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FULL PAPER

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Life cycle of *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis*

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Abstract Field observations and inoculation experiments revealed that *Uromyces appendiculatus* var. *azukicola* has an autoecious and macrocyclic life cycle and produces spermogonia, aecia, uredinia, and telia on *Vigna angularis* var. *angularis* and *V. angularis* var. *nipponensis*. From inoculation experiments, it was suggested that this rust fungus has different host relationships from other varieties. Morphological examinations revealed that the characteristics of urediniospores and teliospores are different among varieties, although aeciospores are morphologically similar to each other.

Key words Life cycle · *Uromyces appendiculatus* var. *azukicola* · *Vigna angularis*

Introduction

A rust fungus on *Vigna angularis* (Willd.) Ohwi & Ohashi var. *angularis* (=*Phaseolus radiatus* L. var. *aurea* Prain.) was reported as *Uromyces appendiculatus* (Pers.) Link. because of morphological similarity with a rust fungus on *Phaseolus vulgaris* L. (Ito 1922, 1950). Hirata (1952) examined the rust fungus on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi, and *V. umbellata* (Thunb.) Ohwi & Ohashi and found morphological differences from *U. appendiculatus*. Therefore, he newly described this fungus as *U. azukicola* S. Hirata (Hirata 1952). However, Hiratsuka et al. (1992) treated this rust as a variety of *U. appendiculatus* (Pers.) Unger. *Uromyces appendiculatus* var. *azukicola* (Hirata) Hiratsuka, f. has been reported to be morphologically different from *U. appendiculatus* var. *appendiculatus* in size of teliospores (Hiratsuka et al. 1992), although the difference is not clear because of overlap in spore size. This fungus is distributed throughout Japan (Hiratsuka 1973; Hiratsuka et al. 1992). However, the spermogonial and aecial stages of *U. appendiculatus* var. *azukicola* have not been reported, whereas other varieties of *U. appendiculatus* have been reported to have an autoecious and macrocyclic life cycle (Hiratsuka 1973; Hiratsuka et al. 1992).

In May 2002, we found the spermogonia and aecia of a rust fungus on leaves of *V. angularis* var. *nipponensis* on Mt. Nantai, Ibaraki Prefecture, Japan. The fungus was suspected to be *U. appendiculatus* var. *azukicola* from its morphology and host plants. We carried out inoculation experiments to clarify the life cycle and host plants of the rust fungus. We also report the morphology of the fungus and discuss its taxonomy.

Materials and methods

Basidiospore inoculation

Leaves of *V. angularis* var. *nipponensis* with abundant telia of *Uromyces* sp. were collected from Mt. Nantai, Ibaraki Pref., on September 1, 2002. The leaves were kept in a refrigerator at 4–5°C until use. The leaves were immersed in running tap water (about 20°C) for about 2–3 weeks to induce germination of teliospores. The teliospores germinated to produce numerous basidiospores within several days. For inoculation, small pieces of the leaves with germinating teliospores were placed on young, healthy leaves of *V. angularis* var. *nipponensis*. The inoculated plants were sprayed with distilled water and placed in a dark, moist chamber at about 20°C for 3 or 4 days, then transferred to a growth cabinet at about 20°C with controlled illumination

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(Kakishima et al. 1999; Kondo et al. 1997; Ono and Kakishima 1981).

Furthermore, teliospores formed on *V. angularis* var. *nipponensis* by aeciospore inoculation were also used as inocula to confirm the life cycle.

Aeciospore inoculation

Aeciospores formed on *V. angularis* var. *nipponensis* by the basidiospores inoculation, and those on the same host collected from Mt. Nantai, Ibaraki Pref. on May 27, 2002, were used as inocula. Aeciospores collected from aecia on the leaves were dusted with a scalpel onto pieces of wet filter paper (about 5 × 5 mm), which were then placed on the lower surface of young, healthy leaves of *V. angularis* var. *nipponensis* planted in clay pots. The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Urediniospore inoculation

Urediniospores from *V. angularis* var. *nipponensis* were dusted with a scalpel onto pieces of wet filter paper (~5 × 5 mm), which were then placed on the lower surface of healthy leaves of *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis*, *V. unguiculata* ssp. *unguiculata*, and *Phaseolus vulgaris.* The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Furthermore, urediniospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref., from June to August of 2002 were inoculated onto *Vigna radiata* (L.) Wilczek, *V. mungo* (L.) Hepper, *Phaseolus vulgaris*, *Vicia cracca* L., *V. faba* L., *V. amoena* Fisch., *V. unijuga* Al. Br., *Lathyrus maritimus* Bigel., *L. palustris* L., and *Pisum sativum* L.

Morphological observations

Specimens on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref., and obtained from inoculation experiments were used for morphological observations. These specimens were deposited as dry herbarium specimens in the Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba (TSH).

For light microscopy, hand sections of spermogonia, aecia, uredinia or telia, and aeciospores, urediniospores, or teliospores were mounted in a drop of lactophenol solution on glass slides. Fifty spores of each spore state were measured with an Image Analyzer (Leica Qwin).

For scanning electron microscopy (SEM), spores were dusted onto double-sided adhesive tape on specimen holders, and then coated with platinum-palladium with a Hitachi E-1030 Ion Sputterer. The spores were examined with a Hitachi S-4200 SEM operating at 15 kV.

Results and discussion

Life cycle

About 12–13 days after leaves of *V. angularis* var. *nipponensis* were inoculated with basidiospores (Fig. 1), yellow and yellow-brown spermogonia appeared on the surface of the leaves, and 8–9 days later, aecia were mostly produced on the lower surface of the leaves (Table 1).

About 4–5 days after leaves of *V. angularis* var. *nipponensis* were inoculated with aeciospores, yellow spots appeared on the leaves. Five to 6 days later, the spots turned brown and brownish and uredinia were produced. After 12–13 days, telia started to appear on the inoculated leaves of *V. angularis* var. *nipponensis* (Table 2).

From the inoculation experiments it was proved that the rust fungus on *V. angularis* var. *nipponensis* has an autoecious and macrocyclic life cycle. Hiratsuka et al. (1992) reported that *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus* are autoecious and macrocyclic rust fungi. Therefore, the three varieties have the same life cycle. We also found in the field that uredinia and telia occurred on *V. angularis* var. *nipponensis* on which spermogonia and aecia had been produced in spring (April to May).

Urediniospores formed on *V. angularis* var. *nipponensis* with aeciospore inoculation and those collected from Mt. Nantai, Ibaraki Pref., on the same host were used in

Table 1. Results of inoculation experiments with basidiospores taken from teliospores on *Vigna angularis* var. *nipponensis*

Inoculum[a]	Plant inoculated	Date of inoculation	Days required for the first appearance of	
			Spermogonia	Aecia
I	*V. angularis* var. *nipponensis*	Oct. 11, 2002	12	20
		Oct. 22, 2002	12	21
II	*V. angularis* var. *nipponensis*	Sept. 3, 2002	13	21

[a] I, Basidiospores from teliospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref.; II, basidiospores from teliospores formed on *V. angularis* var. *nipponensis* by aeciospore inoculation in June 2002

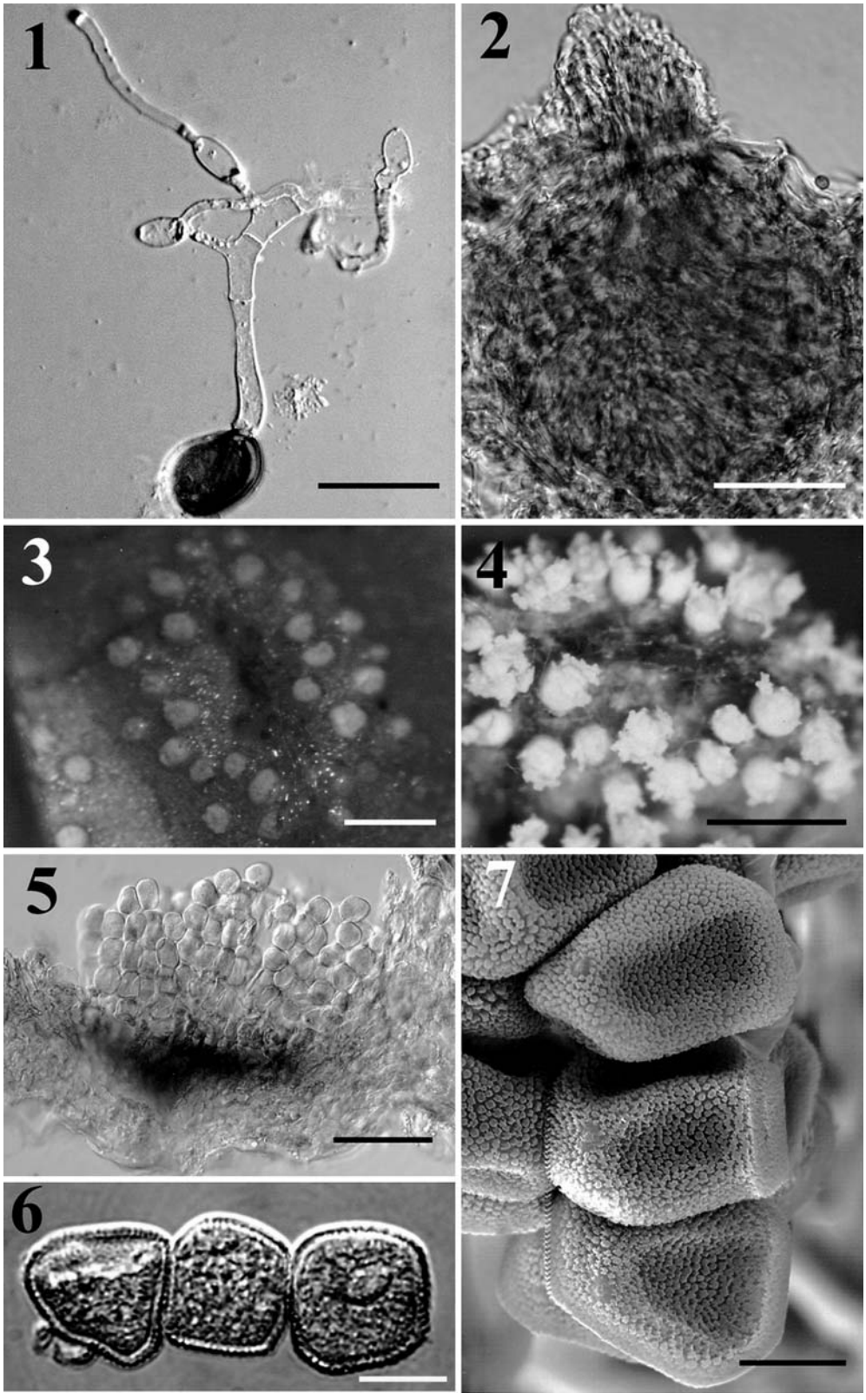

Fig. 1–7. *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis* var. *nipponensis*. **1** A basidium and basidiospores from a teliospore. **2** A cross section of a spermogonium produced by inoculation experiment. **3, 4** Aecia resulting from basidiospore inoculation. **5** A cross section of an aecium. **6, 7** Aeciospores under light **(6)** and scanning electron **(7)** microscopes. *Bars* **1** 30 μm; **2** 50 μm; **3, 4** 1 mm; **5** 50 μm; **6** 10 μm; **7** 5 μm

inoculation for possible host plants of the fungus. Uredinia and telia were produced on *V. angularis* var. *nipponensis* and *V. angularis* var. *angularis* only. *Vicia amoena*, *V. cracca*, *V. faba*, *V. unijuga*, *Lathyrus maritimus*, *L. palustris*, *Pisum sativum*, *Vigna radiata*, *V. mungo*, *V. unguiculata* ssp. *unguiculata*, and *Phaseolus vulgaris* are not infected with this rust fungus (Table 3). Ito (1922, 1950) reported that the rust fungus on *V. angularis* was morphologically similar to

Table 2. Results of inoculation experiments with aeciospores on *Vigna angularis* var. *nipponensis*

Inoculum[a]	Plant inoculated	Date of inoculation	Days required for the first appearance of	
			Uredinia	Telia
I	*V. angularis* var. *nipponensis*	June 8, 2002	10	22
		June 10, 2002	10	23
II	*V. angularis* var. *nipponensis*	Oct. 10, 2002	10	22

[a] I, Aeciospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref.; II, aeciospores formed on *V. angularis* var. *nipponensis* by basidiospore inoculation in September 2002

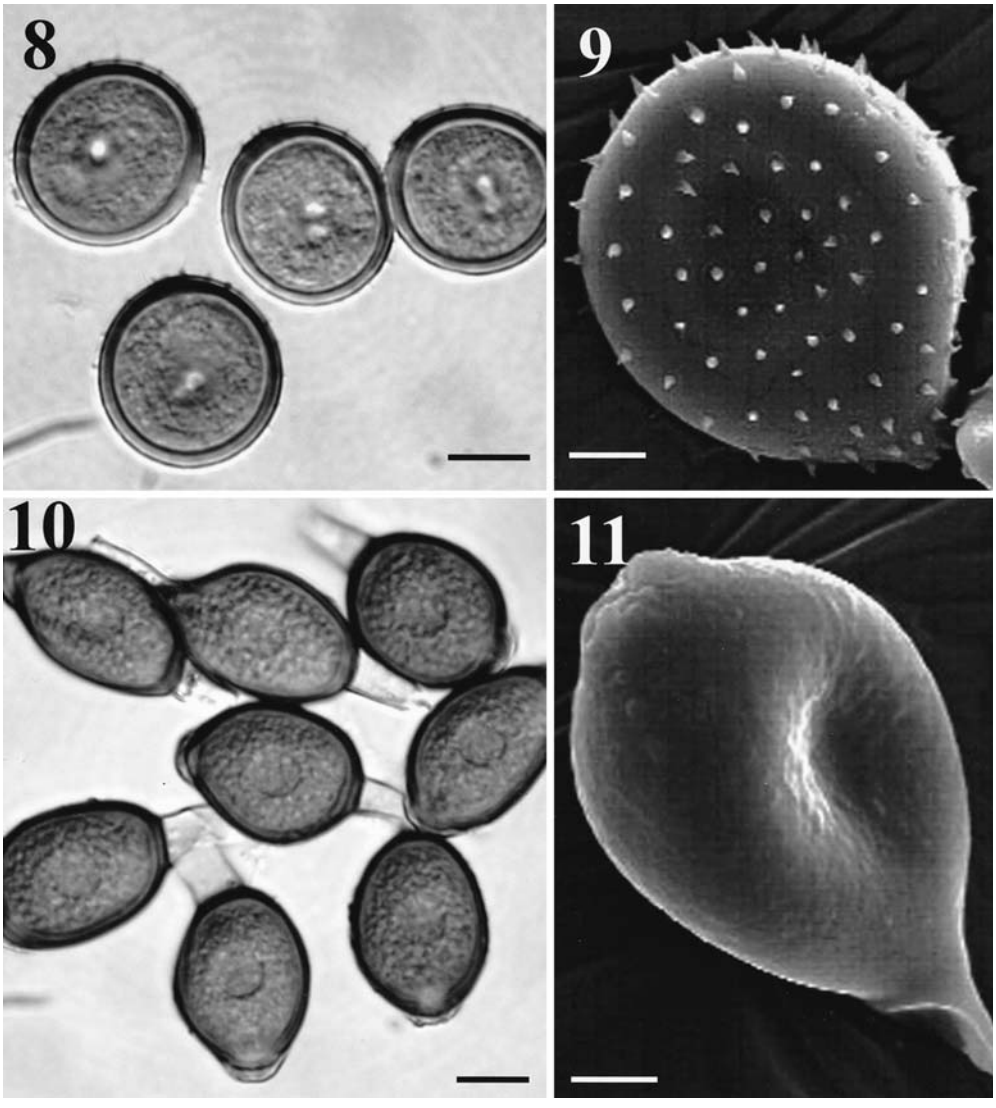

Fig. 8–11. *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis* var. *nipponensis*. Urediniospores under light (**8**) and scanning electron (**9**) microscopes. Teliospores under light (**10**) and scanning electron (**11**) microscopes. *Bars* **8,10** 10 μm; **9,11** 4 μm

U. appendiculatus on *P. vulgaris*, and Duke (1981) also stated that *V. unguiculata* ssp. *unguiculata*, *V. mungo*, and *V. radiata* were host plants of *U. appendiculatus*. In our inoculation, however, the rust fungus from *V. angularis* var. *nipponensis* was not able to infect them. This result confirms that the rust fungus is specific to *V. angularis*, as reported by Hirata (1952). On the other hand, *Vicia*, *Lathyrus*, and *Pisum* are known as host plants of *U. viciae-fabae*. From these results, it is suggested that the rust fungus on *V. angularis* is different from these rust fungi in host relations.

Morphology

The spermogonia on *V. angularis* var. *nipponensis* were amphigenous or epiphyllous, surrounded by yellow lesions,

Table 3. Results of inoculation experiments with urediniospores on *Vigna angularis* var. *nipponensis*

Inoculum[a]	Plant inoculated	Appearance of spore stages	
		Uredinia	Telia
I	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	−	−
	V. radiata	−	−
	V. mungo	−	−
	Phaseolus vulgaris	−	−
	Vicia cracca	−	−
	V. faba	−	−
	V. amoena	−	−
	V. unijuga	−	−
	Lathyrus maritimus	−	−
	L. palustris	−	−
	Pisum sativum	−	−
II	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	−	−
	V. radiata	−	−
	V. mungo	−	−
	Phaseolus vulgaris	−	−
	Vicia cracca	−	−
	V. faba	−	−
	V. amoena	−	−
	V. unijuga	−	−
	Lathyrus maritimus	−	−
	L. palustris	−	−
	Pisum sativum	−	−
III	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	−	−
	V. radiata	−	−
	V. mungo	−	−
	Phaseolus vulgaris	−	−
	Vicia cracca	−	−
	V. faba	−	−
	V. amoena	−	−
	V. unijuga	−	−
	Lathyrus maritimus	−	−
	L. palustris	−	−
	Pisum sativum	−	−
IV	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	−	−
	Phaseolus vulgaris	−	−

[a] I–III, Urediniospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref. in June (I), July (II), and August (III) 2002; IV, urediniospores formed on *V. angulris* var. *nipponensis* by aeciospore inoculations

scattered or aggregated, yellow to yellow-brown, subepidermal, and flask-shaped (type 4 of Cummins and Hiratsuka 2003) (Fig. 2). Spermogonia and aecia also appeared on the petioles of *V. angularis* var. *nipponensis*.

Aecia were amphigenous or hypophyllous, aggregated (rarely scattered), cupulate with peridia, and pale yellow (Figs. 3–5). Aeciospores were globose, subglobose, or angular, and measured 16.4–26.6 × 14.2–21.2 μm. Their walls were 0.9–1.3 μm thick, hyaline, and verrucose (Figs. 6, 7).

The uredinia on *V. angularis* var. *nipponensis* were amphigenous, scattered or aggregated, erumpent, and brown. Urediniospores were subglobose and obovoid, and measured 20.5–27.3 × 16.8–23.1 μm. Their walls were 0.9–1.5 μm thick, brown or light brown, and echinulate. The two germ pores were equatorial (superequatorial) (Figs. 8, 9). Telia were similar to uredinia. Teliospores were ellipsoid, apical papilla pale, mostly cuspidate, and 24.2–33.6 × 17.2–21.8 μm. Walls were brown or brownish, 1.3–2.0 μm thick, and smooth (Figs. 10, 11).

The morphology of the uredinial and telial stages of the specimens on *V. angularis* var. *nipponensis* is identical with the description of *U. appendiculatus* var. *azukicola* [=*U. azukicola*] described by Hirata (1952) and Hiratsuka et al. (1992). Therefore, we identified these specimens as *U. appendiculatus* var. *azukicola*. The morphology of the spermogonia and aecia on *V. angularis* var. *nipponensis* is described for the first time in the present study, and is similar to that of *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus* (Hiratsuka et al. 1992). Size, shape, and wall structure of the aeciospores on *V. angularis* var. *nipponensis* are not distinguishable in these two varieties. Urediniospores, spore size, and wall structure are indistinguishable from other varieties of *U. appendiculatus*, but germ pore position and number are different from *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus*. Although the rust fungus on *V. angularis* var. *nipponensis* has two equatorial or superequatorial pores, *Uromyces appendiculatus* var. *appendiculatus* and var. *dispersus* have two at the equatorial or subequatorial positions and two or three equatorial (Hiratsuka et al. 1992), respectively. Teliospore size is indistinguishable from other varieties of *U. appendiculatus*. However, teliospore wall thickness of the rust fungus on *V. angularis* var. *nipponensis* (1.3–2.0 μm) is different from *U. appendiculatus* var. *appendiculatus* (3.5 μm) and *U. appendiculatus* var. *dispersus* (1.5–3.0 μm). Therefore, we consider the morphological characteristics of the urediniospores and teliospores to be important in separating these varieties.

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