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Identification of Aecial Host Ranges of Four Korean *Gymnosporangium* Species Based on the Artificial Inoculation with Teliospores Obtained from Various Forms of Telia

Hye Young Yun[1], Seung Kyu Lee[2]* and Kyung Joon Lee[1]

[1]*Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea*

[2]*Korea Forest Research Institute, Seoul 130-712, Korea*

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The objectives of this study were to identify the aecial host ranges of four Korean *Gymnosporangium* species, *G. asiaticum*, *G. cornutum*, *G. japonicum* and *G. yamadae*, and to verify the morphological characteristics of telia as diagnostic keys to the species. Thirteen Korean Rosaceous woody species were artificially inoculated with teliospores obtained from *Juniperus* species. There was high specificity between telial and aecial hosts and the fungal species, providing the first experimental proof on host alternation of these rust fungi in Korea. Telia on the witches' broom and on the small galls were identified as new telial characteristics in *G. asiaticum* and in *G. yamadae*, respectively. Aecial hosts of *G. asiaticum* and *G. yamadae* showed varying responses in their susceptibility and in the days required for formation and duration of spermogonia and aecia after inoculation. Four telial host species in *Juniperus* were confirmed for the first time in Korea, which include *J. chinensis* var. *kaizuka*, *J. chinensis* var. *horizontalis* and *J. chinensis* var. *globosa* for *G. asiaticum*; and *J. chinensis* var. *kaizuka* for *G. yamadae*.

Keywords : artificial inoculation, cedar-apple rust, *Gymnosporangium*, host range, host specificity

Gymnosporangium species (Cedar-apple rust fungi) are known to infect a wide range of economically important woody plants of Cupressaceae and Rosaceae (Kern, 1973; Agrios, 1997). They are heteroecious, so that they make a telial stage on plants of Cupressaceae and their aecial stage on pomaceous plants of Rosaceae to complete their life cycle (Kern, 1973). It is known that a definite close relationship exists between rust and their host plants (Agrios, 1997; Cummins and Hiratsuka, 2003). Hence, verification of the aecial and telial host ranges of a given cedar-apple rust fungus is a fundamental step for the correct identification of the given species and also for the subsequent studies on the relationship between host and rust fungi. Such information is vital in the determination of appropriate disease control strategies for cedar-apple rust diseases (Scott and Chakravoty, 1982).

Up to now eight *Gymnosporangium* species have been recorded to the present in Korea (Hiratsuka, 1935, 1940, 1942; Korean Society of Plant Pathology, 2004; Park, 1958; Takimoto, 1916; Yun et al., 2003). However, very few studies have been conducted on the host range of cedar-apple rust fungi in Korea, and these studies have simply listed their host plant species without inoculation experiments (Chung et al., 1977; Hiratsuka, 1935, 1940, 1942; Kim, 1963; Lee, 2001; Park, 1958, 1961). Two *Gymnosporangium* species such as *G. asiaticum* and *G. yamadae* are critical in the production of pear and apple in Korea (Kim and Kim, 1980; Lee, 1990). Nevertheless, there is no report on the inoculation experiment on these species except a report by Lee and Lim (1984). Yun et al. (2003) reported first a full description of two species, *G. cornutum* and *G. japonicum*, with artificial inoculation results. The other four Korean *Gymnosporangium* species, *G. clavariiforme*, *G. juniperi*, *G. miyabei* and *G. shiraianum*, were recorded only with a list of aecial or telial hosts (Chung et al., 1977; Hiratsuka, 1935, 1940, 1942; Park, 1958). In the monographic study on Korean Pucciniaceae, Lee (2001) reported first aecial and telial hosts of *G. asiaticum* with some morphological descriptions. However his report was not based on the strict experimental data to show the host alteration of the species.

Cedar-apple rust fungi have unique forms of telia and aecia compared to other rust fungi, and telial morphology was known as one of the important characters for the diagnostic key to *Gymnosporangium*. Telia of *Gymnosporangium* species develop on the various forms of structures such as galls with different sizes, on green parts of witches' broom, and on leaves singly or collectively, etc (Hiratsuka et al., 1992; Kern, 1973; Yun et al., 2003). Also, telia grow on various parts of trees such as leaves, twigs, branches and trunks (Hiratsuka et al., 1992; Kern, 1973). However, this diverse telial morphology sometimes led the description of a *Gymnosporangium* species ambiguous, making the

*Corresponding author.
Phone) +82-2-961-2625, FAX) +82-2-961-2595
E-mail) leesk77@foa.go.kr

correct identification impossible or difficult.

As a work for the revision of Korean *Gymnosporangium*, this study was carried out to confirm the aecial host range of four *Gymnosporangium* species of *G. asiaticum*, *G. yamadae*, *G. japonicum* and *G. cornutum* in Korea, and to verify the morphological characteristics of their telia as a diagnostic key of these species.

Materials and Methods

Inoculum preparation. During the spring season of 2001, fresh telia were collected from five species of *Juniperus* as shown in Table 1 and stored for several days in the refrigerator before being used for inoculation. Before inoculation, the collected telia were classified into nine telial types based on telial hosts, shapes and positions on trees (Table 1), and identified as *G. asiaticum*, *G. cornutum*, *G. japonicum*, and *G. yamadae* based on the data from previous works (Hiratsuka et al., 1992; Kern, 1973; Lee and Kakishima, 1999a, b; Yun et al., 2003).

Test plants and inoculation. Thirteen host trees of eight genera in the Rosaceae recorded as aecial host plants of *Gymnosporangium* in Korea were used for this inoculation study (Hiratsuka et al., 1992; Kern, 1973; Lee, 1999) (Table 2); *Amelanchier asiatica* (Siebold et Zucc.) Endlicher, *Chaenomeles sinensis* Koehne, *Ch. lagenaria* (Loiselur) Koidzumi, *Crataegus pinnatifida* Bunge, *Eribotrya japonica* (Thunb. ex Murray) Lindley, *Pyrus pyrifolia* var. *culta* Nakai, *P. ussuriensis* Maxim., *Sorbus commixta* Hedl., *S. alnifolia* (Siebold et Zucc.) K. Koch, *Photinia glabra* (Thunb. ex Murray) Maxim., *Ph. villosa* (Thunb. ex Murray) DC., *Malus sieboldii* (Regel) Rehder and *M. pumila* Mill.

Dormant tree seedlings were planted in early February in a green house in 30 cm diameter pots with 15 L capacity and grown until they developed several leaves. In late April they were placed in dark inoculation chambers made of transparent polyethylene film with a dimension of 1.5 m wide, 1.5 m deep and 1.5 m high.

The artificial inoculation generally followed the procedure by Pearson et al. (1977) and Aldwinkle et al. (1977). Two inoculation methods were employed. Firstly, teliospores from telia on leaves, branches and trunks of *Juniperus* trees were suspended in sterile water, and sprayed onto the leaves.

Each inoculation chamber contained 13 tree species with two or three replications each. Of the nine chambers used for this study, four chambers were inoculated with *G. asiaticum*, two chambers with *G. japonicum* and two chambers with *G. yamadae*, while remaining one chamber was inoculated with *G. cornutum*.

Inoculation chambers were humidified at 19 to 21 C for four days to stimulate the germinations of the spores. The first appearance of spermogonia and aecia was observed 3 to 17 days and 48 to 65 days, respectively, after the initial inoculation. The inoculation experiment was terminated in early July.

In 2002, the above same experiment was repeated using only three inoculation chambers with one isolate each to reconfirm the new hosts and new telial characters observed in 2001. The two fungal species used in 2002 experiment were *Gymnosporangium asiaticum* (GA-4, and GA-3) and *G. yamadae* (GY-2). No fungicides were applied to the seedlings at any stage.

The obtained aecia were compared with natural ones and identified based on the existing descriptions (Hiratsuka et al., 1992; Kern, 1973; Lee and Kakishima, 1999a, b). Response of each *Gymnosporangium* species to different aecial host species and days needed for the appearance and maturation of spermogonia and aecia were checked. Sci-

Table 1. Summary on nine telial types of *Gymnosporangium* species used in the present inoculation experiment

Rust species	Telial type[a]	Telial morphology	Telial host
G. asiaticum	GA-1	collective, bluntly conical	*Juniperus chinensis*
	GA-2	singular, bluntly conical	*J. chinensis* var. *globosa*[b]
	GA-3	hemispherical	*J. chinensis* var. *horizontalis*[b]
	GA-4	conical on witches' broom[c]	*J. chinensis* var. *kaizuka*[b]
G. cornutum	GC-1	slightly fusiform, sori applanate low	*J. rigida*
G. japonicum	GJ-1	wedge-shaped on trunk[c]	*J. chinensis*
	GJ-2	wedge-shaped on branch	*J. chinensis* var. *horizontalis*
G. yamadae	GY-1	big gall, sori tongue-shaped	*J. chinensis* var. *kaizuka*[b]
	GY-2	small galls, sori cylindric-acuminate[c]	*J. chinensis* var. *kaizuka*[b]

[a] All telial types tested were characterized and identified according to procedure by Kern (1973).
[b] Telial hosts that were unrecorded previously.
[c] New description of *Gymnosporangium* in Korea.

entific names of host plants were referred to Lee (1999) and Chang (1994).

Results and Discussion

Host alternation of four Korean *Gymnosporangium* species and the morphological characters of telial structures as a diagnostic key. Table 2 reveals the heteroecious nature of the four Korean cedar-apple rust fungi, as they produced spermogonia and aecia on plants artificially inoculated with telia from *Juniperus* trees. The present result is the first experimental proof on the host alternation between telial and aecial host plants of Korean *Gymnosporangium* species, *G. asiaticum*, *G. cornutum*, *G. japonicum*, and *G. yamadae*.

G. asiaticum infected five aecial Rosaceae species, *Ch. lagenaria*, *Ch. sinensis*, *P. pyrifolia* var. *culta* and *P. ussuriensis*, producing symptoms and signs on these plants.

Also three new telial host species in the Cupressaceae were confirmed in Korea, which include *J. chinensis* var. *kaizuka*, *J. chinensis* var. *horizontalis* and *J. chinensis* var. *globosa*.

A host range of *G. asiaticum* was first recorded by Hiratsuka (1935) on *Pyrus sinensis* var. *culta* and on *J. chinensis* as aecial and telial hosts in Korea, respectively. Later, additional host plants of *G. asiaticum* have been reported; aecial stage on *Ch. japonica*, *Ch. lagenaria*, *Ch. sinensis*, *C. pinnatifida*, *C. pinnatifida* var. *psilosa*, *Ph. villosa* var. *longipes*, *Ph. villosa* var. *brunnea*, *P. calleryana* var. *fauriei*, *P. communis*, *P. serotina*, *P. sinensis* var. *culta*, *P. pyrifolia*, *P. pyrifolia* var. *culta*, *P. ussuriensis* and *P. ussuriensis* var. *seoulensis*, and telial stage on *J. chinensis* and *J. chinensis* var. *sargentii* (Chung, 1977; Hiratsuka, 1940, 1942; Kim, 1963; Korean Society of Plant Pathology, 2004; Korean Soc. Plant Prot. 1986; Lee, 2001; Lee and Lim, 1984; Park, 1958, 1961). Although *G. asiaticum* is wide spread and has critical importance in the pear production (Lee and Lim, 1984), there have been no reports on the mycological interaction between telial and aecial hosts of *G. asiaticum* in Korea.

As shown in Table 2, all of four telia types of *G. asiaticum* successfully infected three aecial hosts such as *Ch. lagenaria*, *P. pyrifolia* var. *culta* and *P. ussuriensis*.

Fig. 1. Aecia of *Gymnosporangium asiaticum* on artificially inoculated *Crataegus pinnatifida*.

Table 2. Results of the artificial inoculation of thirteen Rosaceous woody plants with telial types of four Korean *Gymnosporangium* species

Aecial host species[a]	*G. asiaticum*				*G. cornutum*	*G. japonicum*		*G. yamadae*	
	GA-1	GA-2	GA-3	GA-4	GC-1	GJ-1	GJ-2	GY-1	GY-2
Amelanchier asiatica	–[b]	–	–	–	–	–	–	–	–
Chaenomeles sinensis	–	–	+	+	–	–	–	–	–
Ch. lagenaria	+	+	+	+	–	–	–	–	–
Crataegus pinnatifida	–	+	–	–	–	–	–	–	–
Eriobotrya japonica	–	–	–	–	–	–	–	–	–
Malus sileboldii	–	–	–	–	–	–	–	+	+
M. pumila	–	–	–	–	–	–	–	+	+
Photinia villosa	–	–	–	–	–	+	+	–	–
Ph. glabra	–	–	–	–	–	–	–	–	–
Pyrus pyrifolia var. *culta*	+	+	+	+	–	–	–	–	–
P. ussuriensis	+	+	+	+	–	–	–	–	–
Sorbus commixta	–	–	–	–	–	–	–	–	–
S. alnifolia	–	–	–	–	+	–	–	–	–

[a] Thirteen Rosaceae woody species known as alternate hosts of *Gymnosporangium* spp. in Korea.
[b] Symbols (+) and (–) indicate compatible and incompatible reactions to each rust species inoculated.

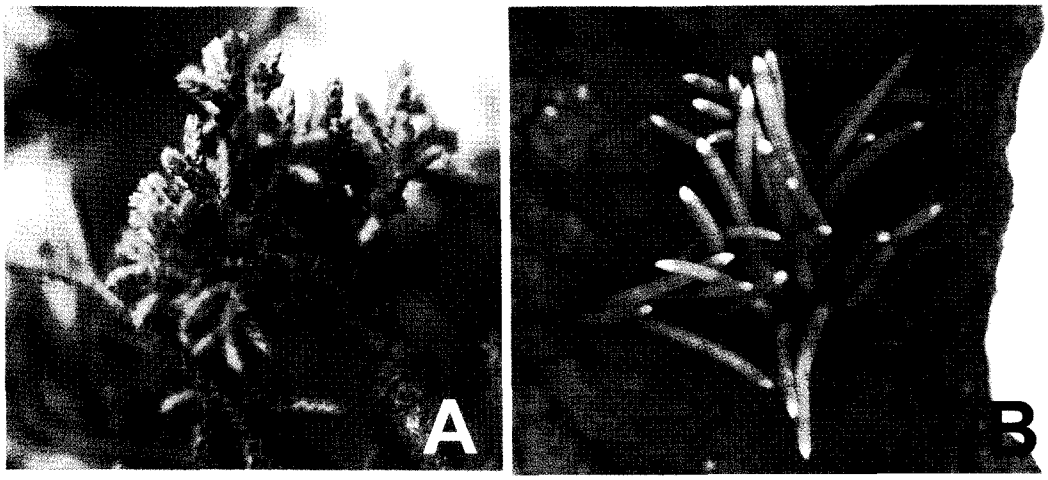

Fig. 2. New description of *Gymnosporangium asiaticum*. (A) Telial type GA-4 of *Gymnosporangium asiaticum* on *Juniperus chinensis* var. *kaizuka* (on Witches' broom) (B) Aecia on artificially inoculated *Pyrus pyrifolia* var. *culta*.

However, telial type GA-2 with singular and bluntly conical shape of telial horn, collected from *J. chinensis* var. *globosa* and identified as *G. asiaticum*, produced aecia only on *C. pinnatifida* (Figure 1). Although Chung et al. (1977) listed the aecial stage of the fungus without morphological descriptions, the present study experimentally confirmed that *C. pinnatifida* was an aecial host for *G. asiaticum* in Korea. However, further taxonomic research on the telial type GA-2 would be needed because this telial type among four inoculated telial types infected only *C. pinnatifida*.

Also in the present inoculation test, telial type GA-4, collected from the witches' broom symptom of *J. chinensis* var. *kaizuka*, was confirmed as the telial stage of *G. asiaticum*, and provided first one of descriptive characteristics of telia for identifying *G. asiaticum* (Figure 2). However, telial horn formed on witches' broom has not been described on *G. asiaticum* in previous literatures (Hiratsuka et al., 1992; Kern, 1973). We suggest that this type of telial formation is a new diagnostic feature of *G. asiaticum*.

Artificial inoculation of *G. cornutum* collected from *J. rigida* produced spermogonia and aecia only on leaves of *S. alnifolia*, but not on other plants tested (Table 2). This is the first report on host alternation of *G. cornutum* between aecial and telial hosts in Korea. *G. cornutum* was one of the first species in the genus to be cultured successfully (Oersted, 1866). Hiratsuka et al. (1992) listed two Rosaceous species, *S. commixta* and *S. sambucifolia* as aecial hosts, and following four species, *J. communis* var. *montana*, *J. communis* var. *nipponica*, *J. conferta* and *J. rigida* as telial hosts. In the present study, telia of *G. cornutum* on *J. rigida* successfully produced spermogonia and aecia on the leaves of *S. alnifolia* (Table 2), which was consistent with previous studies (Hiratsuka et al., 1992; Kern, 1973).

From the inoculation experiment with teliospores of *G. japonicum* collected from the twigs and branches of *J.*

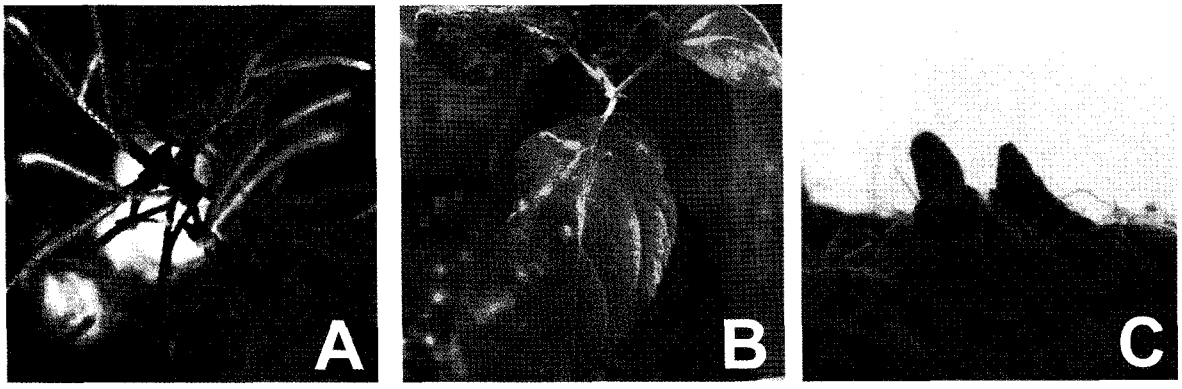

Fig. 3. New description of *Gymnosporangium yamadae*. (A) Telia (GY-2, small galls, sori cylindric-acuminate) of *Gymnosporangium yamadae* on *Juniperus chinensis* var. *kaizuka*. (B) Spermogonia on artificially inoculated *Malus sieboldii*. (C) Aecia on artificially inoculated *M. sieboldii*.

chinensis in Suwon and *J. chinensis* var. *horizontalis* in Jeju, a positive response was obtained only on *Ph. villosa* out of thirteen tree species tested, producing spermogonia and aecia on the plant leaves (Table 2). Therefore, the results indicated that *G. japonicum* was a heteroecious fungus, with the telial state on *J. chinensis* var. *sargentii.* and *J. chinensis*, and with the aecial stage only on *Ph. villosa* in Korea. In *G. japonicum*, artificial infections were successfully made first by Ito in 1913 by spraying teliospores from *J. chinensis* on *Ph. villosa*. Later the same results were obtained by Hara (1926) and Hiratsuka (1936). Our observations for several years showed that *J. chinensis* var. *horizontalis* and *J. chinensis* var. *sargentii* were highly susceptible to this rust fungus, *G. japonicum.* The fungus occasionally killed the hosts (unpublished data). In the pathological aspects, *G. japonicum* is distinctively different from the other *Gymnosporangium* species, since *Gymnosporangium* species have been considered to give little impact on their telial host, *Juniperus*. In Korea, this species was newly identified by Yun et al. (2003).

Telia from two types of galls (small and big in size) of *G. yamadae* from *J. chinensis* var. *kaizuka* infected and formed spermogonia and aecia on aecial hosts, *M. pumila* and *M. sileboldii* (Table 2, Figure 3). In the present inoculation test, it was confirmed that *G. yamadae* formed its telial horns on various sizes of galls. Also *J. chinensis* var. *kaizuka* was identified for the first time as a new aecial host for *G. yamadae* in Korea.

Symptom development and aecial formation in the aecial hosts. A variety of telial structures was described on different positions of *Juniperus* trees (Hiratsuka et al., 1992; Kern, 1973). As shown in Table 3, development pattern of spermogonia and aecia on host plants examined varied depending on telial types of a *Gymnosporangium* species.

In case of *G. asiaticum*, *P. pyrifolia* var. *culta* and *P.*

Table 3. The relationships between days of required for formation of spermogonia and aecia for telial characters (telial host, telial shape and telial position on trees)

Rust species and telial type[a]	Aecial Host	Days required for			Symptoms produced on	Spermogonial formation[b]	Aecial formation[b]
		Spermogonia	Aecia	Maturity			
G. asiaticum							
GA-1[a]	*Chaenomeles sinensis*	10	65	72	leaves	++	++
	Pyrus pyrifolia var. *culta*	11	48	58	leaves	+++++	+++++
	P. ussuriensis	11	48	58	leaves	+++++	+++++
GA-2	*Ch. lagenaria*	03	58	70	leaves	+++	+++
	Crataegus pinnatifida	03	58	70	leaves	++++	++++
	P. pyrifolia var. *culta*	11	49	59	leaves	+++++	+++++
	P. ussuriensis	11	48	56	leaves	+++++	+++++
GA-3	*Ch. lagenaria*	14	–*		leaves	++	–*
	Ch. sinensis	17	–*		leaves	++	–*
	P. ussuriensis	17	–*		leaves	+++	–*
GA-4	*Ch. lagenaria*	11	58	70	leaves	++	++
	Ch. sinensis	02	–*		leaves	++	++
	P. pyrifolia var. *culta*	11	56	72	leaves, twigs	+++++	+++++
	P. ussuriensis	11	48	54	leaves, twigs	+++++	+++++
G. cornutum							
GC-1	*Sorbus alnifolia*	05	48	58	leaves	+++++	+++++
G. japonicum							
GJ-1	*Photonia villosa*	11	58	70	leaves	+++++	+++++
GJ-2	*Ph. villosa*	03	58	72	leaves, twigs	+++++	+++++
G. yamadae							
GY-1	*Malus pumila*	12	–*		leaves	+++	+++
	M. sileboldii	11	48	72	leaves	+++	–*
GY-2	*M. pumila*	05	–*		leaves	++++	–*

[a] Telia shape and position: GA-1, collective bluntly conical; GA-2, singular, bluntly conical; GA-3, hemispheric; GA-4, witches' brooms; GC-1, slight fusiform, sori applanate low; GJ-1, wedge shaped on trunk; GJ-2, wedge shaped on branch; GY-1, big gall, sori tongue-shaped; GY-2, small galls, sori cylindric-acuminate.

[b] Number of "plus" indicates the degree of spermogonial and aecial formation: +++++, severe; +++, moderate; ++ and +, mild. –*: insect infestation.

ussuriensis were most severely infected regardless of telial types (GA-1~GA-4), resulting in abundant aecia and in the rupture of the leaf epidermis (Table 3). However aecial formation, as shown in Table 3, was particularly mild to moderate on *Ch. lagenaria* and *Ch. sinensis* regardless of the telial types inoculated, which suggested that this aecial hosts were more or less resistant to the infection of *G. asiaticum* compared to *Pyrus* spp. For *G. asiaticum*, the shortest period of spermogonial formation was two days after inoculation of telial type GA-4 to *Ch. sinensis*, followed by three days in *Ch. lagenaria* and *C. pinnatifida* by telial type GA-2. In contrast, the longest time for spermogonial formation occurred in *Ch. sinensis* and *P. ussuriensis* by telial type GA-3. It took 48 days in average after inoculation for aecial formation, and about 54 to 72 days for the aecial maturation. More careful and detailed researches would be needed to verify the degree of response of aecial hosts based on the telial types.

In *G. cornutum*, aecia developed in 48 days and matured in 58 days after inoculation, which was relatively shorter than in *G. japonicum* and *G. yamadae*. Aecial formation of *G. cornutum* on the aecial host was evaluated as severe as that of *G. japonicum*, producing abundant formation of aecia (Table 3).

G. japonicum (both telial type GJ-1 and GJ-2) infected only *Ph. villosa,* forming spermogonia and aecia on leaves or sometimes on twigs (by telial type GJ-2). From these results it was confirmed that this species produced its telia both on branches and trunks of *Juniperus* trees. Spermogonia appeared on the leaves of *Ph. villosa* from 3 to 11 days after inoculation, but aecial development and maturation took longer time, about 70 days after inoculation (Table 3). Similar responses were reported in Japan (Kern, 1973; Hiratsuka et al., 1992). During a field trip to Jeju for collecting the telial materials, it was found that *J. chinensis* var. *horizontalis* and *J. chinensis* var. *sargentii* were highly susceptible to this fungus in the field, with occasionally being killed by the fungal infection (unpublished data). This means *G. japonicum* may be pathologically quite distinct from other *Gymnosporangium* species, causing a high disease impact on the telial hosts.

Regardless of gall sizes (big or small), telia of *G. yamadae* from *J. chinensis* var. *kaizuka* successfully infected two host plants of Rosaceae, *M. pumila* and *M. sileboldii* with mild and moderate aecial formation (Table 3). In *G. yamadae*, the days required for the first spermagonium appearance were about 5-12 days after inoculation and the period required for aecial maturity was 72 days (Table 3).

In the present study, it was revealed that telial characteristics such as shapes and forming positions on trees would be useful as diagnostic keys; witches' brooms for *G. asiaticum,* small galls for *G. yamadae* and telia on trunk for *G. japonicum*. These characters may be included in the revision of Korean *Gymnosporangium* as new morphological features. This would be quite essential character for the diagnostic key to *Gymnosporangium*.

Mycological re-examination including the host alteration should be carried out for the other Korean *Gymnosporangium* species such as *G. clavariiforme*, *G. globosum*, *G. miyabei* and *G. shiraianum*, which have been listed or reported without descriptions.

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