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Identification of an Aecidial Rust on *Fallopia japonica*

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Fallopia japonica, commonly known as Japanese knotweed or "itadori", is an increasingly invasive and troublesome weed in Europe and North America. Classical biological control is being assessed as a potential management strategy in the UK. During surveys in its native Japan, a distinctive rust fungus which commonly appeared in spring was recorded. Based on spore morphology, this was identified as *Aecidium polygoni–cuspidati*. Field observation throughout 2003 at Kusu (Oita Pref., Japan) showed that the rust occurred mainly from April to June but it was not associated with severe host damage or defoliation. No secondary infection in the form of uredinia was observed. Attempts to infect *F. japonica* with aeciospores in the laboratory failed. Literature searches confirmed this to be the heteroecious rust, *Puccinia phragmitis*, which alternates between *F. japonica* and *Phragmites communis*. Thus, this species has no potential as a classical biological control agent of Japanese knotweed.

INTRODUCTION

Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decr., is an invasive, alien, perennial weed of now widespread in Europe and North America. The plant was introduced into Europe in the 19th century from Japan by Philip von Siebold as an ornamental garden plant, but has since become problematic there, imposing a significant cost for urban development as well as posing a threat to biodiversity (Bailey and Conolly, 2000). It is listed in "100 of the World's Worst Invasive Alien Species" by the Invasive Species Specialist Group of the Species Survival Commission of the IUCN (the International Union for Conservation of Nature) –World Conservation Union (http://www.issg.org/database/welcome/). It has also described as the most feared plant in the British Isles (Mabey, 1998), and an official study by the UK Government's Department for Environment, Food and Rural Affairs Defra indicated that it would cost upwards of £1.5 billion to bring this weed under control in the UK alone (Defra, UK, 2003). Current chemical control and cutting treatment have been unable to eradicate Japanese knotweed or control it in the long term (Djeddour *et al*., 2008). Therefore, classical biological control for sustainable or long–term management of Japanese knotweed is now being considered.

Field surveys were carried out from 2003–2007 throughout the natural range of Japanese knotweed in Japan. It was shown that Japanese knotweed in Japan has a guild of specialized natural enemies which can severely impact on its vigor and population dynamics (Kurose *et al*., 2006). From the surveys, three fungal pathogens were prioritized: two types of rusts, one common in spring, the other in autumn, and a leaf spot fungus (Kurose *et al*., 2008).

The rust fungi, which are obligate pathogens in nature, have a good long history of success in classical biological control of weeds. The majority of pathogens belong to the rust fungi since they possess all the traits demanded of a classical agent: high specificity; virulence; and efficient, long–distance dispersal (Evans, 2002). For example, *Puccinia chondrillina*, an autoecious, macrocylic rust fungus from Italy was released into Australia in 1971 against skeleton weed, *Chondrilla juncea* L., a native Mediterranean plant which became an invasive weed of annual crops, particularly wheat, in southeast Australia and rapidly reached epidemic proportions (Cullen *et al*., 1972). The rust fungus *Maravalia cryptostegiae*, from south–west Madagascar, was introduced into Australia in 1995 against the highly invasive rubber–vine weed *Cryptostegia grandiflora*, a woody climber endemic to Madagascar and, similarly, quickly impacted on its host (Tomley and Evans, 2004).

The aims of this paper are to identify the rust species which is common in spring; to examine disease development in the field and to assess this pathogen as a potential classical biological control agent for *F. japonica* in the UK.

MATERIALS AND METHODS

Collection of materials

Surveys were undertaken in Japan from 2003–2007 throughout the natural range of *F. japonica* and its close relatives on the islands of Honshu, Kyushu and

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Shikoku. Leaf samples with disease symptoms were collected from the field and placed in paper bags. Samples were dried in a plant press for 3–5 days, with daily changes of paper, and then transferred to wax packets and kept at room temperature.

Phenotypic characterization

For microscopic examination, spores were mounted in sterile distilled water (SDW) on a glass slide (Ritchie, 2001). The samples were observed using an optical microscope (BX60F5, Olympus, Japan) fitted with a digital camera (C–2000Z, Olympus, Japan).

For scanning electron microscopy (SEM), fungal structures on fresh host tissue were placed in 2.5% glutaraldehide in 0.1 M phosphate buffer (pH 7.4). Samples were transferred to a bell jar attached to a vacuum evaporator, which was evacuated with a rotary pump until all were submerged. The fixed samples were washed in the buffer four times for 1 h and postfixed in 2% osmium tetroxide in the same buffer for 1 h. The material was briefly rinsed in the buffer and dehydrated in a series of washes with 50%, 70%, 90%, 99.5% and up to 100% ethanol. After dehydration, the samples were transferred to *t*–butyl alcohol for three changes at 30 °C. The glass container containing the specimens in *t*–butyl alcohol was placed in a refrigerator at 4 °C (Inoue and Osatake, 1988). The *t*–butyl alcohol was then frozen within 10 min, and dried in freeze dryer (FD–1, Tokyo Rikakikai Co., Ltd., Japan) for 3 h. The dried samples were mounted with carbon tape onto a metal block and coated with gold using an ion sputter (JFC–1100E, JEOL, Japan). Samples were examined with a JEOL JSM–5200 scanning electron microscope linked to a camera (Mamiya Co., Ltd., Japan) at 25 kV accelerating voltage.

Disease development in the field

For observation of disease development of the rust on *F. japonica*, a field site at Kusu (Oita Pref., Japan) was selected. Twenty plants were marked and the disease score was assessed every month as the mean disease score index using the following scale: 0=no symptom; 1=1–20% of infected leaves; 2=21–40% of infected leaves; 3=41–60% of infected leaves; 4=more than 61% of infected leaves and 5=infected leaves defoliated. Disease score was calculated using the following formula; Σ (number of infected leaves per rating × rating value) / total number of infected leaves.

Inoculation test

F. japonica from Omura (Nagasaki Pref., Japan) were used for inoculation tests and the spores of the rust collected from infected leaves originating from Kusu, with a fine paint brush, were mixed these with talc (spores:talc=1:4) or with SDW containing 0.1% Tween 80, and then the suspension was applied to both leaf surfaces using a fine paintbrush. Inoculated plants were placed in a customized dew chamber set at 20 °C, without light for 48 h. Subsequently, all inoculated plants were maintained in the greenhouse, together with inoculated controls, and observed daily.

RESLUTS AND DISCUSSION

Morphology and identification

The pustules are very conspicuous in the field, form-

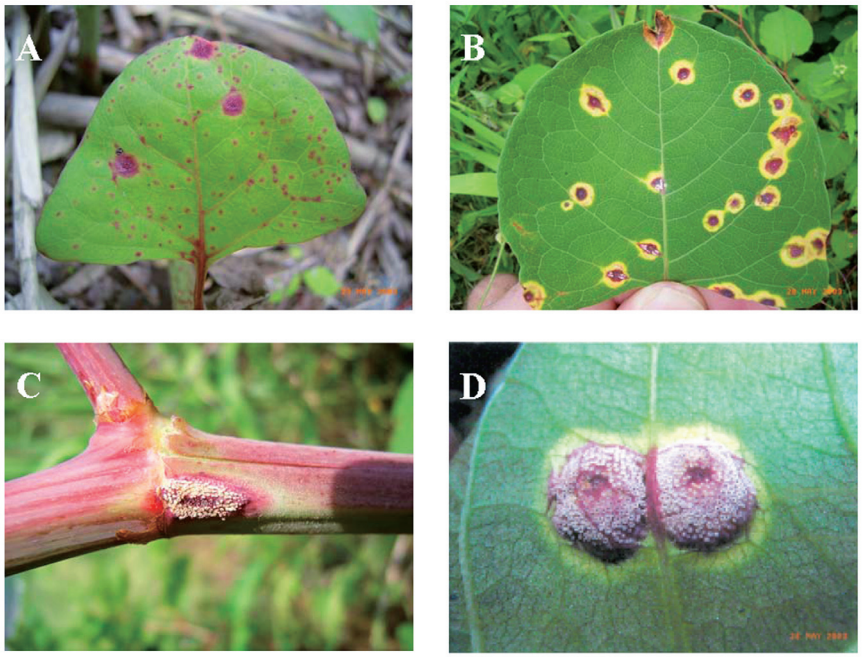

Fig. 1. Disease symptoms of *Fallopia japonica* infected with *Aecidium polygoni–cuspidati* in the field. A; red and rash–like lesions on young leaves (taken at Joetsu, Niigata Pref. on 23 May 2003), B; yellow/purple lesions on older leaves (taken at Kusu, Oita Pref. on 28 May 2003), C; aecia on the stem (taken at Kusu on 30 May 2005) and D; mature aeciospores in the cluster–cup (taken at Kusu on 28 May 2003).

Table 1. Morphological data of *Aecidium polygoni–cuspidati* on *Fallopia japonica*

Specimen[a]	Locality of collection	Aeciospore		
		Shape	Color	Range (μm)
Original[b]	ND[c]	Angularly globose to ellipsoid	Subhyaline	16–21 × 14–19
28–01–03	Kusu, Oita Pref.	Angularly globose to ellipsoid	Subhyaline	16–25 × 16–21
29–01–03	Okawachi, Miyazaki Pref.	Angularly globose to ellipsoid	Subhyaline	16–22 × 16–20
02–03–05	Ueda, Nagano Pref.	Angularly globose to ellipsoid	Subhyaline	16–20 × 15–20

[a] Specimens were stocked in Kyushu University
[b] Data cited from Ito (1950)
[c] Not described

ing red and rash–like infections of pycnia on the upper surface of young leaves (Fig. 1A) which gradually turn reddish purple on older leaves with a distinct yellow ring or halo (Fig. 1B). The mature aecia appear on the underside of the leaf and also on the stem (Fig. 1C) causing the host tissue to become discoloured, raised and thickened. The aeciospores are formed in the cluster–cup (Fig. 1D, 2A) as a pale yellow/white mass of spores, borne in chains. The margin of the cup is crenulate and curled outwards. The aeciospores were angularly globose to ellipsoid, and subhyaline (Fig. 2B), measuring 16–25 × 16–21 μm, with walls about 2 μm thick. This rust pathogen was identified as *Aecidium polygoni–cuspidati* according to spore morphology (Table 1).

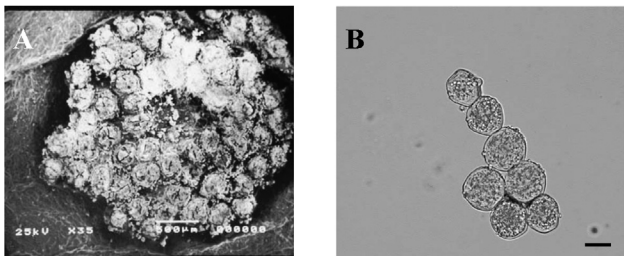

Fig. 2. Morphology of *Aecidium polygoni–cuspidati*. A; aecia by scanning electron microscope and B; aeciospores by optical microscope. Bar indicates = 30 μm.

Disease development in the field

The incidence and development of the rust disease caused by *A. polygoni–cuspidati* were observed at Kusu to help elucidate its life cycle. The rust occurred from April to June but was not associated with severe damage nor defoliation in the field (Fig. 3A–E). The symptoms described above were found on May (Fig.

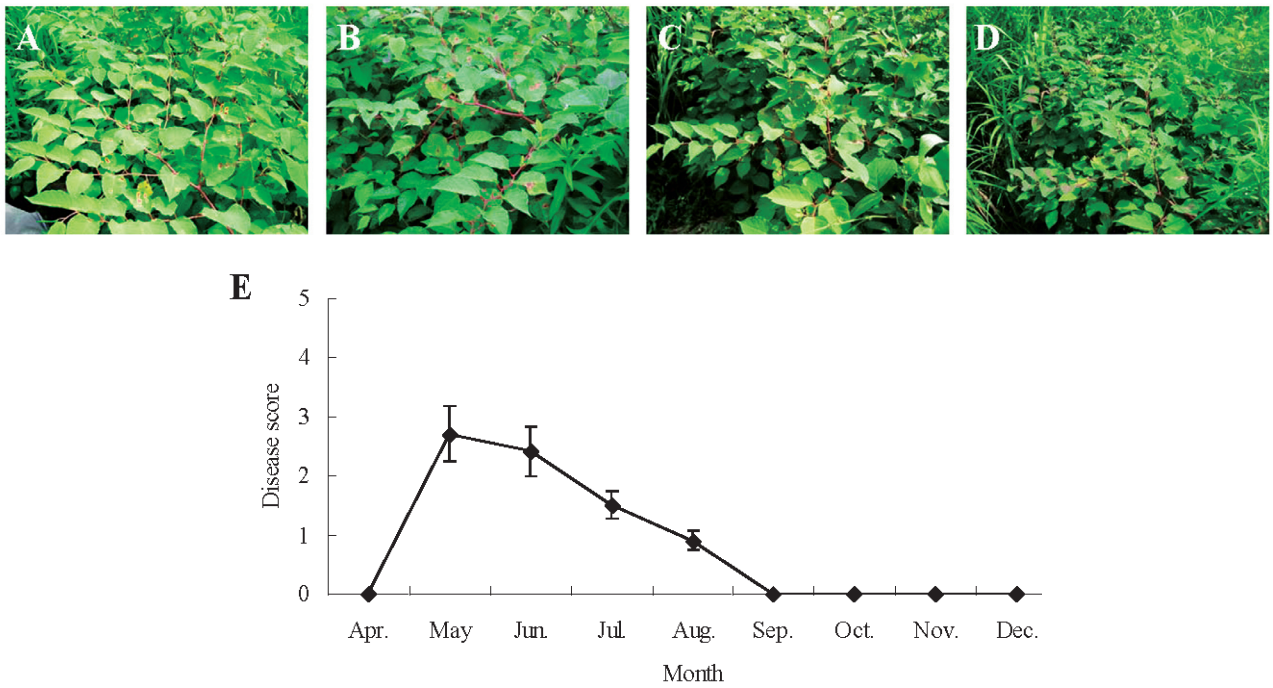

Fig. 3. Disease development of *Aecidium polygoni–cuspidati* on *Fallopia japonica* at Kusu, Oita Pref. on 2003. A; red and rash–like lesions and yellow/purple lesions (taken on 28 May 2003), B; most aeciospores released and aecia were starting to decay (taken on 30 June 2003), C, D; aecia were decayed and new symptoms were not observed (taken on 16 July 2003, C, 6 August 2003, D) and E; graphical representation of disease severity on *A. polygoni–cuspidati*. Disease severity was assessed every month as the mean disease severity index using the following scale: 0=no symptom; 1=1~20% of leaves were infected; 2=21~40% of leaves were infected; 3=41~60% of leaves were infected; 4=> 61% of leaves were infected; 5=infected leaves all defoliated. Disease severity was calculated using the following formula; Σ (number of infected leaves per rating × rating value) / total number of infected leaves. Vertical bars represent standard error of mean.

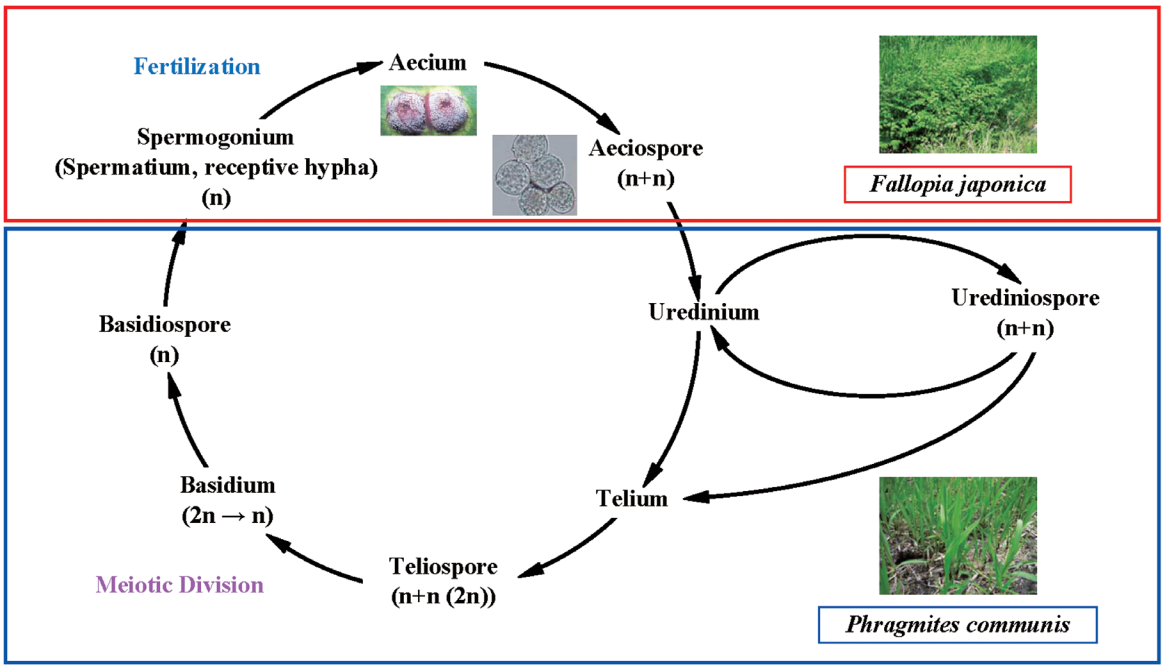

Fig. 4. Schematic life cycle of *Aecidium polygoni-cuspidati*.

3A), when the mean disease score was highest (Fig. 3E), but new symptoms were not observed after July and there was no evidence of further rust development.

Inoculation tests with aeciospores of *A. polygoni–cuspidati* failed to infect *F. japonica*. Uredinia or urediniospores were not observed (data not shown).

Life cycle

The spermogonial and aecial stages on *A. polygoni–cuspidati* were described previously (Hiratsuka *et al*., 1992) but in this study, the aecial stage only was observed in the field. In addition, inoculation experiments revealed that aeciospores failed to achieve any infection are non–infective to *F. japonica*. However, Harada (1978) reported that aeciospores were infective to *Phragmites communis* Trin. and produced uredinia. Futhermore, Hiratsuka *et al*., (1992) also listed *Puccinia phragmitis* as the synonym of *A. polygoni–cuspidati* with the uredinial and telial phases occurring on *P. communis*. Therefore, this species is heteroecious with alternate hosts, *F. japonica* and *P. communis* (Fig. 4).

The rusts considered for classical biological control should have only one host species; they should be autoecious with only one host species (Hajek, 2004). To conclude, *A. polygoni–cuspidati* had to be ruled out as a potential agent, because there was no possibility that it could successfully overwinter in the UK without an alternate host.

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