sup>a)</sup> $\times$ 20-27 (22.9 $\pm$ 2.0)	4-10 (5.5 $\pm$ 1.3)	2-3 (2.8 $\pm$ 0.4)	53-138 (81.2 $\pm$ 22.1)
TSH-R1382 (isotype)	<i>Sedum telephium</i>	Nikko, Tochigi Pref.	32-42 (37.4 $\pm$ 2.8)	6-9 (7.3 $\pm$ 0.8)	3.6-4 (3.7 $\pm$ 0.2)	82-138 (112.9 $\pm$ 15.8)
TSH-R1383	var. <i>purpureum</i> <i>S. kamischaticum</i>	Mt. Fuji, Yamanashi Pref.	$\times$ 22-28 (24.2 $\pm$ 1.5) 32-44 (37.4 $\pm$ 3.2)	6-8 (6.9 $\pm$ 0.7)	3-4 (3.5 $\pm$ 0.3)	80-132 (106.0 $\pm$ 17.6)
TSH-R1384	<i>S. kamischaticum</i>	Mt. Fuji, Yamanashi Pref.	32-39 (34.6 $\pm$ 2.0)	4-7 (6.2 $\pm$ 0.8)	3-4 (3.4 $\pm$ 0.4)	80-128 (95.5 $\pm$ 13.1)
TSH-R1385	<i>S. kamischaticum</i>	Mt. Fuji, Yamanashi Pref.	$\times$ 20-24 (22.1 $\pm$ 1.2) 34-40 (36.7 $\pm$ 1.8)	6-8 (6.9 $\pm$ 0.7)	3-4 (3.5 $\pm$ 0.3)	80-146 (102.3 $\pm$ 15.1)
TSH-R1388	<i>S. aizoon</i>	Mt. Asahi-dake, Yamagata Pref.	$\times$ 20-25 (22.2 $\pm$ 1.2) 32-42 (36.7 $\pm$ 2.7) $\times$ 20-24 (22.3 $\pm$ 1.3)	5-8 (6.5 $\pm$ 0.9)	3-4 (3.6 $\pm$ 0.3)	76-140 (114.4 $\pm$ 20.4)

a) Numbers in parenthesis showed average $\pm$ standard deviation ( $n=20$ ).

days after inoculation. They appeared only on leaves which were very young when inoculated. The spots increased in size and spread to the lower surface of the leaves. Sixteen to 19 days after inoculation, dark brown pustules appeared in the lesions (Table 1). As the lesions enlarged, pustules were produced centripetally in the lesions (Plate I-5) on both sides of the leaves. Some of the lesions turned brown and were dried-up by 30 days after inoculation, but others continued to enlarge and to produce pustules. Maximum size of lesions was 10 mm in diameter 65 days after inoculation.

The pattern of symptom development on *Sedum* sp. was the same as that on *K. blossfeldiana*. Nine days after inoculation, whitish spots appeared on the upper surface of inoculated leaves. Sixteen days after inoculation, dark brown pustules appeared in the lesions (Table 1).

On inoculated leaves of *S. kamtschaticum*, there was no clear discoloration prior to the production of pustules. Nineteen to 20 days after inoculation, dark brown pustules were produced on the inoculated leaves (Table 1). Pustules were produced centripetally in the lesions on both sides of the leaves.

The morphology of teliospores produced in pustules (telia) on leaves of inoculated *K. blossfeldiana*, *S. kamtschaticum*, and *Sedum* sp. was the same as that produced on naturally infected leaves of *K. blossfeldiana* in the greenhouse. No other kinds of spores and sori (spermogonia, aecia, or uredinia) were produced in the lesions.

#### **Taxonomy of the fungus**

From the results of the present study, the pathogen of the new leaf spot disease of *K. blossfeldiana* is considered to be *P. benkei*, a microcyclic rust fungus. One species of *Puccinia*, *P. longissima* Schroeter has been recorded on *Kalanchoe* species in Europe<sup>2)</sup>. However, this fungus is a heteroecious, long-cyclic species with only the spermogonial and aecial stage on *Kalanchoe* species. There is no report on occurrence of telia on *Kalanchoe* species. Two microcyclic species of *Puccinia* have been recorded on plants belonging to Crassulaceae in Japan, i.e., *P. umbilici* Guepin ex Duby (= *P. rhodiolae* Berk. et Br.) and *P. benkei*<sup>3-6)</sup>. The former parasitises *S. rosea* (L.) Scop. and the later *S. aizoon* L., *S. kamtschaticum*, and *S. telephium* L. var. *purpureum* L.<sup>4,5)</sup>. Their teliospores are morphologically different<sup>4,5)</sup>. Teliospores of *P. umbilici* are broadly ellipsoid and often broader than length and have short fragile pedicels. The apex of the teliospores is not thickened. On the other hand, teliospores of *P. benkei* are ellipsoid, fusiform or sometimes irregular in shape and have long persistent pedicels. The apex of the teliospores is thickened. Results from morphological observation and inoculation experiments, in the present study, showed that the fungus causing leaf spot disease of *K. blossfeldiana* is *P. benkei*. Furthermore, a comparison of the fungus on *K. blossfeldiana* with herbarium specimens of *P. benkei*, including the type specimen (Plate I-6), demonstrated that there was no distinguishable difference between them in morphology of teliospores (Table 2). Thus, we conclude that the pathogen of the new leaf spot disease of *K. blossfeldiana* is *P. benkei*. We propose 'Sabi-byo' and 'Rust' as a Japanese common name and an English common name, respectively.

#### **The possible infection source in the field**

*Puccinia benkei* is distributed in Japan and Russia<sup>1,5)</sup>. In Japan, the fungus has been recorded in Central and Northern Honshu, including Tochigi Prefecture<sup>4)</sup>. *P. benkei* was described by Dr. Kusano based on a specimen on *S. telephium* var. *purpureum* collected in Nikko<sup>8)</sup>. We could not find *P. benkei* on *Sedum* species in the present field survey in Nikko and Karuizawa. There were some plants of *Sedum* sp. in a grassland, mainly composed of *Miscanthus sinensis* Anderss., near the field used for the short-day treatment of *Kalanchoe*. These plants were infected with the fungus in the present inoculation experiment. Thus, we speculate that the fungus on *Sedum* sp. had an opportunity to encounter *Kalanchoe* during the short-day treatment, in which the potted plants were covered by a shading sheet for about 16 hr per day. These conditions appear to be suitable for the fungus to infect plants. Further field surveys to find the inoculation source is desired.

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## 和 文 摘 要

山岡裕一・飯田 一・柿島 真：*Puccinia benkei*によるカランコエのさび病（新称）

1991年の夏ごろから、埼玉県鴻巣市周辺のカランコエ (*Kalanchoe blossfeldiana*) の鉢植栽培で、葉に黒色の斑点を生ずる被害が発生した。斑点内には、直径約0.3 mmで黒褐色の冬胞子堆が形成されていた。冬胞子堆には、2細胞の冬胞子の他に1細胞の冬胞子がわずかに混在していた。本菌の冬胞子を発芽させ、カラコエに接種した結果、接種後8~9日で葉上に白色の病斑が形成され、接種後16~19日にはその病斑上に冬胞子堆が形成された。また、本菌はキリンソウ (*Sedum kamtschaticum*) および *Sedum* sp.にも感染することができた。光学顕微鏡による冬胞子の形態観察および接種試験の結果より、本病害の病原菌を *Puccinia benkei* と同定した。本病害を、カラコエさび病 (Rust) と呼称することを提案する。

## Explanation of plate

Plate I *Puccinia benkei*.

1. Two-celled teliospores produced in a telium on a leaf of *Kalanchoe blossfeldiana*. (Scale bar: 20  $\mu$ m)
2. One-celled teliospore (arrow head) produced in a telium on a leaf of *K. blossfeldiana*. (Scale bar: 20  $\mu$ m)
3. A germinating teliospore. Note the production of four basidiospores from the basidium with four cells. (Scale bar: 10  $\mu$ m)
4. Whitish lesions (arrow head) on a leaf of *K. blossfeldiana* 8 days after inoculation with basidiospores.
5. Telia (T) produced on a leaf of *K. blossfeldiana* 23 days after inoculation with basidiospores.
6. Teliospores from the type specimen (TSH-R1382, a part of holotype HH-95803). (Scale bar: 20  $\mu$ m)

Plate I

