Mycoscience 42: 519–533, 2001

Morphological analyses of urediniospores and teliospores in seven *Phragmidium* species parasitic on ornamental roses

Dono Wahyuno[1], Makoto Kakishima[1] and Yoshitaka Ono[2]*

[1] Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan
[2] Faculty of Education, Ibaraki University, Mito, Ibaraki 310–8512, Japan

Received 25 June 2001
Accepted for publication 4 October 2001

Ornamental roses worldwide harbor 10 *Phragmidium* species. Among these, *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum* are frequently reported; however, these species are morphologically similar and difficult to distinguish. For better circumscription and correct identification of the species, this study examined morphological features in urediniospores and teliospores of the seven *Phragmidium* species collected on ornamental rose cultivars and wild species. The results indicated that some host-specific groups could be well circumscribed by the morphological properties of urediniospores and teliospores. However, without the precise identification of hosts, these morphological properties were not effective key characters for the identification of the species.

Key Words——identification; *Phragmidium*; *Rosa*; Rosaceae; taxonomy; Uredinales.

The rust genus *Phragmidium* Link is characterized by linearly arranged multicellular teliospores with a persistent pedicel, the lower half of which is usually thickened and hygroscopic (Cummins and Hiratsuka, 1983). Most species produce subcuticular spermogonium, caeomatoid aecium, uredinium and telium in the autoceious macrocyclic life cycle. Species of the genus are restricted to plants of the family Rosaceae, especially the genera *Rosa, Rubus* and *Potentilla*. Among 60 or more species of *Phragmidium*, some 30 species have been reported to infect wild *Rosa* species and ornamental cultivars.

Ornamental rose cultivars are selections from more than 70 wild species and their hybrids (Beales et al., 1998). Species of the genus *Rosa* are classified in subgenera *Hulthemia*, *Hesperhodes* and *Eurosa*; and the subgenus *Eurosa* is further divided into 10 sections. Domesticated species and hybrid cultivars are distributed in these subgenera and sections, except for *Hulthemia*.

Because of the heterogeneity of rose species and cultivars in their origin and geographic distribution, several *Phragmidium* species are involved in the rust diseases of ornamental roses. Two or more species are reported to occur in any particular area of the world and, overall, 10 species are considered to be the pathogens: *P. americanum* (Peck) Dietel, *P. fusiforme* Schröter, *P. montivagum* Arthur, *P. mucronatum* (Persoon: Persoon) Schlectendal, *P. rosae-californicae* Dietel, *P. rosae-pimpinellifoliae* Dietel, *P. rosae-rugosae* Kasai, *P. rosicola* (Ellis & Everhart) Arthur, *P. speciosum* (Fries) M. C. Cooke and *P. tuberculatum* J. M. Müller (Pirone et al., 1960; Howden and Jacobs, 1973; Horst, 1983).

The causal species of the rose rusts have been variously circumscribed and classified by combinations of the morphological characteristics of a maximum of four spore stages produced in the life cycle (Arthur, 1934; Hiratsuka, 1935; Gäumann, 1959; Wilson and Henderson, 1966; Azbukina, 1984; Wei, 1988; Hiratsuka et al., 1992). Although these *Phragmidium* species are autoecious and macrocyclic in the life cycle, spermogonial-aecial stage is short-lived and quickly replaced by uredinia in the early growing season. Thus, the rose rust species persist in the uredinial or uredinial-telial stage for almost the entire growing season, and either uredinia or a mixture of uredinia and telia can be found at any part of the growing season or on any particular specimen. Because of the prevalence of the uredinial-telial stage and because of the morphological diversity of teliospores, circumscription and identification of the rose rust species have relied heavily on morphological characteristics of the teliospores with supplementary urediniospore characteristics.

Because the morphological circumscription of species does not seem clear and also because a variation range of each morphological feature is broad and continuous, the genus *Phragmidium* has been among the most difficult taxa in the Uredinales to classify and to identify its species, particularly those on *Rosa*. Among the 10 species parasitic on ornamental roses, *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum* are morphologically similar and widespread in the world. Consequently, the identification of the species causing

* Corresponding author: E-mail: onoy@mito.ipc.ibaraki.ac.jp

the rust diseases of ornamental roses has often been problematic.

It is of mycological and horticultural importance to know exactly how many *Phragmidium* species are involved in the rose rusts, what their biological nature is and how they can be correctly identified. This study aims at examining morphological properties in the uredinial and telial stages to find which properties can be reliable key characters for the identification.

Materials and Methods

Specimens examined Herbarium specimens examined were loaned from the Arthur Herbarium, Purdue University (PUR); the Faculty of Agriculture, Hokkaido University (SAPA); the Botanical Garden and Museum, Berlin-Dahlem (B); the National Fungus Collections, the United States Department of Agriculture (BPI); the Herbarium of Systematic Mycology, the Faculty of Education, Ibaraki University (IBA); the Museum of Natural History, Wroclaw University (WRSL); and the Herbarium of the University of Tsukuba (TSH). The specimens had been identified as *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum*. To examine both uredinia and telia in the same life-cycle, 198 specimens with both spore stages were selected from more than 600 specimens (Table 1). Among them, the host species had been identified in 119 specimens. The specimens included the type specimens of *P. montivagum* (holotype on *Rosa acicularis* collected by A. Nelson in Wyoming in 26 July 1895, in Herb. PUR), *P. rosae-pimpinellifoliae* (holotype on *R. pimpinellifolia* collected by Kemmler at Donnstetten no. 1671 in Herb. B) and *P. rosae-rugosae* (one of the syntypes on *R. rugosa* collected by M. Kasai in Sapporo, Japan in 30 October 1908, in Herb. SAPA).

The ontogenic terminology system refined by Hiratsuka (1973) and Cummins and Hiratsuka (1983) was employed in identifying and describing life-cycle stages of the *Phragmidium* specimens throughout the study.

Microscopy Urediniospores and teliospores were scraped from the specimens and mounted in a drop of lactophenol solution on a microscopic slide. For each specimen, 50 spores were randomly chosen and observed under an Olympus BH 100 microscope for the selected morphological features listed. Measurements were made with a Leica Q-Win Image Analyzer.

Spore-wall color was determined under an Olympus BH 100 microscope with a tungsten lamp without filter. The light intensity was adjusted by controlling the voltage of the lamp. The color was described according to Rayner (1970).

Number and distribution of germ-pores in urediniospores were determined by the aniline-blue squash method (Jennings et al., 1989). The distribution pattern of the pores was categorized according to Cummins and Hiratsuka (1983).

For scanning-electron-microscopy, urediniospores and teliospores were dusted on double-adhesive tape on a specimen holder and coated with platinum-palladium at 25 nm thickness under a Hitachi E-1030 ion sputter. The coated specimens were observed under a Hitachi S-4200 operating at 15 kV. The urediniospore-surface type was determined according to Cummins and Hiratsuka (1983).

Statistical analyses Statistics including multivariate analyses of measured continuous numerical variables were performed using the software package Systat™ version 5.2 (Wilkinson, 1989) run on a Macintosh Power Mac G4. Discrete numerical or qualitative attributes or host species were superimposed on two-or three-dimensional scatter diagrams generated from the analyses to detect possible groups.

Results

Morphological analyses of 119 specimens with identified host plants were essentially the same as those of 198 specimens, which included those on unidentified host plants. Therefore, only results from the analyses of host-identified specimens are presented here to facilitate discussion of morphological variations in relation to putative host specificity.

Teliospore morphology Shape and size of teliospores varied both among specimens and within a specimen (Fig. 1A, Table 2). Mean teliospore length in individual specimens ranged from 61.6 to 106.7 μm and mean width from 26.3 to 37.3 μm. Among these specimens, BPI0126320 on *R. macdaugali*, PUR-56692 on *R. manca* (Fig. 4A), PUR7836 on *R. engelmannii*, PUR 8164 on *R. gallica* and PUR7761 on *R. suffulta* bore small spores. The spore length ranged from 50.1 to 83.0 μm and the width from 21.8 to 34.4 μm in the five specimens. PUR44755 on *R. nutkana*, PUR48167 on *R. eastwoodii*, PUR7847 on *R. fendleri* (Fig. 4B), PUR7887 on *R. macounii*, PURF1514 on *R. tomentosa* were among medium-spore bearing specimens. The spore length ranged from 61.0 to 106.4 μm and the width from 26.2 to 37.1 μm in the five specimens. On the other hand, PUR7725 on *R. setigera*, PURF11650 on *R. rugosa*, SAPA (Tokachi) on *R. rugosa*, BPI0126648 on *R. centifolia* and BPI0126642 on *R. alba* (Fig. 4C) were among large-spore bearing specimens. The spore length ranged from 65.0 to 135.6 μm and the width from 29.2 to 45.0 μm in the five specimens. As shown in Fig. 1A, there were many specimens with the spores of various sizes covering all three groups, and no disjunction was detected in the variation range to circumscribe spore-size groups among the specimens.

No correlation was detected between teliospore length and width (Fig. 1A, not significant in Spearman's rank correlation analysis). Thus, the teliospore length/width ratio also varied both among specimens and within a specimen, mean value ranging from 2.0 in BPI0126306 on *R. arkansana*, PURF11649 on *R. canina*, PURF1507 and PURF9504 on *R. gallica* (Fig. 4D) and PURF11645 on *R. rugosa* to 3.3 in PUR 48581 on *R. acicularis*; 3.4 in BPI0126299 on *R. acicularis* and TSH on *R. rugosa*; 3.5 in IBA0311 on *R. acicularis* and 4.0 in

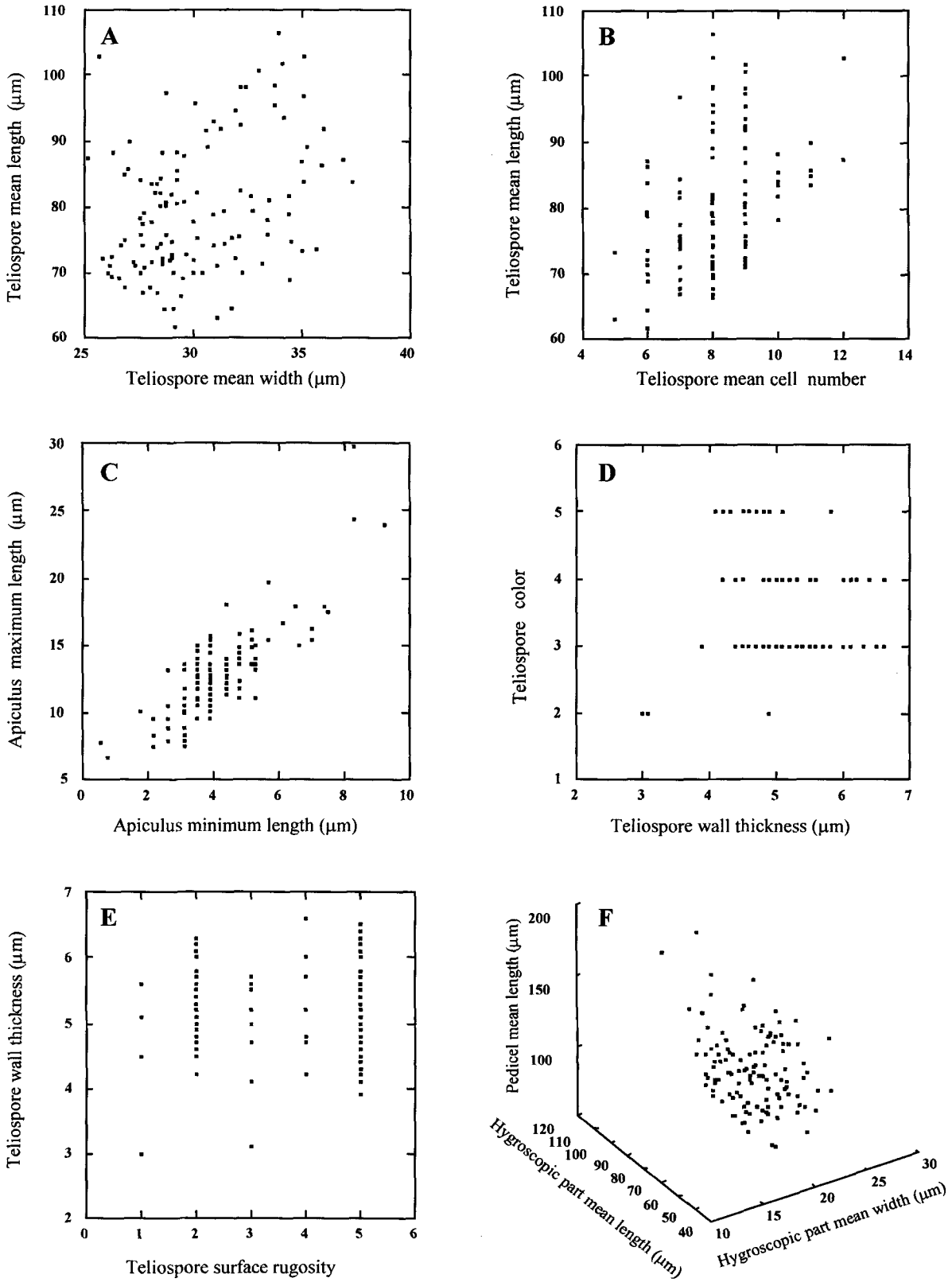

Fig. 1. Variations in the teliospore characteristics observed in the *Phragmidium* specimens. A. Mean lengths against mean widths. B. Mean lengths against mean cell numbers. C. Apiculus maximum lengths against apiculus minimum lengths. D. Darkness of wall color (light brown, 1, to blackish brown, 5) against wall thickness. E. Wall thickness against degrees of wall-surface rugosity (faint, 1, to conspicuous, 5). F. Relationship among the pedicel mean length and mean length and mean width of the hygroscopic part of pedicels.

Table 1. *Phragmidium* specimens examined in this study.

Host	Locality	Accession number[1)]
Rosa acicularis Lindl.	Alaska, U.S.A.	BPI 0126284, PUR F48581
	Colorado, U.S.A.	PUR 7925, 7935
	Michigan, U.S.A.	IBA 0311 and 1387, PUR 7797
	Montana, U.S.A.	BPI 0126338 and 0126270, PUR 55312, 7746, 7927 and 7930
	Russia	BPI 0126299
	Russia	SAPA
	Wyoming, U.S.A.	PUR 61189, 61191, 7934, 7933, 7923 and 7926
	New Mexico, U.S.A.	PUR 61979
R. aciculata Cockerell	Colorado, U.S.A.	PUR 7811
R. alba L.	Alaska, U.S.A.	PUR 8161
	New York, U.S.A.	PUR 8162
	No locality given	BPI 0126642
R. aleutensis Crép.	Alaska U.S.A.	PUR 48583
R. blanda Ait.	Wisconsin, U.S.A.	PUR 65617 and 7660
	Canada	PUR 7657
R. bourgeaniana Crép.	U.S.A.	PUR 44750
	Indiana, U.S.A.	PUR 44752
R. canina L.	Germany	PUR F1567 and F1561
	Belgium	PUR F11649
	Turkey	PUR F16657
R. carolina L.	Indiana, U.S.A.	PUR 88322
	New York, U.S.A.	PUR 7708
	Wisconsin, U.S.A.	PUR 44733
R. centifolia L.	No locality given	BPI 0126648
R. cinnamomea L.	Germany	PUR F1552
R. damascena Mill.	California, U.S.A.	PUR 59483
	Iowa, U.S.A.	PUR 7705
R. davurica Pall.	China	SAPA
	Japan	SAPA
R. eastwoodii Rydb.	California, U.S.A.	PUR 48167
R. engelmannii S. Wats	U.S.A.	PUR 7836 and 7845
	Wyoming, U.S.A.	PUR 7826
R. fendleri Crép	Colorado, U.S.A.	PUR 7847, 7853, and 7860
	New Mexico, U.S.A.	PUR 7861
	Wyoming, U.S.A.	PUR 7868
	Montana, U.S.A.	PUR 7910
	Nevada, U.S.A.	PUR 48561
	New Mexico, U.S.A.	PUR 48092 and 7908
	Utah, U.S.A.	PUR 51058 and 7914
	Wyoming, U.S.A.	PUR 63194, 7904 and 7105
R. gallica L.	Argentina	PUR F9504
	Ecuador	PUR F1507
	Alaska, U.S.A.	PUR 8164
R. gymnocarpa Nutt.	Wyoming, U.S.A.	BPI 0126317
	Oregon, U.S.A.	PUR 7877 and 7878
R. macdaugali Holz	Idaho, U.S.A.	PUR 49821
	Montana, U.S.A.	BPI 0126320
R. macounii Greene	Columbia, U.S.A.	PUR 7887
	Idaho, U.S.A.	PUR 7875
	Nevada, U.S.A.	PUR 7892
	Utah, U.S.A.	PUR 7872
R. manca Greene	Arizona, U.S.A.	PUR 56689 and 56692

Table 1. (Continued)

R. marretii Lev.	Kushiro, Japan	SAPA and SAPA
R. mirifica Cockerell	New Mexico, U.S.A.	PUR 51945 and 51814
R. nutkana Presl.	Alaska, U.S.A.	PUR 48582, 7770 and 7782
	Montana, U.S.A.	PUR 44755
	Oregon, U.S.A.	BPI 0126326
	Washington, U.S.A.	PUR 53448
R. pendulina L.	Germany	PUR F1548
	Switzerland	WRSL
R. pimpinellifolia L.	Wyoming, U.S.A.	BPI 0126625 and B
R. pisocarpa G.Gray.	Idaho, U.S.A.	PUR 7787
R. pulverulenta Rydb.	Nevada, U.S.A.	PUR 7916
R. rubiginosa L.	Nebraska, U.S.A.	PUR 8226
	Switzerland	PUR F1157
R. rugosa Thunb.	Hokkaido, Japan	TSH and SAPA
	Ishikari, Japan	SAPA and SAPA
	Japan	BPI 0126669
	Shiribeshi, Japan	BPI 0126671
	Tokachi, Japan	SAPA
	Tsukuba, Japan	TSH
R. setigera Michx.	Indiana, U.S.A.	PUR 44734, 7731 and 7725
	Ohio, U.S.A.	PUR 44740
	Tennessee, U.S.A.	PUR 44735 and 44737
	Wisconsin, U.S.A.	PUR 53303
R. stellata Wooton	U.S.A.	PUR 59705
R. suffulta Greene	Canada	PUR 44758
	Montana, U.S.A.	BPI 0126643 and 0126306
	North Dakota, U.S.A.	PUR 7761 and BPI 0126318
R. tomentosa Sm.	Germany	PUR F1514
R. virginiana Mill.	Maine, U.S.A.	PUR 7669
	New Hampshire, U.S.A.	PUR 44741
Rosa sp.	Alaska, U.S.A.	PUR 48491, 48497, 7789 and 53449
	California, U.S.A.	PUR 44893, 7975, 8182, and 50718, BPI 0125302
	Colorado, U.S.A.	PUR 52482
	District of Columbia, U.S.A.	PUR 7703
	Hawaii, U.S.A.	BPI 0126634
	Kansas, U.S.A.	PUR 44796
	Massachusetts, U.S.A.	PUR 49172 and 7696
	Michigan, U.S.A.	PUR 8190, 89295, 51672 and 7796
	Montana, U.S.A.	BPI 0126293, IBA 1320 and 1363, PUR 7808
	New York, U.S.A.	PUR 7697 and 8194
	New Jersey, U.S.A.	PUR 8214, 7963 and 7691, BPI 0126291
	New Mexico, U.S.A.	PUR 7963
	North Dakota, U.S.A.	PUR 7683 and 8173
	Oregon, U.S.A.	PUR 8216
	Pennsylvania, U.S.A.	PUR 61459
	South Dakota, U.S.A.	PUR 44744
	Utah, U.S.A.	PUR 60983
	Vermont, U.S.A.	PUR 8177
	Washington, U.S.A.	PUR 8198, 50845
	West Virginia, U.S.A.	PUR 8208 and 44746
	Wisconsin, U.S.A.	PUR 7798 and 7684
	Wyoming, U.S.A.	PUR 62432
	Argentina	PUR F9501

Table 1. (Continued)

	Bolivia	PUR F1526
	Brazil	PUR F1524
Rosa sp.	Canada	PUR 48594, 8215, 59839, 65693, 49165, 7932, and 7702, BPI 0126627
	Canary Island	PUR F1516
	China	PUR F14181
	Chile	PUR F1523
	Denmark	BPI 0125304
	Ethiopia	PUR F16304
	Ecuador	PUR F1528 and BPI 0125309
	Germany	BPI 0126632, 0126633 and 0125308; PUR F1521 and F 1484
	Guatemala	BPI 0126290, 0126292 and 0126288
	Himalaya	PUR F1527
	Italy	PUR F9485
	Japan	JSH
	Mexico	BPI 0125310 and 0125301
	Netherlands	PUR F11834 and 11650
	New Zealand	BPI 0125306
	Peru	PUR F1530 and F1529
	Sweden	BPI 0126631

[1] B: The Botanical Garden and Museum Berlin-Dahlem, Germany.
BPI: The U.S. National Fungus Collection, USA.
IBA: The Herbarium of Systematic Mycology, Faculty Education, Ibaraki University, Japan.
WRSL: The Museum of Natural History, Wroclaw University.
PUR: The Arthur Herbarium, Purdue University, West Lafayette, USA.
SAPA: The Herbarium Botanical Institute, Faculty of Agriculture, Hokkaido University, Japan.
TSH: The Mycological Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba, Japan.

PURF1548 on *R. pendulina* (Fig. 4E). Between the extremes, a continuum of ratios was found, with a median value of 3.0 in BPI0126284 on *R. acicularis*, PUR7877 on *R. gymnocarpa*, PUR7925 on *R. acicularis* (Fig. 4F) and PUR44735 on *R. setigera* among others.

The teliospore length/width ratio was believed to be a numerical indicator of the teliospore shape, i.e., small values indicate broadly ellipsoidal shape while large

Table 2. Size variation in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Length (μm)		Width (μm)	
		Mean	Range	Mean	Range
BPI0126320	*R. macdaugali*	70.8	51.6–83.0	26.3	23.6–28.8
PUR56692	*R. manca*	67.6	55.2–79.3	26.9	23.7–29.8
PUR7836	*R. engelmannii*	66.8	50.8–80.6	27.6	24.5–27.7
PUR8164	*R. gallica*	64.3	50.1–79.4	28.7	24.9–31.8
PUR7761	*R. suffulta*	61.6	50.1–75.4	29.2	21.8–34.4
PUR44755	*R. nutkana*	80.9	63.7–103.3	33.5	29.7–36.6
PUR48167	*R. eastwoodii*	82.4	64.0–99.0	32.2	28.9–35.5
PUR7847	*R. fendleri*	79.3	64.4–94.6	32.7	25.8–32.7
PUR7887	*R. macounii*	75.3	61.8–92.9	31.8	28.9–34.6
PURF1514	*R. tomentosa*	79.2	61.0–106.4	31.4	26.2–37.1
PUR7725	*R. setigera*	106.4	65.0–135.6	33.9	29.2–37.9
PURF11650	*R. rugosa*	87.2	65.8–103.8	36.9	33.1–44.0
SAPA (Tokachi)	*R. rugosa*	101.8	79.1–121.5	35.2	29.7–38.9
BPI0126648	*R. centifolia*	91.8	70.8–116.2	36.0	32.3–39.3
BPI0126642	*R. alba*	83.9	60.7–105.3	37.3	32.8–45.0

values indicate oblong or oblong-ellipsoidal shape. However, the mean ratio value of a specimen did not represent the general shape of its teliospores. Within individual specimens, the length/width ratio, and thus shapes of the spores varied. Among the specimens with mean ratios of less than 2.0, the ratio range was 1.5–2.4 in PURF9504 on *R. gallica* and in PURF11645 on *R. canina*. Among those with mean ratios of more than 3.4, the ratio range was 2.4–4.4 in BPI0126299 on *R. acicularis*, 3.0–4.1 in IBA0311 on *R. acicularis* and 3.1–5.5 in PURF1548 on *R. pendulina*. Among those with mean ratios with a median value of 3.0, the ratios ranged 2.4–3.9 in BPI0126284 on *R. acicularis*, 2.1–3.9 in PUR7877 on *R. gymnocarpa*, 2.6–3.6 in PUR7925 on *R. acicularis* and 2.5–3.8 in PUR44735 on *R. setigera*.

Teliospore length and cell numbers were not correlated (Fig. 1B, not significant in Spearman's rank correlation analysis): teliospores with larger numbers of cells were not necessary longer than those with fewer numbers of cells and vice versa (Table 3, Fig. 1B). IBA0311 on *R. acicularis* (mean cell number of 12 and range of 10–14 cells; mean spore length of 87.4 μm) (Fig. 4G), PURF1548 on *R. pendulina* (12, 8–14 cells; 103 μm), PUR48581 on *R. acicularis* (11, 8–13 cells; 90 μm) and WRSL on *R. pendulina* (11, 8–12 cells; 84.8 μm) were among the specimens bearing teliospores with the largest number of cells. In contrast, PURF1557 on *R. rugosa* (5, 4–7 cells; 73.2 μm), PURF1507 on *R. gallica* (5, 4–6 cells; 62.9 μm) (Fig. 4H) and PUR7761 on *R. suffulta* (5, 3–5 cells; 61.6 μm) were the specimens bearing teliospores with fewest cells.

Significant disequilibrium in the relationship between the cell number and the length of teliospores was observed in those specimens that bore teliospores with 8 or 9 cells on average (Fig. 1B, Table 3). PUR7725 on *R. setigera* (9, 6–11 cells; 106.4 μm), SAPA (Tokachi) on *R. rugosa* (8, 6–10 cells; 101.8 μm), SAPA (Ishikari) on *R. rugosa* (8, 6–10 cells; 100.8 μm) (Fig. 5A) and BPI0126671 on *R. rugosa* (8, 7–9 cells; 98.5 μm) bore longer teliospores, being distributed in the middle top in Fig. 1B. In contrast, PUR48092 on *R. fendleri* (9, 8–10 cells; 70.9 μm) (Fig. 5B), PUR7878 on *R. gymnocarpa* (9, 6–10 cells, 72.0 μm), PUR7836 on *R. engelmannii* (8, 6–10 cells; 66.8 μm), PUR7853 on *R. fendleri* (8, 7–9 cells; 67.6 μm) and BPI0126625 on *R. pimpinellifolia* (8, 6–10 cells; 69.8 μm) bore shorter teliospores, being distributed in the middle bottom in Fig. 1B. Between these extremes, specimens bearing teliospores with different cell numbers and lengths were scattered across the entire range of variation.

In addition to the teliospore length/width ratios, degree of attenuation or roundness of the teliospore apex and base was considered as an important determinant of the general shape of teliospores. The teliospore shape was generally cylindrical. However, there were two extremes in the attenuation of the teliospore apex, i.e., abruptly rounded with the acute apiculus (Fig. 4B) vs. continuously attenuate toward the apiculus (Fig. 4G). In each specimen, the degree of attenuation in both teliospore ends seemed fairly constant, but it varied greatly and continuously among specimens.

The apical wall of teliospores elongated to form an apiculus. Teliospore apiculi were acute, acuminate or obtuse, their appearance being determined by the length and degree of gradation from the wall of teliospore apical cell. The mean length of apiculi varied from 3.9 μm (with minimum length of 0.8 μm and maximum length of 6.6 μm) in BPI0126669 on *R. rugosa* (Fig. 5C, Table 4) to

Table 3. Variation in the cell number and length in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Cell number		Length (μm)	
		Mean	Range	Mean	Range
IBA0311	*R. acicularis*	12	10–14	87.4	71.7–104.9
PURF1548	*R. pendulina*	12	8–14	103.0	76.7–133.0
PUR48581	*R. acicularis*	11	8–13	90.0	72.8–106.4
WRSL	*R. pendulina*	11	8–12	84.8	67.6–101.2
PUR7725	*R. setigera*	9	6–11	106.4	65.0–135.6
SAPA (Tokachi)	*R. rugosa*	8	6–10	101.8	79.1–121.5
SAPA (Ishikari)	*R. rugosa*	8	6–10	100.8	69.5–122.8
BPI0126671	*R. rugosa*	8	7–9	98.5	83.0–127.4
PUR48092	*R. fendleri*	9	8–10	70.9	54.8–83.2
PUR7878	*R. gymnocarpa*	9	6–10	72.0	52.1–85.9
PUR7836	*R. engelmannii*	8	6–10	66.8	50.8–80.6
PUR7853	*R. fendleri*	8	7–9	67.6	60.4–77.5
BPI0126625	*R. pimpinellifolia*	8	6–10	69.8	56.8–83.5
PURF1557	*R. rugosa*	5	4–7	73.2	56.2–95.5
PURF1507	*R. gallica*	5	4–6	62.9	48.4–77.2
PUR7761	*R. suffulta*	5	3–5	61.6	50.1–75.4

Table 4. Extreme values in the apiculus length in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Mean (μm)	Range (μm)
PURF1552	*R. cinnamomea*	15.3	9.2–24.0
PUR7892	*R. pendulina*	13.3	7.5–17.5
IBA0311	*R. acicularis*	12.8	7.4–17.9
PURF1557	*R. rugosa*	12.0	5.7–19.6
WRSL	*R. pendulina*	11.0	6.5–17.9
PUR7669	*R. lucida*	4.9	2.2–8.3
PUR7761	*R. suffulta*	4.7	2.6–7.9
SAPA (Tokachi)	*R. rugosa*	4.0	2.2–7.4
BPI0126671	*R. rugosa*	4.0	0.6–7.7
PUR44741	*R. virginiana*	3.9	2.2–7.5
BPI0126669	*R. rugosa*	3.9	0.8–6.6

15.3 μm (with minimum length of 8.3 μm and maximum length of 24.4 μm) in PURF11834 on *R. rugosa* (Fig. 5D, Table 4). Among specimens examined (Fig. 1C, Table 4), those bearing teliospores with short apiculi included PUR44741 on *R. virginiana* (mean 3.9 μm, range 2.2–7.5 μm), BPI0126671 on *R. rugosa* (4.0 μm, 0.6–7.7 μm), SAPA (Tokachi) on *R. rugosa* (4.0 μm, 1.8–7.4 μm), and PUR7761 on *R. suffulta* (4.7 μm, 2.6–7.9 μm) and PUR7669 on *R. lucida* (4.9 μm, 2.2–8.3 μm). In contrast, specimens bearing teliospores with long apiculi were PURF1552 on *R. cinnamomea* (15.3 μm, 9.2–24.0 μm), PUR7892 on *R. macounii* (13.3 μm, 7.5–17.5 μm), IBA0311 on *R. acicularis* (12.8 μm, 7.4–17.9 μm), PURF1557 on *R. rugosa* (12.0 μm, 5.7–19.6 μm) and WRSL on *R. pendulina* (11.0 μm, 6.5–17.9 μm).

Teliospore-wall color graded continuously from apricot (II9'b), bay (17km), rust (II7'k), sienna (II3b) to chestnut (II7'm) among specimens, although the color did not vary significantly within individual specimens. The wall color was arbitrarily divided into five grades (light brown, 1, to blackish brown, 5). However, the wall color was not correlated with the wall thickness (Fig. 1D, not significant in Spearman's rank correlation analysis) or other teliospore attributes (data not shown).

Similarly, the teliospore-wall thickness was not correlated with the wall-surface rugosity (faint, 1, to conspicuous, 5) (Fig. 1E, not significant in Spearman's rank correlation analysis). Degrees of variation in the wall-surface rugosity of teliospores varied continuously even within a single specimen, the rugosity ranging from conspicuous (as in PUR7892 on *R. macounii*, Fig. 5E) to faint (as in BPI0126284 on *R. acicularis*, Fig. 5F).

Teliospore-pedicel length variation was small within individual specimens. However, large and continuous variations were observed among specimens. The lower half of the pedicel in all specimens was enlarged in both wet and dry conditions as observed under LM and SEM, respectively. The pedicel enlargement was greater in a wet condition due to hygroscopicity than in a dry condition. The degree of pedicel hygroscopicity varied greatly and continuously among specimens, while it did not vary significantly within individual specimens. Length and width of the swollen part were not correlated, giving various shapes to the pedicel from subglobose, broadly ellipsoid to lanceolate among specimens (Fig. 1F, not significant in Spearman's rank correlation analysis), while the shape of the swollen part appeared fairly constant in individual specimens.

The pedicel length was not correlated with the teliospore length among specimens. Similarly, the shapes of teliospores and swollen parts of pedicels were not correlated (Fig. 2A, not significant in Spearman's rank correlation analysis), giving diverse gross morphology of the spore-pedicel complex.

Urediniospore morphology Urediniospores were subglobose to broadly ellipsoid; and the degree of variation in urediniospores, as shown by the length/width ratio, with mean values ranging from 1.0 to 1.4 (–1.5) (Fig. 2B), was smaller than that of teliospores or enlarged parts of pedicels. However, the size of urediniospores varied significantly and continuously among specimens. Specimens bearing large-sized urediniospores were PUR7746 on *R. acicularis* (mean length 24.8 μm, range 21.2–30.7 μm; mean width 21.9 μm, range 18.6–27.2 μm), BPI0126671 on *R. rugosa* (23.6 μm, 15.7–28.9 μm; 19.5 μm, 13.4–24.2 μm), SAPA (Tokachi) on *R. rugosa* (23.4 μm, 18.2–28.3 μm; 21.3 μm, 17.0–23.8 μm), PUR53303 on *R. setigera* (22.9 μm, 20.4–25.8 μm; 18.2 μm, 20.4–22.1 μm) and PUR7933 on *R. acicularis* (22.9 μm, 18.8–27.4 μm; 21.4 μm, 16.0–24.3 μm). In contrast, small urediniospores were observed in PUR51508 on *R. fendleri* (17.5 μm, 15.8–20.2 μm; 16.0 μm, 14.5–17.5 μm), SAPA (Kushiro) on *R. davurica* (18.0 μm, 15.0–20.4 μm; 15.8 μm, 12.8–18.7 μm), PUR61191 on *R. acicularis* (18.6 μm, 16.2–20.6 μm; 15.4 μm, 13.4–17.1 μm), PUR65617 on *R. blanda* (18.8 μm, 16.2–21.5 μm; 16.1 μm, 14.7–18.2 μm) and BPI0126320 on *R. macdougali* (18.9 μm, 14.4–23.0 μm; 15.6 μm, 12.7–18.8 μm). Between these extremes, medium-sized urediniospores were observed, among many others, in BPI0126648 on *R. centifolia* (21.0 μm, 17.5–24.5 μm; 17.4 μm, 15.4–19.5 μm), PUR7910 on *R. fendleri* (21.2 μm, 19.1–24.1 μm; 17.6 μm, 14.7–22.1 μm), PUR44750 on *R. bourgeaniana* (20.7 μm, 17.3–23.0 μm; 17.6 μm, 15.3–20.0 μm), PUR7657 on *R. blanda* (20.4 μm, 17.3–23.4 μm; 18.0 μm, 14.7–20.6 μm) and IBA1387 on *R. acicularis* (19.8 μm, 16.4–23.7 μm; 18.0 μm, 11.9–22.1 μm).

The urediniospore-wall was almost colorless or pale yellowish and did not show significant difference both within a specimen and among specimens. Similarly, the urediniospore-wall thickness was fairly uniform, mean values ranging from 1.1 to 2.2 μm (mostly 1.5–2.0 μm), and thus showed no correlation with the urediniospore size and other attributes.

The urediniospore-wall surface was echinulate in all specimens observed. Density of echinae was measured and found to be different among specimens, mean values ranging from 4.9 (as in PUR55312 on *R. acicularis*, Fig. 5G) to 9.8 (as in PUR59483 on *R. damascena*, Fig. 5H) echinae/10 μm^2. However, the density variation was

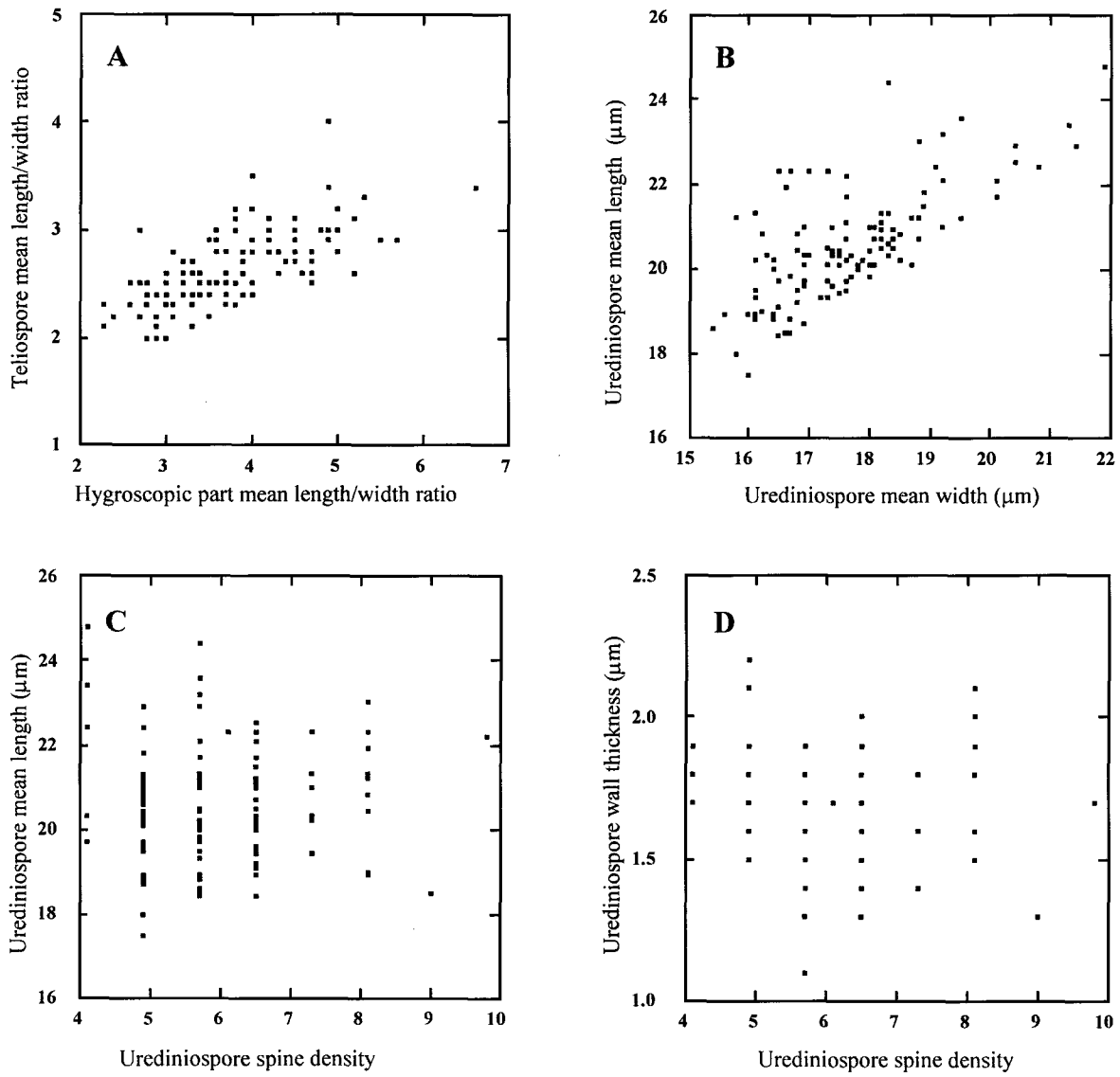

Fig. 2. Variations in the teliospore and urediniospore characteristics observed in the *Phragmidium* specimens. A. Relationship between the mean length/width ratio of teliospores and the mean length/width ratio of hygroscopic part of pedicels. B. Urediniospore mean lengths against urediniospore mean widths. C. Urediniospore mean lengths against mean spine density (echinulae/10 μm^2). D. Mean spine density (echinulae/10 μm^2) against mean urediniospore-wall thickness.

continuous and was not correlated with urediniospore size (Fig. 2C, not significant in Spearman's rank correlation analysis), wall thickness (Fig. 2D, not significant in Spearman's rank correlation analysis) or other urediniospore characteristics observed (data not shown).

Germ pores ranged from 5 (rarely 4) to 10 (rarely 11) with mean values of 6, 7 or 8 depending on specimens. Mean germ-pore numbers did not correlate with urediniospore size or wall thickness (data not shown). The pores were scattered over the wall. In certain specimens, the wall at the germ pores appeared to intrude into the urediniospore lumen. However, this attribute was not correlated with urediniospore size or wall thickness.

Uredinial-telial paraphyses were cylindrical to weakly clavate, weakly to strongly incurved. The wall was colorless, ventrally thin-walled (1.0–2.0 μm) and dorsally thick-walled (1.5–6.6 μm). The size of paraphyses varied from 34.8–74.9 × 12.8–26.4 μm in BPI0126671 on *R. rugosa* to 23.0–39.4 × 7.9–14.9 μm in PUR51814 on *R. mirifica*; mean length varied from 53.8 to 30.3 μm and mean width from 12.7 to 19.1 μm.

Principal component analyses Principal component analyses were undertaken with various combinations of numerical variables in urediniospores and teliospore morphology. Figure 3A is one of representative results. In this analysis, mean values of the following variables were used: paraphysis length and width, paraphysis-wall thickness, urediniospore length and width, urediniospore-wall thickness, teliospore-apiculus length, teliospore length and width, teliospore-wall thickness, pedicel length and width, and length and width of enlarged pedicel part. After the Varimax rotation, the calculated factors one

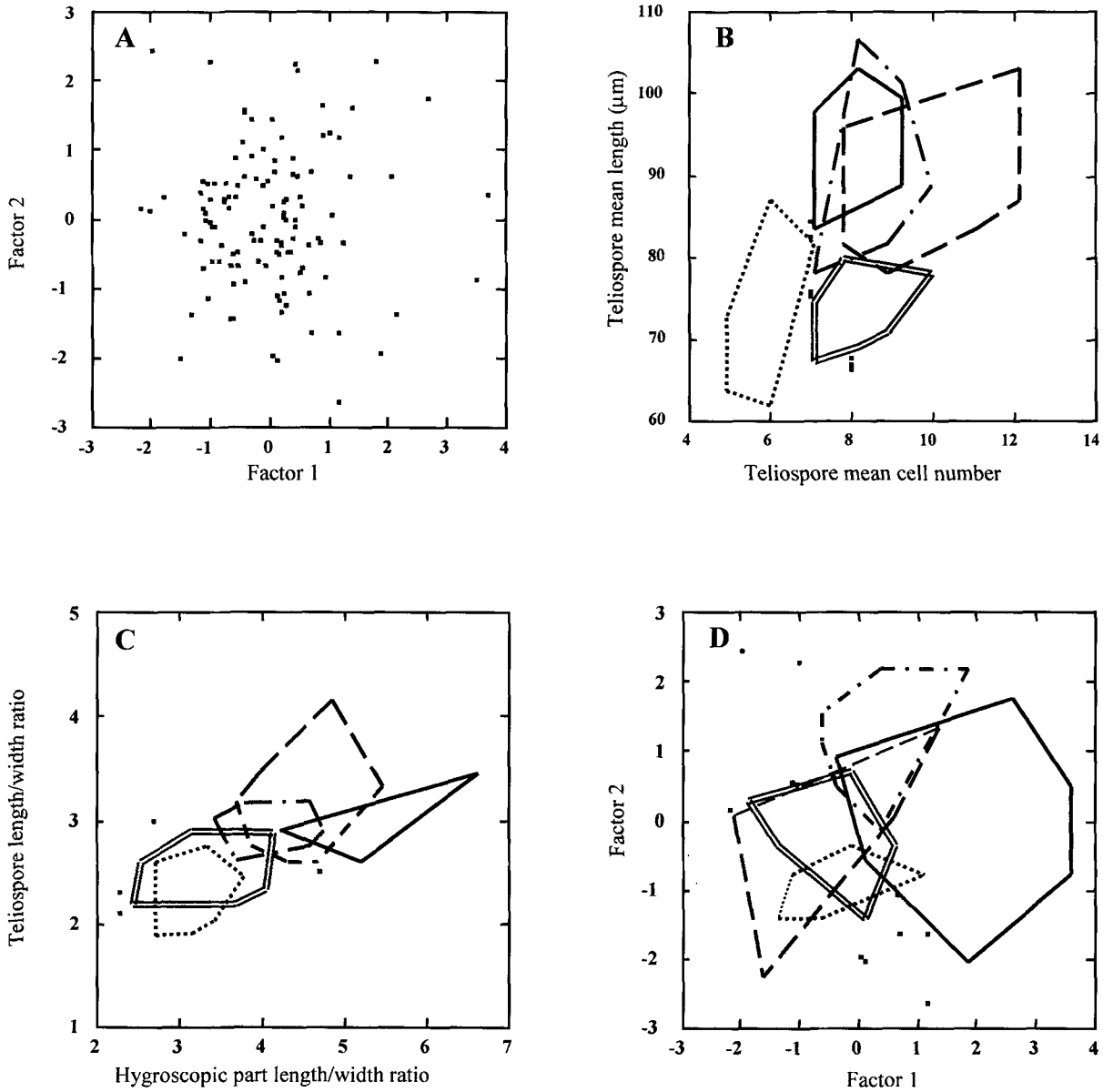

Fig. 3. Variations in the telial and uredinial characteristics observed in the *Phragmidium* specimens. A. A result of a principal component analysis. See the text for a detailed explanation. B. Teliospore dimensions observed among possible host-specific groups. C. Length/width ratios of teliospores and hygroscopic part of pedicels observed among possible host-specific groups. D. Distribution of possible host-specific groups in a scatter diagram generated by a principal component analysis. See text for a detailed explanation. –·–·: *Phragmidium americanum*; — —: *P. fusiforme*; ═══: *P. montivagum*; ·····: *P. mucronatum*; ——: *P. rosae-rugosae*.

and two explained 70.4% and 8.3% of the total variance, respectively.

The scatter diagram with factor one as the horizontal axis and the factor two as the vertical axis did not reveal discrete groups even after qualitative attributes, e.g., teliospore-wall color or teliospore-wall rugosity, were superimposed. The three-dimensional scatter diagram (not shown) with factors one, two and three also did not reveal any discrete groups.

Discussion

The causal species of the rose rusts under discussion have been circumscribed variously and classified by combinations of morphological characteristics of a maximum of four spore stages, which are produced in a sequence in the macrocyclic, autoecious life cycle. However, a uredinial or telial stage or both stages are frequently encountered in most herbarium specimens, and thus their features have been employed as taxonomic and key characters in the *Phragmidium* classification (Dietel, 1905, 1906; Arthur, 1934; Cummins, 1931), because a sper-

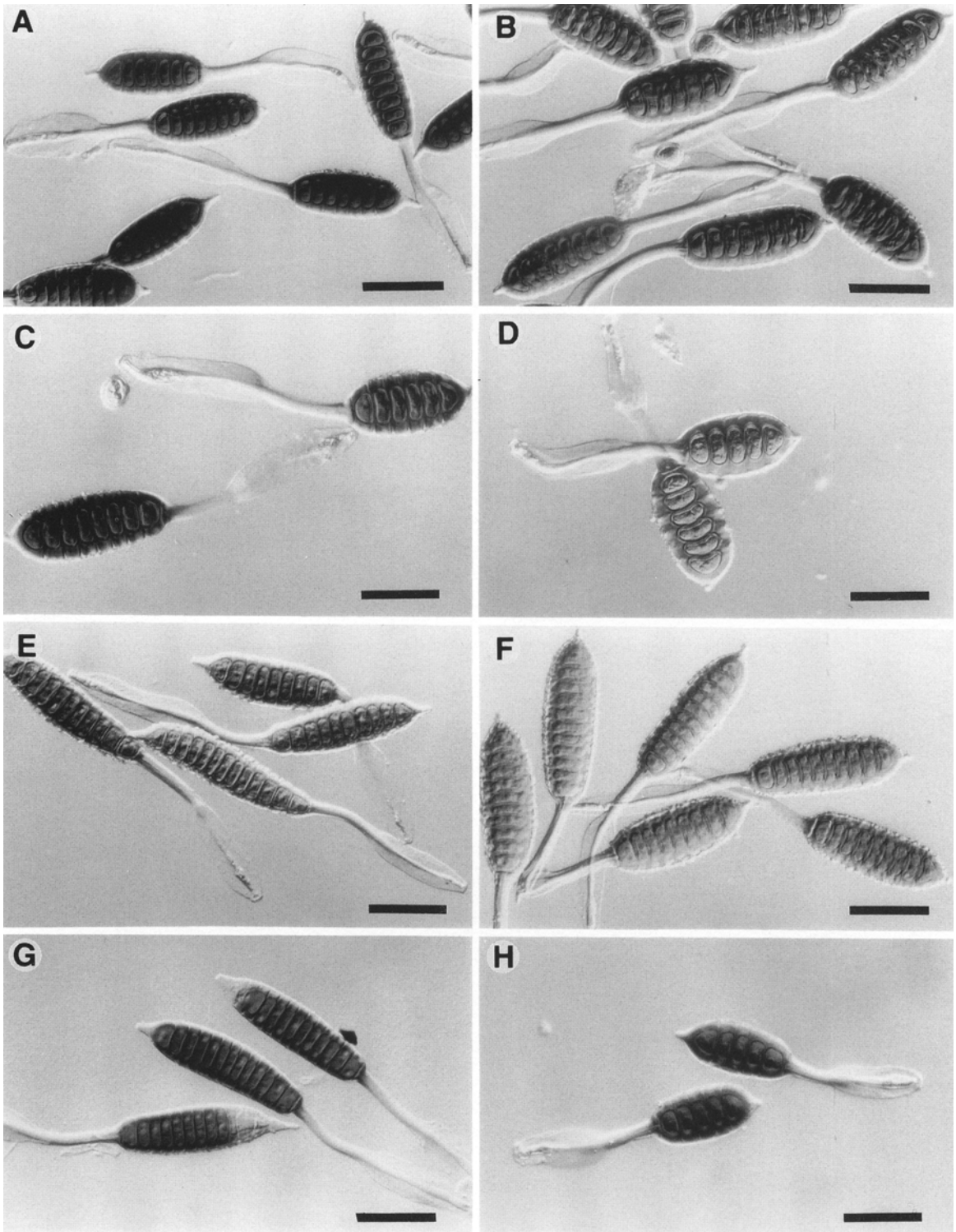

Fig. 4. Teliospore morphology observed in the *Phragmidium* specimens. A. PUR56692 on *Rosa manca*. B. PUR7847 on *R. fendleri*. C. BPI0126642 on *R. alba*. D. PURF9504 on *R. gallica*. E. PURF1548 on *R. pendulina*. F. PUR7925 on *R. acicularis*. G. IBA0311 on *R. acicularis*. H. PURF1507 on *R. gallica*. Scale bar = 50 μm.

mogonial-aecial stage is produced in a short period during the early growing season.

Length, width, degrees of tapering toward both ends and apiculus length were believed to determine gross morphology of the teliospores; and these have been considered important taxonomic characters. The length/

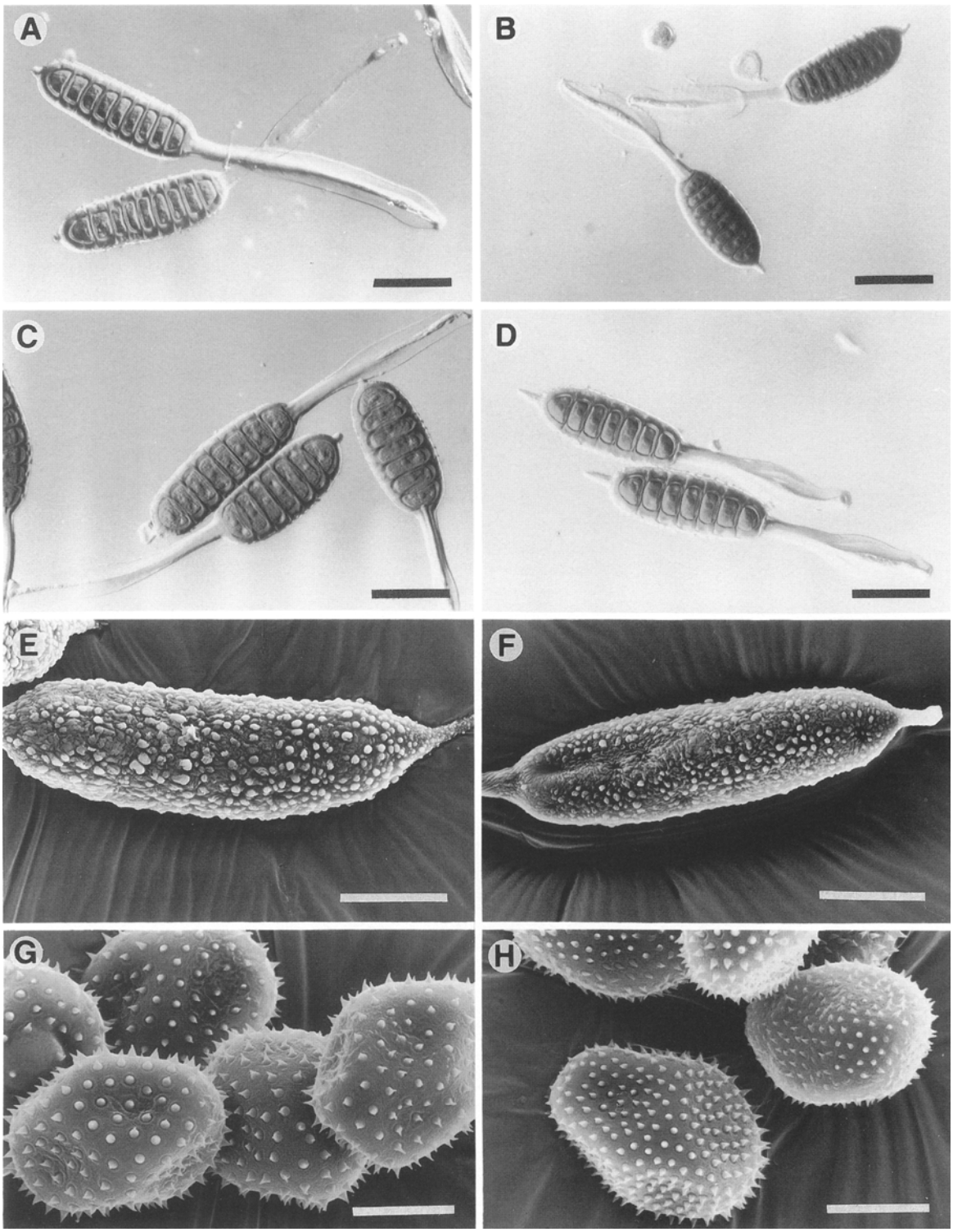

Fig. 5. Teliospore and urediniospore morphology observed in the *Phragmidium* specimens. A. Teliospores from SAPA (Ishikari) on *Rosa rugosa*. B. Teliospores from PUR48092 on *R. fendleri*. C. Teliospores from BPI0126669 on *R. rugosa*. D. Teliospores from PUR11834 on *R. rugosa*. E. Teliospore (SEM) from PUR7892 on *R. pendulina*. F. Teliospore (SEM) from BPI0126284 on *R. acicularis*. G. Urediniospores (SEM) from PUR55312 on *R. acicularis*. H. Urediniospores (SEM) from PUR59483 on *R. damascena*. Scale bars: A, B, C, D=50 μm; E, F=25 μm; G, H=10 μm.

width ratio was also considered as important. In addition to the gross morphology, cell number, wall color and surface rugosity have been used as taxonomic characters at the telial stage. Equally important was hygroscopicity of lower part of the pedicel. In contrast, only minuteness and density of echinae-verrucae on the wall surface, and sometimes wall thickness, have been stressed for urediniospores in distinguishing the species.

Thus, *P. mucronatum* was characterized by cylindrical teliospores composed of 5–9 cells and the size of 64–90 × 22–33 μm (Arthur, 1934; Cummins, 1931). Both ends of the teliospores were described as being rounded with a prominent apiculus of 7–13 μm long. The wall was dark chocolate-brown and coarsely verrucose. The pedicel length was about one and a half times the teliospore length, and the lower part of the pedicel becomes abruptly swollen to become broadly clavate or globose. Among related species, conspicuous hygroscopicity was reported only in this species (Cummins, 1931). The urediniospores were described as obovate or obovate-globoid and 20–26 × 16–19 μm (Arthur, 1934; Wilson and Henderson, 1966). The wall was closely echinulate with 8 or more indistinct, scattered pores.

Phragmidium mucronatum var. *americanum* was segregated from var. *mucronatum* because it possessed dark-walled teliospores with 8–10 cells (Peck, 1876). Var. *americanum* was raised to the species rank by Dietel (1905). This species has been characterized by long teliospores (80–100 μm) composed of 8–11 cells (Arthur, 1909) or by long telispores (64–125 μm) with dark chocolate-brown walls and thin-walled, moderately and finely verrucose aeciospres (Cummins, 1931).

Narrowly fusoid-cylindrical teliospores (7–13-celled, 90–110 × 19–30 μm) were said to be characteristic of *P. fusiforme* (Schröter, 1872). The wall was described as most finely verrucose among related species (Cummins, 1931).

Phragmidium tuberculatum was characterized by forming (3–)5(–6)-celled teliospores (54–81 × 27–35 μm) with an acute apiculus on a rounded apex (Müller, 1886). The wall was dark brown and verrucose. The lower half of the pedicel was swollen to clavate. Although not mentioned in the original description, the urediniospore wall at germ pores was stated to be conspicuously intruding in the spore lumen (Wilson and Henderson, 1966). This character of urediniospores was unique to this species among related species.

A fungus on *R. pimpinellifolia*, which was included in the "*P. subcorticium* group", was segregated and named as a new species, *P. rosae-pimpinellifoliae* (Dietel, 1906). This species was characterized by teliospores (6–8-celled, 65–87 × 28–30 μm) with a non-opaque, chestnut-brown wall. This is only the species having a translucent teliospore wall among related species (Cummins, 1931).

On the other hand, *P. montivagum* was described as distinct because it possessed 6–9-celled teliospores of 64–96 μm in length and 24–29 μm in width, with a conical subhyaline papilla (7–10 μm long) and a coarsely and moderately verrucose wall (Arthur, 1909). The urediniospores were stated to be obovate-globoid and 19–23 × 16–19 μm with a pale yellow and closely verrucose-echinulate wall (1–1.5 μm thick). Arthur (1909) stated that this species was distributed only in the Rocky Mountains and most variable morphologically among the species distributed in North America.

In comparison with *P. rosae-pimpinellifoliae*, Cummins (1931) stated that *P. montivagum* was similar to *P. rosae-pimpinellifoliae* in the teliospore size, measuring 64–95 × 24–32 μm. However, the two species were different in the teliospore-cell number (5–7 in *P. rosae-pimpinellifoliae* vs. 5–9 in *P. montivagum*) and the teliospore-wall color (dark chocolate-brown in the former and chestnut-brown and non-opaque in the latter).

Phragmidium rosae-rugosae was characterized by large cylindrical teliospores (7–11-celled, 72–128 × 28–32 μm) with lighter (yellowish brown) walls than those of related species (Kasai, 1910). The lower half of the pedicel was described as being only slightly swollen, while related species possessed pedicels with abruptly or gradually and moderately swollen lower parts.

As briefly described above, each of the seven species under consideration seems to be distinguishable by comparing the described characters. Thus, combinations of a few characters seem to be sufficient to separate these species, i.e., *P. americanum* by the large teliospores (up to 11-celled, up to 125 μm long), *P. fusiforme* by the fusoid-spindle-shaped teliospores, *P. mucronatum* by the abruptly swollen pedicels and the coarsely verrucose teliospore wall, *P. rosae-pimpinellifoliae* by the non-opaque chestnut-brown and finely verrucose teliospore wall, *P. rosae-rugosae* by the yellowish brown teliospore wall and the least hygroscopic pedicels and *P. tuberculatum* by the acute and long apiculi (up to 22 μm long) and the intruding wall at germ pores in the urediniospores.

However, it appears from the examinations of the uredinial and telial features in 119 specimens identified as one of the seven species, that no single morphological property or combination of properties is sufficient to distinguish morphological groups of the specimens that seem to correspond with the seven *Phragmidium* species under discussion. For example, mean size (Fig. 1A), cell number (Fig. 1B) and length/width ratio of the teliospores and pedicels (Fig. 2A) among others, continuously vary among the specimens and are not correlated with any other morphological features. Similarly, the mean apiculus length (Fig. 1C) continuously varies among the specimens. These results show that, using telial and uredinial features either singly or in combination, it is very difficult, if not impossible, to identify the seven causal species of the ornamental rose rusts.

Nevertheless, the non-discrete distribution patterns of uredinial-telial morphological features as described by continuous and/or discrete numerical variables do not necessarily mean that the specimens examined in the study comprise a single morphological taxon rather than two or more species. Each of the closely related but distinct rust taxa maintains characteristic biological properties. The properties of these taxa may or may not be

unique or distinct to each taxon. If a descriptor of the properties, particularly morphological properties is a continuous numerical variable, the properties of the taxa thus described are likely to overlap. When these morphological properties are employed as taxonomic characters, which is a common practice in the taxonomy of rust and other fungi, the distinction of taxa circumscribed in this way is likely to be difficult (see Stuessy (1990) for taxonomic characters and related terminology). Thus, it is not rare in the rust fungi that are reproductively isolated, host-restricted species are morphologically similar or even indistinguishable (Ono, 2001; Pfunder et al., 2001; Roy et al., 1998).

To overcome the current difficulties in distinguishing the *Phragmidium* species on ornamental roses, biological properties other than those described by a continuous numerical variable must be taken into account as taxonomic characters. One possible property that is currently available is the putative host specificity of the *Phragmidium* species. Assuming that the host identification and the putative host specificity are correct, certain *Rosa* species of the specimens examined are considered to harbor only one or two rust species. Accordingly, *R. blanda* and *R. setigera* harbor *P. americanum*; *R. acicularis*, *R. nutkana* and *R. pendulina* harbor *P. fusiforme*; *R. acicularis*, *R. engelmannii*, *R. fendleri*, *R. macounii*, *R. manca* and *R. mirifica* harbor *P. montivagum*; *R. alba*, *R. blanda* and *R. gallica* harbor *P. mucronatum*; *R. pimpinellifolia* harbors *P. rosae-pimpinellifoliae*; *R. canina* and *R. cinnamomea* harbor *P. tuberculatum*; and *R. rugosa* harbors *P. rosae-rugosae*. When these host species are plotted over the specimen scatter diagrams, host-specific morphological groups seem to be detected in the scatter diagrams of some teliospore-feature combinations, even though the ranges of groups overlap. In the scatter diagram with the teliospore cell number as a horizontal axis and the mean teliospore length as a vertical axis, the specimens that seem to correspond to *P. mucronatum*, *P. montivagum* and *P. fusiforme* are separated (Fig. 3B). However, those that seem to correspond to *P. americanum* and *P. rosae-rugosae* are not separated well and locate between those of *P. mucronatum* and *P. fusiforme*. The specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are scattered over the range of *P. mucronatum*, *P. montivagum* and *P. fusiforme*.

In the scatter diagram with the length/width ratio of the enlargement in the pedicels as a horizontal axis and the teliospore length/width ratio as a vertical axis, five host-specific groups which seem to correspond to *P. fusiforme*, *P. montivagum* and *P. rosae-rugosae* are detected (Fig. 3C), while the distribution range of those specimens that seem to correspond to *P. americanum* and *P. mucronatum* overlap the specimens of *P. fusiforme*, *P. montivagum* and *P. rosae-rugosae*. The specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are again enclosed in the range of both *P. montivagum* and *P. mucronatum*.

Similarly, in the scatter diagram generated from a principal component analysis, five host-specific groups, which are considered to correspond to *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum* and *P. rosae-rugosae*, appear to be morphologically circumscribed even though their distribution ranges overlap (Fig. 3D). Specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are scattered over the whole range.

In contrast to the teliospore features, no host-specific groups are detected by urediniospore features, except for the wall-thickening over the urediniospore germ-pores, which seems to be characteristic of *P. tuberculatum*. Minuteness and density of verrucae-echinae in the urediniospores have been considered to be key characters in the taxonomy of the seven *Phragmidium* species. However, the urediniospore-surface ornamentation is exclusively echinulate and the density of echinae does not separate the host-specific groups.

Our results indicate that host-specific groups of the specimens that are considered to correspond to *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum* and *P. rosae-rugosae* can be circumscribed well by size, shape, cell number of teliospores, size and shape of pedicels and wall-thickening over urediniospore germpores. However, these morphological properties are considered not to be suitable as taxonomic characters for species identification. In order to find mutually exclusive morphological groups that correspond to the well-established species, further detailed analyses of the uredinial-telial properties together with the spermogonial-aecial properties of the *Phragmidium* species are now being undertaken.

Acknowledgments—A number of specimens examined in this study were loaned from the Arthur Herbarium, Purdue University (PUR); the Faculty of Agriculture, Hokkaido University (SAPA); the Botanical Garden and Museum, Berlin-Dahlem (B); the National Fungus Collections, the United States Department of Agriculture (BPI); and the Museum of Natural History, Wroclaw University (WRSL), for which we are grateful.

Contribution No. 158, Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba.

Literature cited

Arthur, J. C. 1909. North America rose rusts. Torreya **9**: 21–28.

Arthur, J. C. 1934. Manual of the rust in United States and Canada, Hafner Publ. Co., New York.

Azbukina, Z. M. 1984. Key to the rust fungi of the Soviet Far East. Nauka, Moscow. (In Russian.)

Beales, P., Cairns, T., Duncan, W., Fagan, G., Grant, W., Grapes, K., Harkness, P., Hughes, K., Mattock, J. and Ruston, D. 1998. Botanica's roses. Randomhouse Australia Pty. Ltd., Sydney.

Cummins, G. B. 1931. *Phragmidium* species of North America: Differential teliospore and aecial characters. Mycologia **23**: 433–445.

Cummins, G. B. and Hiratsuka, Y. 1983. Illustrated genera of rust fungi. Revised edn. Amer. Phytopathol. Soc., St. Paul, Minnesota.

Dietel, P. 1905. Uber die arten der Gattung *Phragmidium*.

Hedwigia **44**: 112–132.
Dietel, P. 1906. Uber die arten der Gattung *Phragmidium* II. Hedwigia **44**: 330–346.
Gäumann, E. 1959. Die Rostpilze Metteleuropas. Beiträge zur Kryptogamenflora der Schweiz **12**: 1–1407.
Hiratsuka, N. 1935. *Phragmidium* of Japan. Jpn. J. Bot. **7**: 227–299.
Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T. and Nakayama, K. 1992. The rust flora of Japan. Tsukuba Shuppankai, Tsukuba.
Hiratsuka, Y. 1973. The nuclear cycle and terminology of spore state in Uredinales. Mycologia **65**: 432–443.
Horst, R. K. 1983. Compendium of rose disease. Amer. Phytopathol. Soc., St. Paul, Minnesota.
Howden, J. C. W. and Jacobs, L. 1973. Report on the rust work at Bath. Ann. Natl. Rose Soc. England **1973**: 113–119.
Jennings, D. M., Ford-llyoid, B. V. and Butler, G. M. 1989. An aniline blue squash technique for observation of urediniospore germ pores. Mycol. Res. **92**: 230–251.
Kasai, M. 1910. On the Japanese species of *Phragmidium*. Trans. Sapporo Nat. Hist. Soc. **3**: 25–51.
Laundon, G. F. 1970. Additions to the rust fungi of New Zealand-5. New Zealand J. Bot. **8**: 310–319.
Müller, J. 1886. Die Rostpilze der *Rosa*- und *Rubus*- Arten und die auf ihnen vorkomenden parasiten. Ber. Deutsch. Bot. Ges. **3**: 391–395.
Ono, Y. 2000. Taxonomy of the *Phakopsora ampelopsidis* species complex on vitaceous hosts in Asia including a new species, *P. euvitis*. Mycologia **92**: 154–173.
Phillips, R. and M. Rix. 1994. Roses. Pan Books Ltd., London.
Peck, C. H. 1876. Report of the botanist. Ann. Rep. New York State Mus. **28**: 31–88.
Pirone, P. P., Dodge, B. O. and Ricket, H. W. 1960. Disease and pests of ornamental plants. 3rd edn. Ronald Press Co., New York.
Pfunder, M., Schürch, S. and Roy, B. A. 2001. Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol. Res. **105**: 57–66.
Rayner, R. W. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew.
Roy, B. A., Vogler, D. R., Bruns, T. D. and Szaro, T. M. 1998. Cryptic species in the *Puccinia monoica* complex. Mycologia **90**: 846–853.
Schröter, J. 1872. Die Brand- und Rostpilze Schlesiens. Abh. Schles. Ges. Nat. Med. **1869–72**: 1–31.
Stuessy, T. F. 1990. Plant taxonomy. The systematic evaluation of comparative data. Columbia Univ. Press, New York.
Wei, S. 1988. A taxonomic study of the genus *Phragmidium* of China. Mycosystema **1**: 179–210.
Wilkinson, L. 1989. SYSTAT: the system for statistics.Version 5.2 edition. Systat, Inc., Evanston, Illinois.
Wilson, M. and Henderson, D. M. 1966. British rust fungi. Cambridge Univ. Press, London.