Techniques

Teliospore Germination in Some Rust Fungi

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ABSTRACT

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Germination of 4,000 teliospore samples isolated from 59 hosts and representing 27 rust species of the genera *Puccinia*, *Uromyces*, *Tranzschelia*, *Frommea* (*Phragmidium*), and *Melampsora* was successfully induced by floating the spores on distilled or tap water at 16–18 C for 24–144 hr. In some instances, longer floating periods were required. After floating, drops of teliospore suspension were dispersed on 2% water agar. Presoaking of telia-bearing plant segments at 4 C considerably enhanced germination. Optimal germination was obtained at 16 C, with 12 and 25 C the lower and upper limits, respectively. Teliospores formed on summer crops appeared to have a higher optimum germination temperature. Ordinarily, light had no influence on teliospore germination. The spores lost germinability when maintained outdoors for a year in shade or when exposed to sun. Teliospores stored at 5 C under dry conditions in partial vacuum retained viability for more than 14 yr.

An understanding of teliospore biology provides insight into the problems of perennation of the fungus and facilitates studies on its genetics and pathogenicity. The significance of basidia and their nuclear history in the evolution of rusts (4,8) and the importance of pycnia in taxonomy (5) make exploration of teliospore germination imperative.

In numerous fungi teliospores germinate only after periods of dormancy. The dormancy is constitutional, an inherent property of the spore, requiring an activation process (9). In nature, dormancy is often an asset, carrying the fungus over a period unfavorable for growth (9,10). However, tardiness in germination may handicap experimentation, and a method to control germination under experimental conditions is desirable (10). A comprehensive review of methods for breaking rust teliospore dormancy is provided by Mengden (9).

The purpose of this study was to develop methods and conditions conducive to teliospore germination and preservation.

MATERIALS AND METHODS

The fungus. The study involved 4,060 different teliospore samples isolated from 59 hosts representing 27 rust species of five genera and four families, viz., *Puccinia* and *Uromyces* of the family Pucciniaceae, *Tranzschelia* of the family Uropyxioidaceae,

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Frommea (*Phragmidium*) of the Phragmidiaceae family, and *Melampsora* of the Melampsoraceae family. The taxonomic designation of the mentioned rust families is from Cummins and Hiratsuka (5), who classified the genus *Frommea* as *Phragmidium*.

The majority of teliospore samples were collected in Israel from 58 different hosts. Twenty-four teliospore collections were procured from the United States, two from Australia, and one from West Germany (Table 1). In Israel, small leaf segments harboring

TABLE 1. Rust pathogen species with induced teliospore germination

Rust pathogen species	Source host	Origin
Frommea obtusa (Str.) Arth.	*Potentilla simplex* Michx.	USA (3)[a]
Melampsora lini (Schum.) Lev.	*Linum pubescens* Russ.	Israel (2)
Melampsora sp.	*Euphorbia* sp.	Israel (1)
Puccinia allii Rud.	*Allium ampeloprasum* L.	Israel (16)
	A. cepa L.	Israel (2)
	A. orientale Boiss.	Israel (6)
	A. sativum L.	Israel (4)
P. antirrhini Diet. & Holw.	*Antirrhinum majus* L.	Israel (20)
P. cacabata (*Stakmanii*) Arth. & Holw.	*Bouteloua* sp.	USA (1)
P. carthami Cda.	*Carthamus glaucus* M.B.	Israel (3)
	C. tenuis (Boiss.) Bornm.	Israel (3)
	C. tinctorius L.	Israel (8)
P. coronata Cda.	*Avena barbata* Pott.	Israel (2)
	A. sativa L.	Israel (4)
	A. sterilis L.	Israel (7)
	Phalaris bulbosa L.	Israel (22)
P. graminis Pers.	*Agropyron repens* (L.) Pal.	USA (4)
	Avena sativa L.	USA (4)
	A. sterilis L.	Israel (2)
	Triticum aestivum L.	Australia (1), USA (1)
	Triticale	Australia (1)
P. helianthi Schw.	*Helianthus annuus* L.	USA (5), Israel (2)
P. hordei Otth.	*Hordeum bulbosum* L.	Israel (362)
	H. murinum Huds.	Israel (21)
	H. spontaneum C. Koch	Israel (2314)
	H. vulgare L.	Israel (105), USA (6), W. Germany (1)
P. lagenophorae (Cooke)	*Senecio joppensis* Dinsm.	Israel (25)
	S. vernalis Waldst. & Kit.	Israel (17)
	S. vulgaris L.	Israel (7)
P. malvacearum Bert.	*Althaea setosa* Boiss.	Israel (7)
	Malva parviflora L.	Israel (2)
P. mesnieriana Thum.	*Rhamnus palaestina* Boiss.	Israel (39)
	R. punctata Boiss.	Israel (2)
P. recondita Rob. & Desm.	*Aegilops longissima* Schw. & Muschl.	Israel (20)
	A. ovata L.	Israel (2)
	A. peregrina (Hacks) Eig (*A. variabilis* Eig)	Israel (32)
	A. sharonensis Eig	Israel (77)
	Triticum aestivum L.	Israel (85)
	T. dicoccoides Koern.	Israel (34)
	T. durum Desf.	Israel (6)
P. smyrnii Biv.-Bernh.	*Smyrnium connatum* Boiss. et Ky.	Israel (4)
	S. olusatrum L.	Israel (4)
P. striiformis Westend.	*Hordeum vulgare* L.	Israel (3)
	Triticum aestivum L.	Israel (12)
P. xanthii Schw.	*Xanthium strumarium* L.	Israel (13)
Tranzschelia pruni-spinosae (Pers.) Diet.	*Prunus amygdalus* Stokes	Israel (2)
	P. persica (L.) Sieb. et Zucc.	Israel (2)
Uromyces christensenii Anikst. & Wahl	*Hordeum bulbosum* L.	Israel (11)
U. hordeastri Guyot	*H. bulbosum* L.	Israel (136)
	H. marinum Huds.	Israel (19)
U. oliveirae Anikst. & Wahl	*Bellevalia eigii* Feinbr.	Israel (46)
U. rayssii Anikst. & Wahl	*Scilla hyacinthoides* L.	Israel (14)
U. reichertii Anikst. & Wahl	*H. bulbosum* L.	Israel (17)
U. scillarum (Grev.) Wint.	*Bellevalia desertorum* Eig & Feinbr.	Israel (21)
	B. flexuosa Boiss.	Israel (31)
	Dipcadi erythraeum Webb & Bert.	Israel (7)
	Leopoldia eburnea Eig & Feinbr.	Israel (24)
	L. maritima Desf.	Israel (31)
	Muscari commutatum Guss.	Israel (5)
	M. parviflorum Desf.	Israel (116)
	M. racemosum (L.) Mill.	Israel (5)
	Ornithogalum eigii Feinbr.	Israel (52)
	O. trichophyllum Boiss. & Feinbr.	Israel (36)
	Pancratium parviflorum Dec.	Israel (21)
	Urginea maritima (L.) Bak.	Israel (38)
	U. undulata (Desf.) Steinh.	Israel (11)
U. vesicatorius (Bub.) Natrass	*Leontice leontopetalum* L.	Israel (5)
U. viennot-bourginii Wahl & Anikst.	*Hordeum spontaneum* C. Koch	Israel (81)

[a] Figures in brackets denote the number of isolates tested in the specified rust species.

teliospores were collected mainly from fields in winter and spring, when spores are formed. The specimens were routinely stored in paper bags at 2–4 C. Some teliospore samples were produced on plants grown in the greenhouse.

For long-term storage, teliospores were preserved in glass vials 5 mm in diameter. Teliospores placed at the bottom of the vial were covered with two thin layers of cotton containing 0.5 g $CaCl_2$ between them. The vials were vacuumed for at least 10 min, then sealed while connected to the vacuum pump.

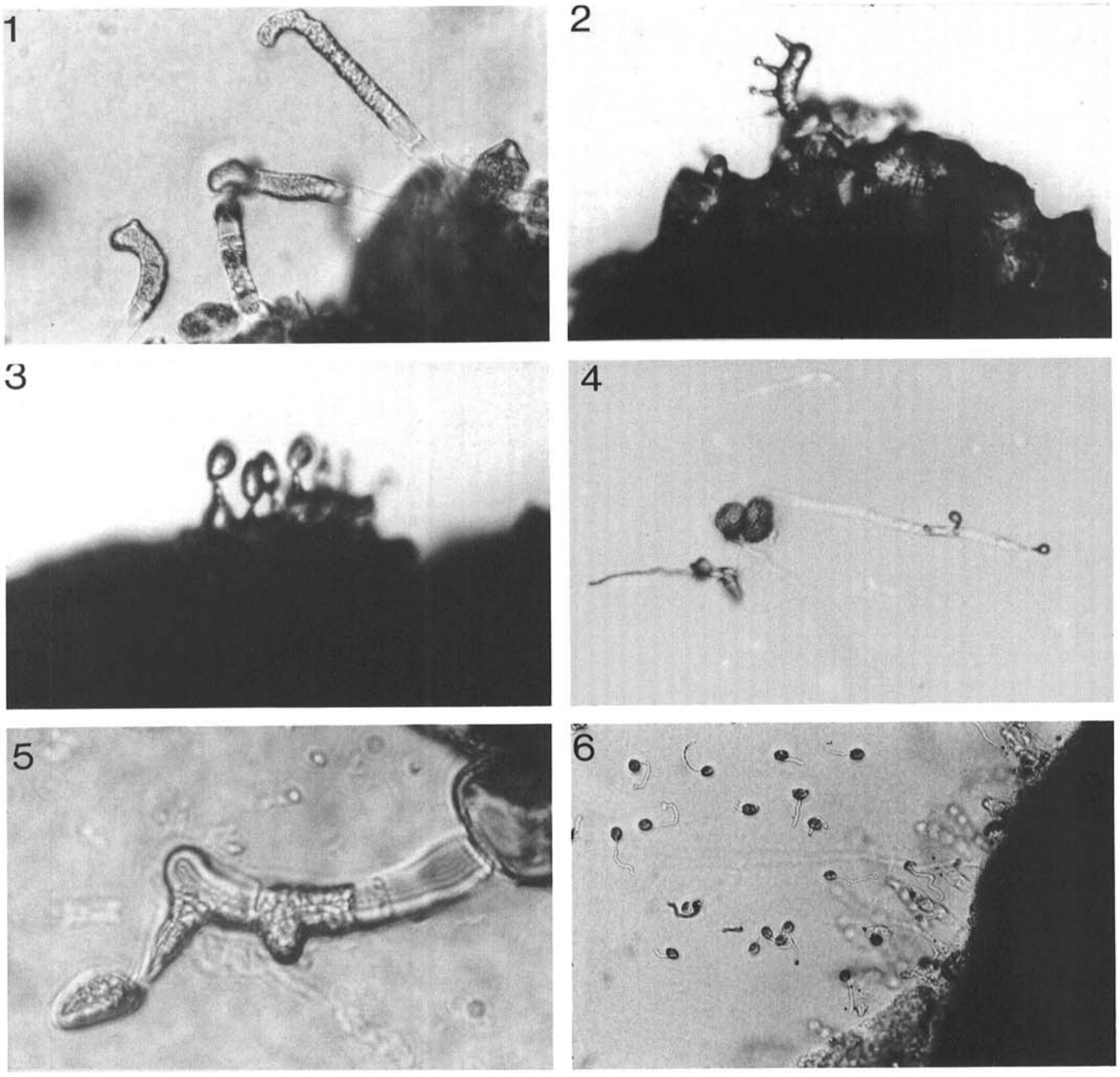
Growth chambers. Numerous tests were carried out in a growth chamber (walk-in type, model 5.5E + 5 JU-PK, K. Weiss, West Germany) with illumination supply of 16,000 lx.

Teliospore activation and germination. Usually, activation of dormant teliospores was achieved with the aid of Anikster's method (1–3). Small leaf segments bearing telia, or teliospore scraped from green leaves or straw, were floated on distilled or tap water for 24 hr or more at 16 C. Then the material was distributed on 2% water agar in petri dishes. Similar results were recorded when 40 ppm chloramphenicol was added to water and/or to water agar to reduce bacterial contamination.

The onset and rate of germination were assessed by direct examination under the microscope by the appearance of promycelia and basidiospores (Figs. 1–6).

RESULTS

Germination usually commenced after 24–144 hr of transferring to 16 C. Optimal germination was obtained at 16–18 C, whereas the



Figs. 1–6. Rust fungus spores. **1,** Promycelia emerging from a telium of *Uromyces scillarum* isolated from *Urginea maritima* (×500). **2,** Promycelium with sterigmata and the beginning of formation of basidiospores of *Puccinia recondita* isolated from *Triticum aestivum* (×200). **3,** Basidiospores of *Puccinia hordei* isolated from *Hordeum vulgare*, just before ejection (×600). **4,** Germination of a single teliospore of *Tranzschelia pruni spinosae* (×160). **5,** Promycelium of *Uromyces scillarum* bearing one basidiospore and forming a new sterigma. The fungus was isolated from *Pancratium parviflorum* (×1,250). **6,** Germinating basidiospores of *Puccinia striiformis* secured from *Triticum aestivum* after exposure to 16 C for 24 hr (×160).

minimum and maximum limits of germination was obtained at 12 and 25 C, respectively. In the case of cotton rust, *P. cacabata* (*P. stakmanii*) Arth. & Hollw., isolated from *Bouteloua* sp., optimal teliospore germination was observed at 25 C, and traces of germination were recorded at 32 C. It is noteworthy that the fungus develops on a summer crop at high temperatures. The effect of temperature on the rate of teliospore germination is shown in Table 2.

Effect of presoaking on teliospore germination. Teliospores of the rust fungi listed in Table 3 were stored in dry conditions at 4 C in darkness. The teliospore were divided into two groups, and those of one group were used in germination tests according to the method described above. In parallel tests, teliospores of the second group were presoaked in water at 4 C for 2 wk, and included in germination tests with teliospores of the first group. The tests were performed at 16 C. The results summarized in Table 3 demonstrate that a 2-wk presoaking increased germination intensity. In contrast, teliospores presoaked for 4–5 wk or more lost germinability.

Influence of light on teliospore germination. Gold and Mengden (7) reported that teliospore germination is affected by light and alternating periods of light and darkness. In this study, the influence of light on teliospore germination was investigated in the rust fungi listed in Table 4. Teliospores were stored for 2–3 wk at 2–4 C in small petri dishes containing 40 ppm chloramphenicol solidified with 2% water agar. They were then exposed at 16 C for

TABLE 2. Influence of temperature on teliospore germination[a] of the listed rust fungi[b]

Fungus	Source host	Isolates (no.)	Temperature					
			10 C	12 C	16 C	20 C	25 C	30 C
Puccinia hordei	*Hordeum spontaneum*	16	−	++	+++	+++	+	−
P. recondita tritici	*Triticum dicoccoides*	14	−	+++	+++	++	−	−
P. lagenophorae	*Senecio vernalis*	11	−	++	+++	++	+	−
P. cacabata (*P. stakmanii*)	*Bouteloua* sp.	1	−	−	++	+++	++++	+
P. malvacearum	*Malva silvestris*	8	−	++	+++	+++	−	−
Uromyces hordeastri	*Hordeum.bulbosum*	5	−	++	+++	++	−	−
U. viennot-bourginii	*Hordeum spontaneum*	8	+	+	++	+	+	−
U. scillarum	*Urginea maritima*	14	−	−	++	+	−	−
U. scillarum	*Pancratium parviflorum*	16	−	+	++	++	−	−

[a] Symbols: + = germination scanty, 1–2 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++ = germination moderate, 10 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; +++ = germination very profuse, 100 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++++ = germination very profuse, almost all teliospores germinated.

[b] Results recorded after 100 hr of teliospore exposure to the specified temperatures at 12 hr light/12 hr darkness alternate schedule. Teliospores previously stored in dry conditions at 2–4 C.

TABLE 3. Effect of presoaking for 2 wk on teliospore germination at 16 C and 12 hr diurnal illumination

Fungus	Source host	Intensity of germination after									
		6 hr		24 hr		48 hr		76 hr		96 hr	
		P[a]	N[b]	P	N	P	N	P	N	P	N
Puccinia hordei	*Hordeum spontaneum*	++[c]	−	+++	+	+++	++	++++	+++	++++	++++
P. hordei	*H. bulbosum*	++	−	+++	+	+++	++	++++	+++	++++	+++
P. recondita	*Aegilops variabilis*	+++	−	+++	+	+++	+++	++++	+++	++++	+++
P. graminis avenae	*Avena sativa*	++	−	++	−	+++	++	++++	+++	++++	+++
P. lagenophorae	*Senecio vernalis*	−	−	+++	−	+++	+	+++	+++	+++	+++
Uromyces scillarum	*Muscari parviflorum*	−	−	++	−	+++	++	++++	+++	++++	+++
U. scillarum	*Urginea undulata*	−	−	++	−	+++	++	++++	+++	++++	+++
U. viennot-bourginii	*Hordeum spontaneum*	−	−	++	−	+++	+	+++	++	+++	+++

[a] Teliospores presoaked.

[b] Teliospores not presoaked.

[c] Symbols: + = germination scanty, 1–2 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++ = germination moderate, 10 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; +++ = germination very profuse, 100 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++++ = germination very profuse, almost all teliospores germinated.

TABLE 4. Influence of light on teliospore germination at 16 C after 100 and 120 hr of incubation on 40 ppm chloramphenicol solidified with 2% agar

Fungus	Source host	Teliospore germination intensity					
		Continuous light		12 hr light/ 12 hr darkness		Continuous darkness	
		100 hr	120 hr	100 hr	120 hr	100 hr	120 hr
Puccinia hordei	*Hordeum spontaneum*	++[a]	+++	++	+++	++	+++
P. graminis avenae	*Avena sativa*	++	+++	++	+++	+	++
P. recondita	*Aegilops variabilis*	+++	+++	++	+++	++	+++
P. lagenophorae	*Senecio vernalis*	++	+++	++	+++	++	+++
Uromyces viennot-bourginii	*H. spontaneum*	++	+++	++	+++	+	+++
U. scillarum	*Muscari parviflorum*	++	+++	++	+++	++	+++
U. scillarum	*Pancratium parviflorum*	++	+++	++	+++	++	+++

[a] Symbols: + = germination scanty, 1–2 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++ = germination moderate, 10 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; +++ = germination very profuse, 100 teliospores germinating per microscopic field of low-power magnification containing approximately 1,000–2,000 teliospores; ++++ = germination very profuse, almost all teliospores germinated.

100 or 120 hr under the following illumination regimes. One group of petri plates with teliospores was maintained to continual darkness, and the other group was exposed to continual illumination. Teliospores of the third group were incubated at 12 hr light and 12 hr darkness. Light intensity in all tests was 16,000 lx. Results show that in the investigated fungi, light and light/darkness alternation do not influence teliospore germination, except in the case of *P. graminis* Pers. f. sp. *avenae* Eriks. where darkness seems to depress germination intensity (Table 4).

Longevity of teliospores. In numerous rust species teliospores are important means of perennation of the fungus, and in tiding it over a period unfavorable for development (9,10). Such a situation occurs in some rusts of annual plants in the Mediterranean region, where the host desiccates during the rainless summer. The duration of teliospore viability was studied in most of the species listed in Table 4 except that teliospores of *U. scillarum* (Grev.) Wint. isolated from *Pancratium parviflorum* Dec. were substituted by teliospores of *U. scillarum* harbored on *Urginea maritima* (L.) Bak. In addition, teliospores of the microcyclic *P. mesnieriana* Thum. borne on *Rhamnus palaestina* Boiss. were included in the trials. Each of the investigated species was represented by three isolates.

Results of the tests showed that teliospores of all investigated samples lost germinability after exposure for 1 yr outdoors at sites unprotected from direct sunlight, as well as at shaded sites. Teliospores preserved in paper bags in dry conditions at 2–4 C retained good germinability after 1 yr, lost some germinability after 2 yr, and were incapable of germination after 3 yr. Teliospores maintained at 5 C in sealed glass vials in partial vacuum and dry conditions retained viability over a period of 14 yr. Teliospores of stem rust of wheat, oats, and *Agropyron* sp. preserved germinability after 8 yr of storage at −18 C.

DISCUSSION

Teliospores and basidia are the most essential phases in the life cycle of rust fungi. It is estimated that in half of all Uredinales teliospores are dormant and require a post-ripening period for germination (5). Dormancy often enables survival of the fungus under adverse conditions. On the other hand, dormancy can frequently be a handicap in experimentation.

Israel is in a region where coevolution of some heteroecious rust fungi with their main and alternate hosts has taken place. According to Tranzschel (11), in such regions the implicated organisms develop telia abundantly. Teliospore germination is therefore indispensable for studies of the biology of the rust fungus.

The method for teliospore germination described above has been used in studies. It has proved useful in activating teliospores of 27 rust species of the genera *Puccinia*, *Uromyces*, *Tranzschelia*, *Frommea* (*Phragmidium*), and *Melampsora*. These species embrace macrocyclic heteroecious and autoecious organisms and microcyclic rusts. Most fungi originated in Israel, but promising results were also obtained with a limited number of species sampled in the United States, Australia, and West Germany (Table 1). These results suggest that the procedure can be used with a broad range of rusts. Germination was triggered in heteroecious species with the main and alternate hosts native in Israel as well as in fungi where the alternate hosts have never been identified (*P. striiformis* Westend., *P. antirrhini* Diet. & Holw.) or in heteroecious rusts whose alternate hosts do not occur in Israel (*P. graminis*, *P. recondita* Rob. & Desm.). The method yields reproducible results, enabling the whole process of teliospore germination and nuclear behavior to be studied under controlled conditions (2–4,12). Isolates of a given species can vary in their germinability. Teliospores of some isolates defied all efforts to trigger their germination (Table 5).

TABLE 5. Rate of teliospore germination of some of the investigated rust species

Rust pathogen species	Source host	Origin	Tested isolates (no.)	Germinating isolates (no.)
Puccinia graminis tritici	*Triticum aestivum* L.	Israel	46	0
P. graminis tritici	*T. aestivum* L.	USA	4	1
P. graminis tritici	*T. aestivum* L.	Australia	5	1
P. hordei	*Hordeum* spp.	Israel	2,891	2,802
P. hordei	*H. vulgare* L.	USA	15	6
P. hordei	*H. vulgare* L.	West Germany	5	1

Knowledge of the physiology of teliospore dormancy and germination is scanty and more information is needed (9). Gold and Mengden (7) have discussed the effect of light on teliospore germination and distinguish three groups of rusts. Teliospores of one group germinate equally well in continued light and darkness; teliospores of the second group germinate in continued darkness and germination is inhibited by light; teliospores of the third group need light and darkness alternatively for successful germination. In our studies light has not influenced teliospore germination, except in *P. graminis* f. sp. *avenae*, where darkness postponed germination and reduced its rate.

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