Life cycle of *Melampsora laricis-populina* on *Populus* ×*canadensis* in Jilin, China, and its morphological clarification of spermogonial and aecial stages

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Abstract: *Populus* ×*canadensis* is widely planted in northeast of China and seriously infected with *Melampsora laricis-populina*. The life cycle of this species was confirmed by inoculation experiments. Based on specimens obtained by the inoculations, morphological characteristics of spermogonial and aecial stages were clarified and compared with previous reports. This species has relatively larger aeciospores than those in other species of *Melampsora* producing aecia on *Larix*. The presence of rudimentary peridia in aecia of this species was a useful characteristic to distinguish it from other species of *Melampsora* on *Populus* producing aecia on *Larix*. It was recognized that morphological characteristics of aecial stages in species of *Melampsora* required reconsideration based on specimens obtained from inoculations.

Zusammenfassung: *Populus* ×*canadensis* im Nordosten Chinas großflächig angepflanzt und ist erheblich von *Melampsora laricis-populina* befallen. Der Lebenszyklus dieser Art konnte durch Inokulationsversuche bestätigt werden. Auf der Grundlage von Belegen, die durch die Inokulationen erhalten wurden, wurden morphologische Merkmale der Spermogonien und Aecidien geklärt und mit früheren Beschreibungen verglichen. Diese Art hat größere Aecidiosporen als andere *Melampsora*-Arten, die Aecien auf *Larix* ausbilden. Die Anwesenheit von rudimentären Peridien in den Aecidien war eine nützliches Merkmal, um sie von anderen *Melampsora*-Arten zu unterscheiden, die *Populus* besiedeln und Aecidien auf *Larix* ausbilden. Es wurde erkannt, dass die Überprüfung der morphologischen Merkmale der Aecien der *Melampsora*-Arten anhand von Inokulationsversuchen notwendig ist.

Canadian poplar (*Populus* ×*canadensis*) is a hybrid poplar between *P. deltoides* and *P. nigra* and has been widely planted as roadside or shade trees in China (Fig. 1) because of its strong adaptability and fast growth. The rust disease of this poplar, caused by *Melampsora laricis-populina*, occurs commonly in China, affecting growth because of early defoliation (TIAN & KAKISHIMA 2005, YU & al. 2006). Serious infection of the tree has been observed in Changchun, Jilin Province. The rust has been reported to produce uredinia and telia on many species of *Populus* belonging to *Populus* sect. *Tacamahaca* and *Populus* sect. *Aigeiros*, and spermogonia and aecia on species of

Larix (TAI 1979, HIRATSUKA & al. 1992, BAGYANARAYANA 1998, TIAN & KAKISHIMA 2005). However, the original inoculum of the rust occurrence of *Populus ×canadensis* is still unknown in Jilin Prov., especially its spermogonial and aecial stages were not proved. Therefore, these stages on *Larix* species have been frequently confused with those of other species because several *Melampsora* species also have been known to produce them on *Larix* species. Inoculation experiments were carried out to confirm the life cycle of *Melampsora laricis-populina* based on material collected in Jilin Prov. The morphology of the spermogonial and aecial stages was clarified based on specimens obtained by inoculations compared with those of origin in nature, with other species and previous reports.

Materials and methods

Inoculations

Basidiospore inoculation: Fallen leaves of *Populus ×canadensis* were collected at the campus of Jilin Agricultural University, Changchun, Jilin Province, China (43°48' 33.23'' N, 125°24' 22.01'' E, alt. 238 m) on 15 May 2015 when a yellow and powdery mass of basidiospores was observed on the leaves caused by germinations of the teliospores (Figs. 4–6). Leaves were cut into small pieces (ca. 5 mm^2) and placed on healthy leaves of *Larix olgensis* (Fig. 15). Four seedlings of *L. olgensis* planted in plastic pots were used for inoculations. The inoculated plants were kept in a moist atmosphere in a plastic box in darkness at 18-22ºC for three days after which they were transferred to the place near windows at about 18–22 ºC for observation.

Aeciospore inoculation: Aeciospores produced on *L. olgensis* by basidiospore inoculations were used as inoculua (Figs. 19, 20). Aeciospores were dusted on wet filter papers at ca. 3 mm^2 and spore-dusted papers were placed on young healthy leaves of *Populus ×canadensis* planted in plastic pots. The plants were kept in a plastic box under the same conditions as basidiospore inoculations. A detached leaf inoculation method was also applied for inoculations (NEWCOMBE & CHASTAGNER 1993) (Fig. 9). Young leaves of *Populus ×canadensis* were collected from trees and rinsed twice in distilled water before being placed in 9-cm Petri dishes on filter paper saturated with a 100 µg/ml solution of gibberellic acid. Spore-dusted papers were placed on the surface of an inverted leaf in Petri dishes placed near windows at about 18–22 ºC for observation.

Morphological observations

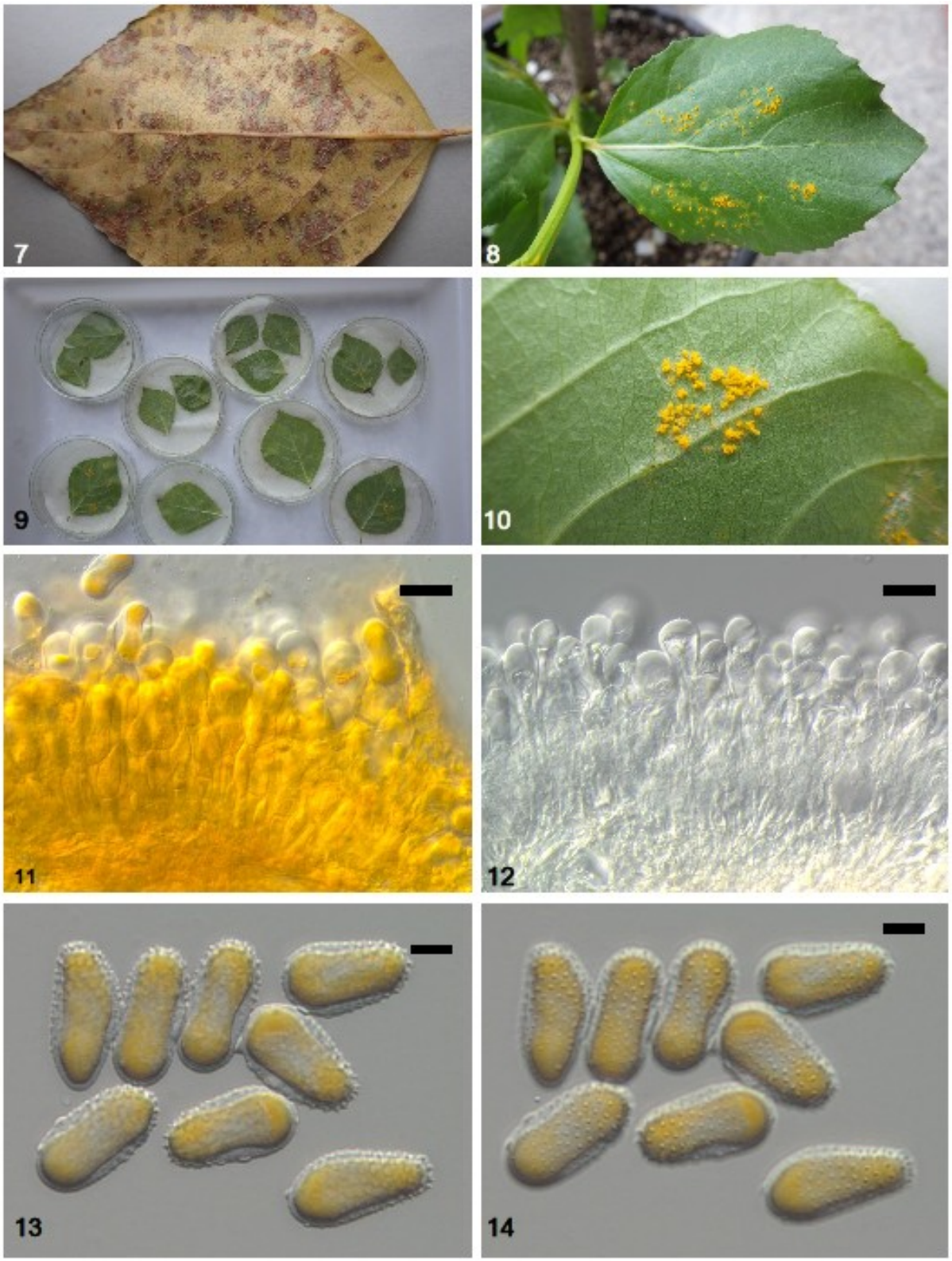
Specimens collected in the field or obtained from inoculations were observed morphologically by light microscope (LM) and scanning electron microscope (SEM). Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Measurements of size and wall thickness were taken from a random sample of 30–50 spores under a DM 2000 microscope (Leica, Germany). For SEM, sori and spores obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using an Ion Sputter Coater (Hitachi MC1000). They were examined with a Hitachi SU8010 SEM operated at 1–10kV. All specimens used in the experiments were deposited in the Fungarium, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Results

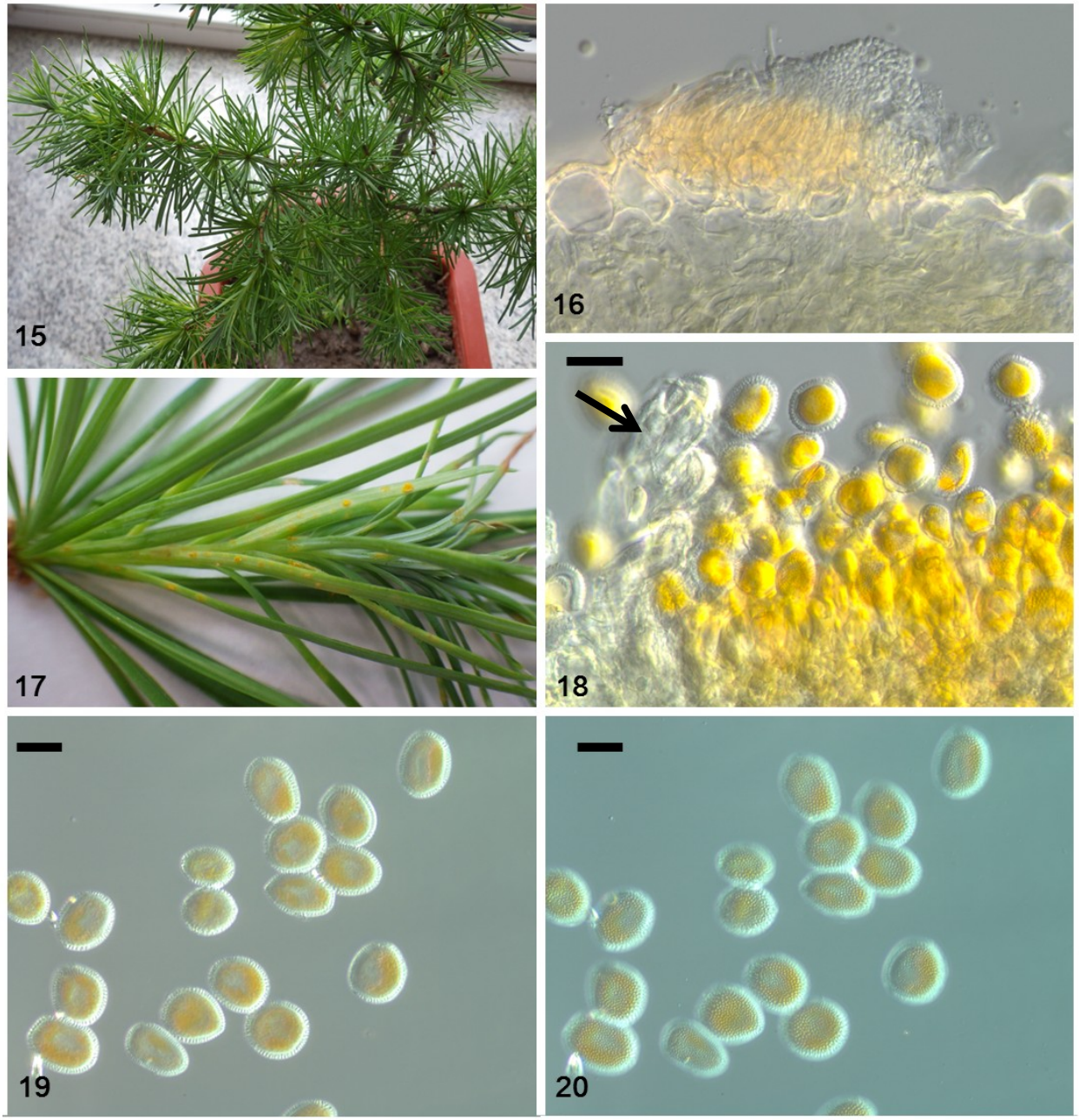
Infections

Six days after inoculation of *Populus ×canadensis* with basidiospores produced by teliospores, small yellow spots of spermogonia appeared on the upper leaf surface of

Figs. 1–6. Telial stage of *Melampsora laricis-populina* on *Populus* ×*canadensis*. – Fig. 1. Canadian popular planted at the campus of Jilin Agricultural University. – Fig. 2. Reddish to dark brown telia produced on the upper side of a leaf. – Fig. 3. Telia of a fallen leaf. – Fig. 4. Yellow mass of basidiospores produced on the surface of a leaf. – Fig. 5. Teliospores produced under epidermal cells. Scale bar 20 µm. – Fig. 6. Basidiospores produced from a telium. Scale bar 5 µm.



Figs. 7–13. Uredinial stage of *Melampsora laricis-populina* on *Populus ×canadensis*. – Fig. 7. Uredinia produced on the underside of a leaf. – Fig. 8. Uredinia produced on the upper side of a leaf by aeciospore inoculation. – Fig. 9. Detached leaf inoculation. Leaves were placed on filter paper saturated with a 100µg/ml solution of gibberellic acid in 9-cm Petri dishes. – Fig. 10. Uredinia produced on underside of a leaf by detached leaf inoculation with aeciospores. – Fig. 11. A vertical section of a uredinium produced under the epidermis of the plant. Urediniospores and paraphyses are intermixed in a uredinium. Scale bar 30 µm. – Fig. 12. Paraphyses in the uredinia. Scale bar 30 µm. – Figs. 13, 14. Urediniospores, echinulate surface except smooth apex (14). Scale bar 20 µm.



Figs. 15–20. Spermogonial and aecial stages of *Melampsora laricis-populina* on *Larix olgensis*. – Fig. 15. *Larix olgensis* used for inoculations with basidiospores – Fig. 16. Aecia produced on underside of leaves. – Fig. 17. Vertical section of a spermogonium (Type 7). Scale bar 20 µm. – Fig. 18. Vertical section of a Caeoma-type aecium with rudimentary peridium (arrow). Scale bar 30 µm. – Figs. 19, 20. Aeciospores. Verrucose surface of aeciospores (20). Scale bar 20 µm.

L. olgensis. About three days later, pale yellow aecia with aeciospores were produced mainly on underside of the leaves (Figs. 15, 17).

In reciprocal inoculations with aeciospores from *L. olgensis* on *Populus* ×*canadensis* yellow uredinia were produced on both upper and lower leaf surfaces five to six days after inoculations (Fig. 8). Detached leaves in Petri-dishes were also infected and produced uredinia (Figs. 9, 10).

Morphology

Spermogonial and aecial stages on *L. olgensis*: Specimens obtained by inoculation were examined.

Spermogonia were amphigenous, subcuticular and type 3 of CUMMINS & HIRATSUKA (2003) (Fig. 16).

Aecia were hypophyllous, erumpent, subepidermal and *Caeoma*-type with rudimentary peridia (Fig. 18, 23, 25).

Aeciospores were catenulate, globose to obovoid and 20–31 × 17–28 µm (av. 25×21 µm). Their walls were hyaline, verrucose and 1.5–3.0 µm thick (Figs. 19, 20, 24).

Specimens examined: HMJAU8120 (3 June 2015, 0, I, inoculated with HMJU8124); HMJAU8121-8123 (4 June 2015, 0, I, inoculated with HMJU8124).

Uredinial and telial stages on *Populus ×canadensis*: In examination of the uredinial stage both specimens collected at the campus of Jilin Agricultural University (Fig. 7) and obtained by aeciospore inoculations (Figs. 8, 10) were used, and their morphology compared. However, no significant difference was observed though urediniospores obtained by detached leaf inoculations were slightly longer and narrower. All telial specimens were collected at the same locality as uredinial specimens (Figs. 2, 3). Basidiospores were observed on the surface of leaves in the specimens collected in May 2015 (Fig. 4).

Uredinia were amphigenous, scattered, subepidermal, erumpent, yellow to orange and *Uredo*-type with abundant paraphyses (Figs. 7–9, 11, 12, 21).

Urediniospores were pedicellate, obovoid or ellipsoid, 21–43 × 12–24 µm (av. 34 × 17 µm) (Figs. 13, 14), Their walls were hyaline, thickened in the equatorial parts, echinulate except smooth at the apex and 1.0-4.0 µm (av. 2.1 µm) thick (Figs. 14, 22).

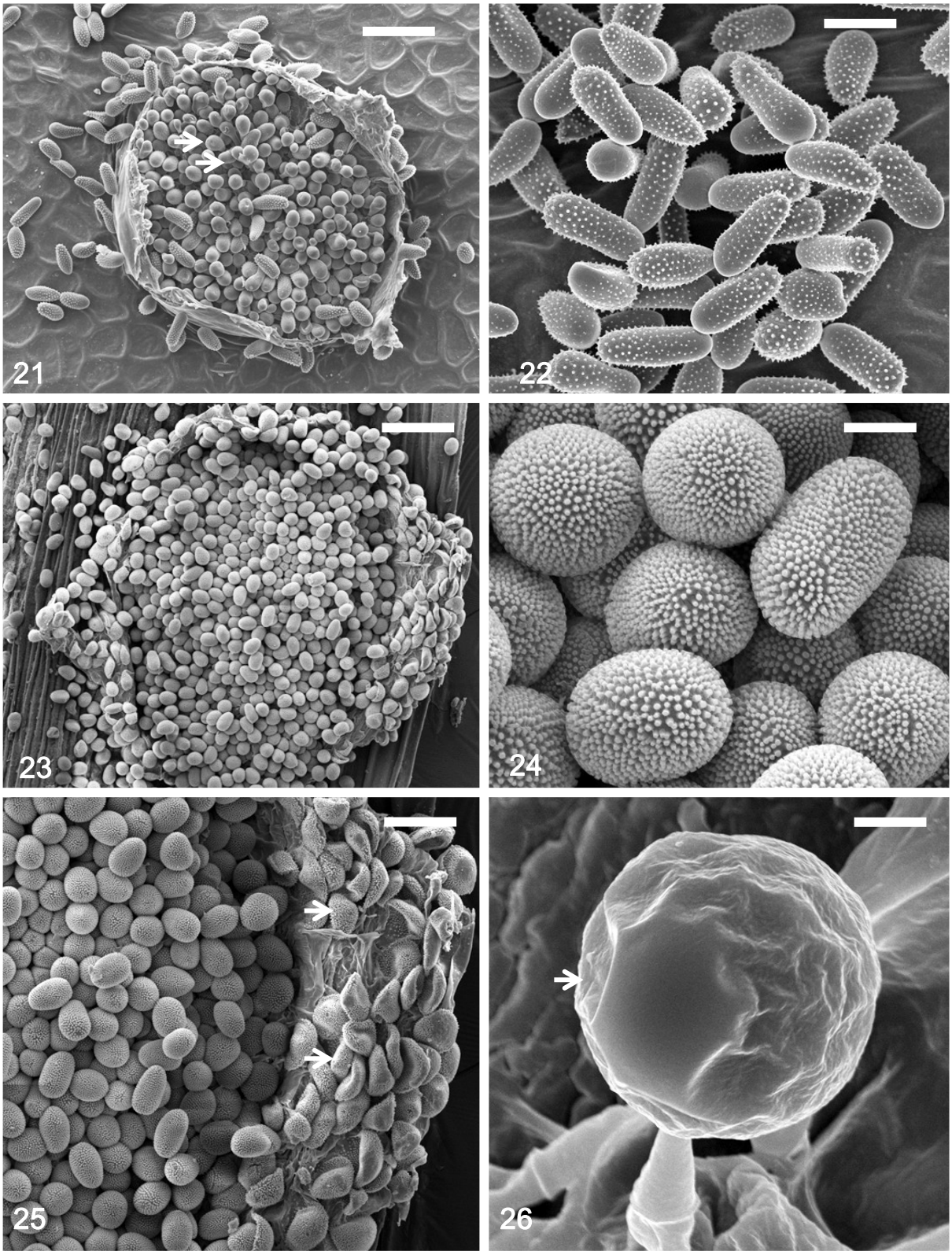
Paraphyses were intermixed in uredinia, clavate to capitate, thickened at the apex and hyaline (Figs. 12, 22).

Telia were mainly epiphyllous, subepidermal, reddish to dark brown consisting laterally adherent crust (Figs. 2, 3).

Teliospores were 1-celled, sessile and 28–61 × 4–19 µm (av. 44 × 10 µm) (Fig. 5). Their walls were not thickend at the apex, brown and 0.5–1.5 µm (av. 0.9 µm) thick. Teliospores germinated after dormancy and produced basidiospores (Figs. 4, 6).

Basidiospores were globose and 7.0–10.0 µm diam (av. 8.8 µm). Their walls were mostly smooth, but several minute projections were observed on their surfaces (Fig. 6, 26).

Specimens examined: HMJAU8126 (26. April 2013, III); HMJAU8127 (14. October 2014, II, III); HMJAU8124 (15. May 2014, III, IV, inoculum to *L. olgensis*); HMJAU8125 (12. June 2015, II, inoculated with aeciospores); HMJAU8128 (15. June 2015, II, inoculated with aeciospores); HMJAU8129 (13. June 2015, II, detached leaf inoculated with aeciospores).



Figs. 21–26. Surface structures of sori and spores of *Melampsora laricis-populina* observed under SEM. – Fig. 21. An uredinium on *Populus* ×*canadensis*. Urediniospores and abundant paraphyses (allow) are observed in an uredinium. Scale bar 40 µm. – Fig. 22. Urediniospores. Their surfaces are echinulate except smooth apex parts. Scale bar 20 µm. – Fig. 23. *Caeoma*-type aecium on *Larix olgensis*. Scale bar 100 µm. – Fig. 24. Verrucose aeciospores. Scale bar 10 µm. – Fig. 25. Rudimentary peridia in a aecium (allow). Scale bar 50 µm. – Fig. 26. A basidiospore. Minute projection (allow) on the surface. Scale bar 2 µm.

Discussion

Identification

Morphological characteristics of uredinial and telial stages on *Populus ×canadensis* were comparable to those found in the descriptions of *M. laricis-populina* by WILSON & HENDERSON (1966), PEI & SHANG (1984), HIRATSUKA & al. (1992), BAGYANARAYANA (1998) and TIAN & KAKISHIMA (2005) although the size of the urediniopsores and teliospores were somewhat variable in these descriptions. The identity of the rust from Jilin Prov. was confirmed as *M. laricis-populina*. This rust has been reported on many species of *Populus* belonging to *Populus* sect. *Tacamahaca* and *Populus* sect. *Aigeiros* and is globally distributed. However, morphological and ecological information relating to the basidiospores is rare, with the exception of the report of PEI & SHANG (1984). They reported that teliospores produced basidiospores under 100% humidity at 13–18 ºC. Basidiospores were obtained from wet fallen-leaves producing teliospores (Fig. 3), but the production period was very short in the experimental site. It may be suggested that optimum condition for basidiospore productions is limited in this site. PEI & SHANG (1984) also reported that basidiospores produced secondary basidiospores, but their function was unclear. In microscopic observations with LM and SEM several minute projections were confirmed on the smooth surface of basidiospores as suggested by PEI & SHANG (1984) (Fig. 26). Most of the rust fungi have been reported to have basidiospores with a smooth wall surface (CUMMINS & HIRATSUKA 2003). Therefore, this surface may be characteristic of this species.

Life cycle

The results of inoculations showed that *M. laricis-populina* on *Populus ×canadensis* collected at our experiment site produced spermogonial and aecial stages on *L. olgensis*. This species has been reported to produce these stages on *Larix* species globally (WILSON & HENDERSON 1966, PEI & SHANG 1984, HIRATSUKA & al. 1992), BAGYANARAYANA 1998, TIAN & KAKISHIMA 2005) and was confirmed in this study. However, these stages could not be found on *Larix* species in fields around our site in the spring and summer seasons though many fallen leaves of *Populus ×canadensis* with telial stages were observed on the surface of the ground. It is suspected that climatic conditions are not suitable for the infections of basidiospores or teliospore germinations, and also new leaf development of species of *Larix* is not synchronized with teliospore germinations. Therefore, we suspect that infections of poplar trees with aeciospores do not occur in Jilin Agricultural University campus and urediniospores from other areas may be the main sources of infections.

Morphology of spermogonial and aecial stages

About 12 species of *Melampsora* have been reported to produce spermogonia and aecia on species of *Larix* (ARTHUR 1934, WILSON & HENDERSON 1966, AZBUKINA 1984, HIRATSUKA & al. 1992, BAGYANARAYANA 1998) (Tab. 1). They have all type 3 spermogonia and *Caeoma*-type aecia (CUMMINS & HIRATSUKA 2003). However, the size and wall thickness of aeciospores within species are sometimes quite different

among references as shown in Tab. 1. This may be the results of observations of specimens mostly collected from fields. Many species produce aeciospores on the same host species of *Larix* (Tab. 1). Therefore, it may be difficult to identify species based on specimens from fields because they all have the same type of aecia (*Caeoma*-type). To clarify, observations of specimens obtained by inoculations are important and more reliable.

Aecial specimens on *L. olgensis* were obtained by inoculations with basidiospores originated from teliospores, which were identified, based on morphology, as *M. laricis-populina*. Data reported here are similar to the description of HIRATSUKA & al. (1992), but quite different from those of WILSON & HENDERSON (1966), AZBUKINA (1984), PEI & SHANG (1984) and BAGYANARAYANA (1998). Therefore, these data should be reevaluated.

Based on the data presented here *M. laricis-populina* has relatively large aeciospores compared to other species of *Melampsora* on *Larix* (Tab. 1). This may be characteristic of this species. In aecia, this species has rudimentary peridia (Figs. 18, 23, 25) as reported by WILSON & HENDERSON (1966) and PEI & SHANG (1984) (Tab. 1). Descriptions of peridia are lacking in other species with the exception of *M. epitea* (*M. laricis-epitea*) on *Salix* (WILSON & HENDERSON 1966). XIE & al. (2015) did not report peridia in aecia of *M. laricis* after observation of specimens obtained by inoculations. Though the presence of peridia in other species on *Populus* awaits reexamination based on specimens obtained by inoculation, their presence or absence will be a useful characteristic to distinguish *M. laricis-populina* from aecia of other species on *Populus*.

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Tab. 1. Comparative aecial morphology of *Melampsora* species producing aecia on *Larix.* (n.d. = no data.) [a] Telial host genus is *Populus* (P), *Salix* is (S). [b] *M. populnea* = *M. laricis-tremulae*, [c] *M. populnea* = *M. populnea* f. sp. *laricis,* [d] *M. bigelowi* = *M. paradoxa*

Species[a]	Aecial host[f]	Peridium[g]	Aeciospore			Reference
			Size(µm)	Wall (µm)	Surface[h]	
M. laricis–populina(P)	L	P	20–31 × 17–28	1.3–2.8	V	The present paper
	D, E	P	17–22 × 14–19	1–1.5	V	WILSON & HENDERSON (1966)
	Larix spp.	n.d	28–38(–40) × 13–18	2	V	AZBUKINA (1984)
	G, E, S, L, P	P	25–39 × 17–37	n.d.	S	PEI & SHANG (1984)
	D, G, A, E, O, S	n.d	22–37 × 18–27	1–1.5	V	HIRASUKA & al. (1992)
	D, G, A, E, O, S	n.d	16–24 × 15–20	1–2	V	BAGYANARAYANA (1998)
M. populnea(P)	E	n.d	14–17 × 12–16	1	V	HIRATSUKA & al. (1992)
M. populnea[b](P)	D	n.d	14–17 × 12–16	1	V	WILSON & HENDERSON (1966)
M. populnea[c](P)	*Larix* spp.	n.d	17–25 × 14–19	2	V	BAGYANARAYANA (1998)
M. laricis(P)	*Larix* spp.	n.d	17–25 × 10–17.2	1.5–2	V	AZBUKINA (1984)
	E, L	N	14–23 × 10–16	0.6–1.3	V	XIE & al. (in press)
M. larici–tremulae(P)	G, E, S, L, P	n.d	15–27 × 13–22	n.d.	S	SHANG & PEI (1984)
M. medusae(P)	A	n.d	17–24 × 17–22	2.5–3	V	ARTHUR (1934)
	Larix spp.	n.d	21–32 × 17–22	2.5–3	V	AZBUKINA (1984)
	D, A, E, O	n.d	17–26(–32) × 13–20(–24)	n.d.	V	BAGYANARAYANA (1998)
M. occidentalis(P)	E, O	n.d	26–35 × 22–27	n.d.	V	BAGYANARAYANA (1998)
M. bigelowi[d](S)	D, A, Y	n.d	18–27 × 15–22	2–3	V	ARTHUR (1934)
M. capraearum(S)	D, E	n.d	15–25 × 12–17	–2	V	WILSON & HENDERSON (1966)
	E	n.d	13–25 × 12–20	2.5–4	V	AZBUKINA (1984)
	E	n.d	15–25 × 13–20	1.5–2	V	HIRATSUKA & AL. (1992)
M. epiphylla(S)	D, E	n.d	12–19 × 11–15	1.5–2	V	AZBUKINA (1984)
	D, E	n.d	15–20 × 12.5–18	1.5–1.8	V	HIRATSUKA & al. (1992)
M. epitea(S)	D, E	n.d	15–22 × 12–16	n.d	n.d	HIRATSUKA & al. (1992)
M. epitea[e](S)	D, E	P	15–25 × 10–21	1.5–2.5 (5)	V	WILSON & HENDERSON (1966)
M. larici–pentandrae(S)	D	n.d	18–26 × 13–10	1.5–2	V	WILSON & HENDERSON (1966)
	Larix spp.	n.d	22–48 × 10–18	2	V	AZBUKINA (1984)
M. larici–urbaniana(S)	E	n.d	15–25 × 13–21	1.5–2	V	HIRATSUKA & al. (1992)

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