Distribution of the Larch Brown Rust Caused by *Triphragmiopsis laricinum* (Uredinales) in Russian Far East

Yoshitaka Ono,*1,1 Zinaida M. Azbukina,*2 Makoto Kakishima,*3 and Shigeru Kaneko*4

*1 Faculty of Education, Ibaraki University, Mito 310, Japan.

*2 Institute of Biology and Pedology, Russian Academy of Sciences. 690022 Vladivostok, Russia.

*3 Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba 305, Japan.

The larch brown rust caused by *Triphragmiopsis laricinum* was found on *Larix cajandri* in Primorsky Territory. Russia. This was a new host and locality record for the pathogen. Preliminary survey of the disease incidence in several localities in East and Northeast Russia and Yakutia-Sakha Republic indicated the restricted distribution of the rust in a few localities in Primorsky Territory. As previously reported in China, *T. laricinum* was proved to form uredinia in nature and to be able to propagate vegetatively by urediniospores. This suggests the fungus could become a devastating pathogen threatening a large-scale larch plantation in Far East of Russia. Field observations and the failure of basidiospore inoculation onto larch needles suggest the heteroecious life cycle of *T. laricinum*.

Key words: Larix, life cycle, Russia, rust disease, Triphragmiopsis

Triphragmiopsis laricinum (Chou) Tai is the only rust fungus that forms the uredinial and telial stages on the needles of Larix species (Chou, 1954; Lohsomboon et al., 1990; Shao et al., 1983). This rust fungus has been reported to occur only in the northeastern provinces of China and the Korean Peninsula with L. gmelini (Rupr.) Kuz., L. olgensis A. Henry (= L. gmelini sensu lato), and L. kaempferi (Lam.) Carr. (= L. leptolepis (Sieb. and Zucc.) Gord.) being reported as the naturally infected hosts (Lohsomboon et al., 1990). In addition to these species, L. principis-rupprechttii Pilger and L. sibirica Ledeb. were successfully infected with the urediniospores in the experimental field in China (Shao et al., 1983). During the rust-flora study in Russia in 1993, we found trees of L. cajandri Mayr heavily infected with T. laricinum at Maisa and Ust-Serebryany, the Sikhote-Alin Reserve, Primorsky Territory and its persistent occurrence was confirmed in 1995 at the same localities.

Species of *Larix* often harbor the aecial stage in the life cycle of several heteroecious rust fungi in the genera *Melampsora* and *Melampsoridium* (Arthur, 1934; Azbukina, 1984; Gäumann, 1959; Hiratsuka *et al.*, 1992; Wilson and Henderson, 1966). The aecial infections of *Melampsora* and *Melampsoridium* rusts are often sparse among needles of the larches and seem not to cause severe needle casts affecting tree growth although quantitative relations between the disease severity and the larch tree growth have not been analysed. Rather, the larches provide these fungi with the place where genetic recombinations take place producing new physiological-pathological "races" of these fungi; and these "races" in turn become pathogenic on such alternate hosts as *Salix* and *Populus* for *Melampsoria* rusts and *Alnus* and *Betula* for *Melampsoridium* rusts.

By contrast, a previous report (Shao *et al.*, 1983) and our field observations showed that *T. laricinum* can infect larch

needles heavily and form dense masses of uredinio- and teliospores causing premature needle casts. Because of this apparent pathogenic potential of the fungus and because the larches are the indispensable component of the Taiga vegetation and economically important in the timber production in Eastern Siberia and Far East of Russia, the study on *T. laricinuum* to be reported here was initiated to determine the geographic distribution of the pathogen in East and Northeast Russia and its possible life cycle in nature. This study was paralleled with the ecological and taxonomic studies of the pine blister rusts caused by *Cronartium* species in Russia in 1992–1994 (Kakishima *et al.*, 1995).

Materials and Methods

1 Localities surveyed (Fig. 1)

Primorsky Territory: Vladivostok (43°10 N, 131°55′ E) in 1992–1995, Mt. Livadiyskaya (43°5′ N, 132°40′ E) in 1992 and 1995, the Ussurisky Reserve (43°45′ N, 132°15′ E) in 1992, 1993 and 1995, the Sikhote-Alin Reserve (Ternei, 45°5′ N, 136°35′ E; Maisa, *ca.* 24 km northwest of Ternei; Ust-Serebryany, *ca.* 25 km west of Ternei) in 1993–1995; Khabarovsk Territory, Bichuha (48°5′ N, 135°E) in 1993 and 1994, the Bolshechechtsirski Reserve (*ca.* 20 km west of Bichuha) in 1993 and 1994; Magadan Region, Magadan (*ca.* 59°N, *ca.* 151°E) in 1993 and 1994, Settlement Contact (61°55′ N, 147°50′ E) in 1993 and 1994; Yakutia-Sakha Republic, Yakutsk (62°N, 129°40′ E) in 1995.

2 Specimens examined

On *L. cajandri* Mayr, Russia, Primorsky Territory. the Sikhote-Alin Reserve, Maisa, 8 Sept. 1993. Y. Ono 93RU-165 (IBA-6885, TSH-R8908); Ust-Serebryany, 13 Sept. 1993. Y. Ono 93RU-253 (IBA-6971, TSH-R8997); Maisa, 26 Aug. 1995. Y. Ono, S. Kaneko, and Z. M. Azbukina 95RU-179 (IBA-7518, TSH-R9488) and 95RU-186 (IBA-7525, TSH-R9494); on *L. kaemfperi* (Lam.) Carr., Japan, Ibaraki, Mito, Bunkyo, Ibaraki University campus (results of inoculation

^{*4} Forestry and Forest Products Research Institute, Ibaraki 305, Japan.

¹ The corresponding author.

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Fig. 1 A map of Northeastern Siberia and Far East of Russia.

experiments), 4 Nov. 1995. Y. Ono 3176 (IBA-7679); 24 Nov. 1995. Y. Ono 3183 (IBA-7686); on *L. gmelini* (Rupr.) Kuz., China, Heilongjiang, Harbin. June 1993. Xu Yu (IBA-7749). All these specimens have been deposited in the Herbarium of Systematic Mycology, Ibaraki University (IBA), the Herbarium of Institute of Biology and Pedology (VLA), and the Herbarium of Institute of Agriculture and Forestry, University of Tsukuba (TSH).

3 Inocula

Cacomatoid and peridermioid aecia were found on *L. cajandri* needles at Maisa in June 1995. Aecidioid aecia were also found on leaves of *Thalictrum tuberiferum* Maxim. and *Jeffersonia dubia* Maxim. grown nearby *L. cajandri* that were infected by *T. laricinum* previously. These aeciumbearing needles and leaves were collected and the spores were subjected to inoculation experiments immediately after the collection. Uredinio- and teliospore-bearing needles of *L. cajandri* were collected at Maisa in August 1995, then airdried at room temperature, and preserved in a refrigerator at *ca*. 5 °C.

4 Inoculations

Aeciospore inoculations were carried out at Maisa in June

1995. Seedlings (unknown age) of *L. cajandri* were taken from the field and transplanted in a plastic bag. Two stocks were used for the inoculation of each aecial fungus on the above-mentioned three plants. Abaxial surface of the larch needles were dusted with aeciospores scraped from the aecia. The spore-dusted larches were sprayed with water and maintained in a sealed plastic bag at room temperature $(12-20^{\circ}C)$ for 48 h. Subsequently, the inoculated larches were transferred to Institute of Biology and Pedology in Vladivostok where further observations were undertaken.

Inoculation experiments with T. laricinum urediniospores and basidiospores were undertaken through the permission of the Minister of Agriculture, Forestry and Fishery (the permission number: 7 YokoShoku 742) at Ibaraki University in Mito. Urediniospore inoculations were carried out in September=October 1995. Three two-year-old seedlings of L. kaempferi were grown in a clay pot (18 cm diam.) with loam soil. Six seedlings in two pots were inoculated with urediniospores. The urediniospores were scraped from the uredinia on L. cajandri needles, that had been preserved in a refrigerator as described above, and dusted on small pieces (1 \times 3 mm) of water-saturated filter paper. The spore-dusted pieces of filter paper were subsequently placed on abaxial surface of the needles of L. kaempferi. The inoculated seedlings were sprayed with distilled water and placed in a dark moist chamber at room temperature (18-22 °C) for 48 h. The seedlings were then removed into a growth chamber (ca. 20 °C) for further observations. The inoculation was repeated twice.

Basidiospore inoculations were carried out in March 1996. The teliospore-bearing needles that had been preserved in a refrigerator as described above were soaked in a running tap water for 10-14 days at room temperature to induce germination. The needles were then placed on a water saturated filter paper in petri dish (9 cm diam.) and incubated under the dark at 18 °C. After a few-day incubation when teliospore germination was confirmed under binocular microscope, the needle-bearing filter papers were stuck on under side of a lid of a moist chamber (60 cm diam. and 100 cm high). Six three-year-old seedlings of L. kaempferi and three seedlings (unknown age) of L. cajandri taken from Maisa, which were grown in clay pots with loam soil, were placed in the chamber and sprayed with distilled water. Then, the lid was gently covered on the chamber such a way that the basidiospores formed on germinating teliospores on the needles fell on the needles of L. kaempferi and L. cajandri to be inoculated. The chamber was maintained at room temperature (18-22 °C) for 48 h. Subsequently, the inoculated larches were removed into a growth chamber (ca. 20 °C) for further observations.

- Fig. 2 A heavily infected tree of *Larix cajandri* with yellowing needles. Scale: 1 m.
- Fig. 3 Black telia formed on the abaxial surface of needles of Larix cajandri. Scale: 3 cm.
- Fig. 4 Adaxial surface view of yellowing needles of Larix cajandri. Scale: 3 cm.

Figs. 2–4 The larch brown rust caused by *Triphragmiopsis laricinum* found at Maisa, the Sikhote-Alin Reserve, Russia.

Fig. 5 Uredinia (orange-brown) and telia (black) formed on L. kaempferi by the inoculation of urediniospore from Larix cajandri. Scale: 1 cm.

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Figs. 2-5

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Results and Discussion 1 Geographic distribution

The larch brown rust was found for the first time at Maisa and Ust-Serebryany in the Sikhote-Alin Reserve, Primorsky Territory, in Russia in August–September 1993 with *L. cajandri* being a new host for this pathogen. In both localities, juvinile trees (less than 10 years old) were heavily infected by *T. laricinum* (Fig. 2) and their needles were covered densely by black masses of teliospores (Fig. 3). These heavily infected needles were yellowing (Fig. 4) and easily casted. Mature trees as evidenced by the presence of cones on the branches were infected as well but less heavily. The rust incidence was negligible in both localities in 1994. However, outbreak of the rust was again observed in 1995 confirming its persistent occurrence in these same localities. However, the rust incidence was not confirmed in the other places described above.

The larch brown rust has been reported several localities in the Provinces of Liaoning, Jirin and Heilongjian in China and two localities in the Korean Peninsula (Lohsomboon *et al.*, 1990; Shao *et al.*, 1983). Now it is known to be distributed in Russian Far East. Despite that the total range of the rust distribution is broad, individual localities where the rust occurs seem to be isolated. Even in the Sikhote-Aline Reserve, the rust incidence was confined only in two localities; larch stands were almost continuous, though stand densities vary, in a large area of the Reserve but no rust was found in larch stands 2-3 km distant from the place where the rust occurred heavily.

Reason(s) for the isolated distribution of the larch brown rust is not clear. This may be reflected by uneven distribution of larch trees that are diverse in their age, physiology and genetically controlled rust resistance. At Maisa in the Sikhote-Alin Reserve, two morphologically variable larches were noticed: one with smaller cones and with heavy infection by the rust fungus and another with larger cones without the infection. The difference in the observed cone size may be of taxonomic importance, while these morphological variants have been identified as L. cajandri (an unpublished flora, the Sikhote-Alin Reserve Office). However, the Reserve is located within the natural distribution range of L. gmelini sensu stricto, L. kamtschatica (Rupr.) Carr., L. × lubarskii Sukacz., L. \times maritima Sukacz., and L. olgensis in addition to L. cajandri (Czerepanov, 1995). As stated previously, L. gmelini and L. olgensis are known to be the host of the rust fungus in China. Consequently, taxonomic identity of the diseased and nondiseased larches which correspond with a cone morphology variation in the area must be carefully restudied. An important point here is that the morphological variation does indicate genetic differences in the physiological attributes and the rust resistance among larch trees distributed in the Reserve. Variable topography and uneven distribution of microclimatical conditions may reinforce field resistance of individual larch trees. These biotic and abiotic factors may synergistically act and eventually determine mosaic or patchy distribution of susceptible/resistant larch populations.

Degree of efficacy at which the pathogen's vegetative

reproduction by uredinospores occurs would also be expected to have a great influence on widespread distribution of the disease. Type of life cycle may affect the rust incidence as well. It has been repeatedly documented (Arthur, 1928; Jackson, 1931; Ono, 1985; Savile, 1953) that both autoecious and heteroecious macrocyclic rust fungi may delete a uredinial stage in the life cycle particularly in arctic and alpine zones due to a short growing season. For the heteroecious rust fungi whose uredinia are deleted or urediniospores are nonfunctional under the cold climate, a close association of aecial host(s) and telial host(s) in a habitat inevitably become essential for their survival and persistent existence. Accordingly, distribution of alternate host(s) automatically determines a possible range of ecogeographic distribution of those fungi. This may explain partly why the rust fungus is not found either in Yakutsk or in Magadan Region.

2 Possible role of urediniospores in the fungus life cycle

Only the telial stage was described for the fungus in the original report (Chou, 1954) and subsequently the uredinial stage was reported to occur in the fungus life cycle (Loh-somboon *et al.*, 1990; Shao *et al.*, 1983). Shao *et al.* (1983) showed that the fungus was able to infect and spread on *L. gmelini*, *L. olgensis*, *L. kaempferi*. *L. principis-rupprechttii*, and *L. sibirica* by the urediniospores in the experimental field. In our study, urediniospores from *L. cajandri* were successfully inoculated on *L. kaempferi* needles each time tried, resulting in reproduction of the uredinia 13–19 days after the inoculation under the experimental condition at *ca.* 20 °C. This latent period is in accordance with that reported by Shao *et al.* (1983). As the result of the urediniospore inoculations, telia were produced on the same inoculated leaves simultaneously with the uredinia (Fig. 5).

The occurrence of the uredinia in the field and the successful inoculations with the urediniospores clearly showed that this fungus was able to persist and spread vegetatively by producing the urediniospores repeatedly if climatic corditions allow. Accordingly, the rust incidence had been epidemic in larch nurseries in the Provinces of Heilongjian, Jiling, and Liaoning in China for more than 30 years (Shao *et al.*, 1983). However, no climatic conditions that directly affect the epidemics were not identified. From the field observations and the inoculation experiments by Shao *et al.* (1983) and ourselves, it is reasonable to speculate that the fungus can reproduce at least 3-5 times vegetatively by the urediniospores in July and August at Maisa and Ust-Serebryany although no detailed meteorological data at these localities is available.

It is not known whether uredinial reproductions repeated less than 10 times in one season is insufficient for the pathogen to become epidemic. However, it should be stressed that the pathogen is a potential threat to large-scale managed larch stands in the temperate zone as in Japan; if the pathogen invades such a zone and if its life cycle can complete, it would become epidemic there where repeated infections and wide-spread by the urediniospores are possible. Therefore, every effort should be paid not to introduce this pathogen to Japan. Ono et al.

3 Life cycle of the fungus

Field observations proved the presence of the uredinia in the life cycle of this fungus but no spermogoinal-aecial stage. None of three species in *Triphragmiopsis* is known the full life cycle (Lohsomboon *et al.*, 1990). If the genus is closely allied to the family Sphaerophragmiaceae (Lohsomboon *et al.*, 1992, 1994), then *Triphragmiopsis* species are likely to have autoecious life cycle.

At Maisa in June 1995, caeomatoid and peridermioid aecia were found on the same trees of *L. cajandri* on which the telia of *T. laricinum* were collected in the previous years. Accidioid aecia were also found on the leaves of *T. tuberiferum* and *J. dubia* that grew nearby the larches. Inoculations with aeciospores from these aecia were unsuccessful indicating that these aecia were not the aecial stage of *T. laricinum*. The caeomatoid aecia seem to be an aecial stage of *Melampsora* sp. and the peridermioid sori *Melampsoridium* sp. The aeica on *T. tuberiferum* may be an aecial stage of *Puccinia recondita* Roberge ex Desm. and those on *J. dubia* is *Triphragmiopsis jeffersoniae* Naumov.

Although the teliospores germinated fairly well forming sufficient amount of basidiospores for the inoculation, the basidiospore inoculations onto the needles of *L. cajandri* and *L. kaempferi* were also unsuccessful. Similarly, Shao *et al.* (1983) failed to obtain a spermogonial-aecial stage of the fungus on five *Larix* species in their field inoculation experiments. Absence of a possible spermogonial-aecial stage of *T. laricimum* on *L. cajandri* in nature and the failure of the basidiospore inoculation onto the needles of *L. cajandri* suggest a heteroecious nature of the life cycle of *T. laricinum*. This may explain partly the isolated geographic distribution of the fungus in eastern Asia. However, further extensive inoculation experiments are required along with careful field observations before any conclusion as to the fungus life cycle is drawn.

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