Factors Influencing Teliospore Germination in *Tilletia fusca*

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ABSTRACT

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The influence of cold temperature storage and exogenous nutrients on teliospore germination of 11 isolates of *Tilletia fusca* was assessed. Storage at 5 C stimulated germination of some isolates when they were incubated at 5 C, both in light and in dark. The relation of cold temperature and light activation to dormancy mechanisms and the significance of dormancy in the life cycle of *T. fusca* was discussed. Isolates of *T. fusca* varied in germination responses to water agar, soil extract agar, and media that contained different concentrations of mineral salts, asparagine, and sucrose. Most isolates germinated best on soil extract agar and media that contained low concentrations of mineral salts. Media prepared from extracts of different soils also affected germination responses of many isolates. These isolates usually required potassium, calcium, or phosphorus for maximal germination. Sucrose or asparagine were not required for teliospore germination in *T. fusca*.

The development of bunt (*Tilletia* spp.) diseases depends on the interaction between the host, the environment, and the pathogen (6). Bunt infection on wheat required a susceptible stage of the host and an infective stage of the fungus (18,19). Soil moisture, temperature, and light optimum for fungal spore germination were also optimum for host infection. Variations in these factors among seasons and locations could account for irregular disease incidence (1,12).

Low temperatures have been used since the turn of the century to break the dormancy of spores of certain fungi (21). In addition to treatments that break dormancy, some fungal spores require exogenous nutrients to germinate (7). Soil extract agar is routinely used to test teliospore germination in several species of *Tilletia* (10,16). However, chemical properties of soils can differ widely depending on the parent material, vegetation, and climate (8). Consequently, spore germination might vary with soil. Soil extract agar is also chemically undefined and will not provide precise information about nutritional requirements for spore germination.

The annual fluctuation in the incidence of *T. fusca* Ell. & Ev. on *Bromus tectorum* L. was probably also related to environmental factors that affect host infection (15). Light and temperature are the only factors that have been reported to influence teliospore germination in *T. fusca* (16), but the relation of these factors to host infection has not been explored. In addition, individual strains of *T. fusca* varied considerably in the time required for spore germination as well as in capacity to germinate at various temperatures (15,16). Dormancy and exogenous nutrients may also be important for spore germination in *T. fusca*; these factors might vary among individual isolates, but these factors have not been investigated. The objectives of this study were to investigate the effects of cold temperature storage, of chemically defined media, and of soil extract agar prepared from different soils on teliospore germination of 11 isolates of *T. fusca*.

MATERIALS AND METHODS

The 11 teliospore collections used in this study were obtained from naturally infected grasses collected from early to late summer of 1982, 1983, and 1984 from several locations in northern Utah and Idaho. Isolates 286, 289, 290, 292, and 296 were from *B. tectorum* obtained from Green Canyon (Cache County), UT; Park Valley (Box Elder County), UT; Boise (Ada County), ID; Preston (Franklin County), ID; and Blue Creek (Box Elder County), UT, respectively. Isolates 285, 291, and 298 were from *B. japonicus* Thunb. obtained from Treasureton Summit (Franklin County), ID; Stewart Pass (Cache County), UT; and Preston, ID, respectively. Isolates 301 and 303 were from *B. brizaeformis* Fisch. & Mey. obtained from Green Canyon, UT, and Pine Canyon (Cache County), UT, respectively. Isolate 300 was from *Festuca reflexa* Buckl. obtained near Boise, ID. Because teliospores of *Tilletia* often lose viability if removed from the sori (Guillemette, *unpublished*), the intact spore material was left on the host and stored in dark envelopes.

Before each experiment, spores were removed from the sori and surface-sterilized by a standard procedure for bunt fungi (16). Approximately 0.2 ml of a teliospore suspension was added to plates of appropriate media. Observations were obtained from three plates per collection per treatment within each experiment. All experiments were duplicated.

To determine the effect of cold temperature storage on teliospore germination, portions of collections of *T. fusca* were

stored at room temperature or 5 C for 2, 4, and 8 mo. In the room temperature storage treatment, collections were stored at 22–25 C for 3–6 mo (one isolate had been stored for 12 mo). After storage, teliospores were plated on soil extract agar (Green Canyon soil) prepared by a standard procedure (24). The plates were then incubated at 5, 10, and 15 C in continuous light or 5 C in the dark.

The effect of different media on teliospore germination was tested on water agar (WA), soil extract agar (SEA), and two chemically defined media used for growth of bunt fungi, T-19 and T-19M (24). The nutritional status of WA was low nutritive, whereas SEA contained low concentrations of mineral salts and undefined components, T-19M contained high concentrations of mineral salts but lacked a carbon and nitrogen source, and T-19 contained high concentrations of mineral salts plus a carbon and nitrogen source (sucrose and asparagine). The effect of salt concentrations on teliospore germination was tested on 3% T-19M (low salts and comparable to those in SEA), 30% T-19M (intermediate salts) and 100% T-19M (high salts). The effect of sucrose and asparagine concentrations on teliospore germination was tested on WA amended with 0.5, 1, 5, 10, and 20 g of sucrose per liter, and 0.1, 0.5, 1, 2, and 3 g of asparagine per liter. The effect of soil extracts on teliospore germination was tested with soils obtained from Green Canyon, UT; Wellsville Mountains (Cache County), UT; Hansel Valley (Box Elder County), UT; Park Valley, UT; Boise, ID; and Togwatee Pass (Fremont County), WY. The Green Canyon soil was a gravelly loam derived from limestone and brownish in color. The Wellsville Mountains soil was a fine sandy loam and black in color. The Hansel Valley soil was a silt loam and brownish in color. The Park Valley soil was saline, a fine silt in texture and whitish in color. The Boise soil was a fine silt and pale reddish in color. The Togwatee Pass soil was a loam, black in color and with a thick organic horizon. The Green Canyon and Wellsville Mountains soils were agricultural soils; the Hansel Valley, Park Valley, and Boise soils were desert soils, whereas the Togwatee Pass soil was a forest soil. SEA was prepared from each soil. All collections had been stored at 5 C for 10–20 mo and teliospores were incubated at 5 C in the light.

Germination percentages were determined weekly for 4 wk per experiment by counting 50–100 spores from five different fields of view per plate. A spore was considered to have germinated if a promycelium had emerged. The highest percentage value attained for the 4-wk period was used for statistical analysis. Because spore germination exhibits sigmoidal kinetics, germination rates were determined using the equation for J-shaped (exponential) curves, $r = [\log_e (X_T / X_0)] / T$ (7). T was the time (in days) at the end of the exponential phase, X_T was the percent germination at time T, X_0 was the percent germination at the time of plating and r was the germination rate. This equation is a modification of that used to determine fungal growth rates (7), but the length of the lag phase was an important component in spore germination rates. The exponential phase (specific growth rate) was when fungal growth was maximal and was a useful parameter for comparing the growth of different fungi or the growth of one species under different environmental conditions (7). Because percent germination at the time of plating was always zero, X_0 was assigned a value of 0.0001 (14). Percentages between 0 and 1 were assigned values of 0.005. Germination responses were analyzed statistically with a completely randomized factorial design coupled with Fisher's least significant difference (LSD) test (14). For the storage temperature experiment, the main effects were storage times, incubation temperature/light regimes, and isolates. For the experiment with different media, the main effects were media and isolates. For the soil extract experiment, the main effects were soil types and isolates. All interactions between main effects in all experiments were significant. The storage temperature experiment was analyzed further by using regression analysis with germination responses regressed across storage times.

TABLE 1. Correlation of germination responses of isolates of *Tilletia fusca* with length of cold temperature storage when spores were incubated at 5 C in light using regression analysis

	Germination (%)			Germination rate		
Collection	r^2	F^a	SD[b]	r^2	F^a	SD[b]
286	0.00	0.00	±12%	0.29	8.25	±0.06
289	0.88	156.45*	±11%	0.76	70.50*	±0.06
290	0.49	21.15*	±12%	0.57	27.89*	±0.09
292	0.49	21.38*	± 7%	0.49	18.63*	±0.09
296	0.61	34.19*	±17%	0.71	55.14*	±0.08
285	0.36	12.28*	± 7%	0.11	2.60	±0.07
291	0.75	65.48*	± 7%	0.07	2.00	±0.05
298	0.55	27.07*	± 8%	0.67	47.50*	±0.06
301	0.01	0.18	± 6%	0.58	31.60*	±0.07
303	0.01	0.13	± 9%	0.31	10.60*	±0.07
300	0.00	0.01	± 7%	0.06	1.40	±0.16

[a] Asterisk (*) indicates a significant F value (7.96), at $P = 0.01$.
[b] Standard deviation (SD) for the regression model (departure from linearity) (17).

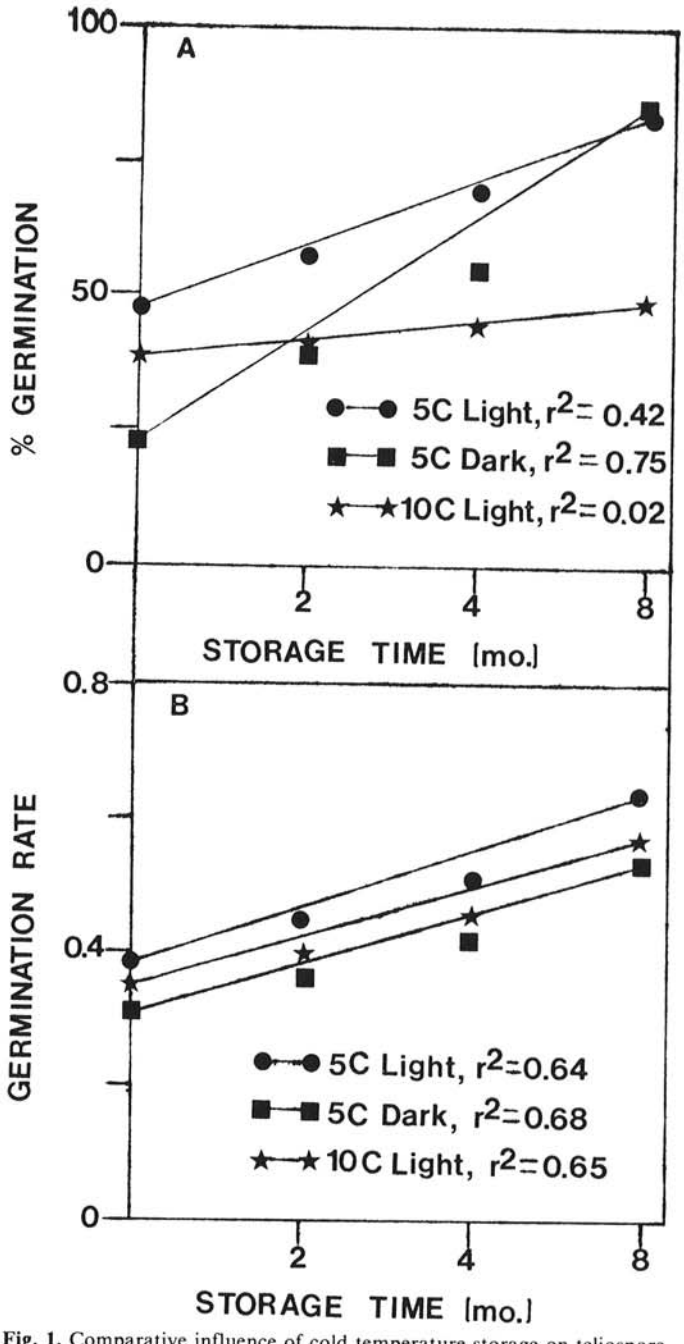

Fig. 1. Comparative influence of cold temperature storage on teliospore germination in *Tilletia fusca* at three temperature/light regimes. Germination values are the average of all isolates whose germination responses were stimulated by cold temperature storage, based on five fields of view per plate and six plates per isolate.

Because there were significant differences in both germination percentages and rates among the six soil types, the soil extracts were analyzed chemically. The extracts were analyzed by the Soils Testing Laboratory, Utah State University, for pH, salinity, available cations, available phosphorus, nitrate-nitrogen, and ammonium-nitrogen.

The effects of the various components in the soil extracts were determined when there were significant differences in germination percentages and rates among soil types. Because collections did not respond identically to the different soils, germination percentages and rates for each collection were compared to the concentration of the various components of the soil extracts. If germination appeared to be related to a particular element, the effect of the respective element was tested using regression analysis to obtain an r^2 value (17). If the r^2 value was 0.36 or higher (14), the concentration of the element was considered to be at least partially correlated with the differences in germination percentages and/or rates. Regression analysis helped to identify components in the soil extracts that may be important in teliospore germination and, thus, saved an enormous amount of experimentation and time. I am not implying a linear relation between germination responses and concentrations of ions in soil extracts; there is just a correlation, not necessarily a direct causal effect. Regression analysis, at best, must be interpreted cautiously (17).

To confirm the importance of the soil extract components predicted by regression analysis, the various elements were omitted from 3% T-19M and teliospores of the respective collections were plated on the deficient medium. When potassium was omitted, NaH_2PO_4 and Na_2HPO_4 were substituted at equimolar concentrations as sources of phosphorus and for buffering. When phosphorus was omitted, KCl was substituted as a source of potassium (in concentrations equal to the amount of available K in 3% T-19M), and the medium was buffered at pH 6 with glycyl-glycine. Water agar and 3% T-19M served as controls. Before use, collections were stored at 5 C for 14–22 mo, and plates with teliospores were incubated at 5 C in the light.

RESULTS

Spore germination of isolates 286 and 300 of *T. fusca* was not affected by storage at 5 C, whereas isolates 289, 290, 292, and 296 were stimulated to germinate by storage at 5 C (Table 1). Germination percentages of isolates 301 and 303 were not affected by length of cold temperature storage, but germination rates were stimulated by the treatment. The opposite was true for isolates 285, 291, and 298.

All collections of *T. fusca* whose germination responses increased with increased storage at cold temperature when incubated at 5 C in the light, also showed the same response in darkness. Likewise, collections that were not affected when incubated at 5 C in the light were also not affected in the dark. The effect of length of storage temperature when spores were incubated at 10 C in the light was highly variable within isolates of *T. fusca*. Teliospore germination for all isolates of *T. fusca* was neglible at 15 C and was not influenced by storage temperature. As cold temperature storage increased from 0 to 8 mo, germination percentages and rates at 5 C, both in light and dark, also increased (Fig. 1). After 8 mo of storage, germination percentages of spores in the light or dark were similar. Cold temperature storage had no effect on germination percentages at 10 C in the light, but germination rates increased as the storage time increased. After 8 mo of storage at cold temperature, germination rates at 5 and 10 C in the light were similar.

Spore germination of *T. fusca* on different media depended on the isolate (Table 2). For a given collection, the range of media that produced the highest germination percentages was not always identical to that which produced the highest rates. For example, germination percentages of isolate 290 did not vary among the different media, but germination rates were maximal on SEA. Teliospores of isolates 286, 290, 292, 296, 285, 291, 298, and 300 germinated best on SEA. Teliospore germination of isolates 301 and 303 was similar on WA, SEA, and T-19M, whereas that of isolate 289 was highest on SEA or WA.

Media made from extracts of the six soil types had similar effects on germination percentages and rates of isolates 289, 292, and 300 of *T. fusca* (Fig. 2). In contrast, there were significant differences in germination percentages and rates associated with soil types for isolates 285, 296, 291, 298, 301, and 303. Although germination rates of 296 did not change in media from different soils, germination percentages differed significantly. Germination rates but not percentages for isolate 290 differed significantly with soil types. Germination of individual isolates of *T. fusca* responded differently to the various soils. For example, the germination percent of 296 was lowest on soil extract from Park Valley, whereas germination percent of 285 was lowest on soil extract from Wellsville Mountains. In general, soil extract of Boise, Wellsville Mountains, and Togwatee Pass resulted in lowest germination responses. The soil extract of Hansel Valley resulted in the highest germination responses.

Individual isolates of *T. fusca* also varied in spore germination responses to different concentrations of mineral salts, sucrose, and asparagine. However, the data had some significant trends. Isolates that germinated best on SEA also germinated maximally on 3% T-19M. Similarly, isolates whose germination responses did not vary among WA, SEA, or T-19M were not influenced by mineral salt concentrations or by low concentrations of sucrose or asparagine. On the average, low concentrations of mineral salts were most conducive to germination of spores of *T. fusca*, as teliospore germination declined at intermediate and high concentrations of mineral salts (Fig. 3). Germination responses were not affected by low concentrations of sucrose or asparagine, but high concentrations of both nutrients reduced teliospore germination compared with WA.

Concentrations of several components of extracts from the six

TABLE 2. Teliospore germination of collections of *Tilletia fusca* on four different media[x]

Collection	Germination (%)[y]				Germination rate[z]			
	WA	SEA	T-19M	T-19	WA	SEA	T-19M	T-19
286	0 a	48 b	0 a	0 a	0.000 a	0.376 b	0.000 a	0.000 a
289	74 b	84 b	32 a	23 a	0.424 a	0.454 a	0.384 a	0.366 a
290	86 a	78 a	80 a	89 a	0.431 a	0.554 b	0.429 a	0.433 a
292	87 a	88 a	88 a	86 a	0.432 a	0.638 c	0.553 b	0.431 a
296	40 b	73 c	31 ab	24 a	0.383 a	0.416 a	0.383 a	0.371 a
285	12 a	80 b	1 a	0 a	0.338 c	0.518 d	0.186 b	0.000 a
291	2 a	68 b	1 a	0.5 a	0.252 a	0.411 b	0.219 a	0.186 a
298	59 b	71 c	48 b	5 a	0.413 b	0.613 c	0.404 ab	0.324 a
301	71 b	69 b	72 b	16 a	0.422 ab	0.445 b	0.424 ab	0.331 a
303	80 b	71 b	83 b	51 a	0.426 a	0.477 a	0.430 a	0.407 a
300	43 b	78 c	44 b	0 a	0.354 b	0.485 c	0.341 b	0.000 a

[x]Media were water agar (WA), soil extract agar (SEA), high concentrations of mineral salts but lacking carbon and nitrogen sources (T-19M) and high concentrations of mineral salts plus sucrose and asparagine (T-19).

[y]Means in a row followed by different letters indicate significant differences (SD = ±9%, LSD = 12%, $P = 0.05$).

[z]The equation for germination rate is described in the text (SD = ±0.063, LSD = 0.084, with $P = 0.05$).

soil types differed (Table 3): the pH, salinity, and concentrations of calcium and sodium in extracts from the Park Valley soil were higher than those from other soils. Potassium concentrations were highest in the Hansel Valley and Park Valley soil extracts, and lowest in those from Boise soil. Phosphorus concentrations were highest in the Hansel Valley soil extracts and lowest in extracts from Green Canyon, Park Valley, and Togwatee Pass soils. The concentrations of ammonium-nitrogen were similar in all soil extracts. Extracts from the Togwatee Pass soil contained the most magnesium and nitrate-nitrogen. The concentrations of potassium, calcium, magnesium, and phosphorus were highest in T-19M.

Regression analysis showed that differences in germination responses of *T. fusca* among the six soil types were most often correlated with concentrations of potassium, phosphorus, and calcium (Table 4). Germination responses on media deficient in the three nutrients supported the correlation (Table 5). When the appropriate ions were omitted from the 3% T-19M medium, germination responses of most isolates that had been highest on that medium decreased to levels on WA. The percentage germination of isolate 296 appeared to be adversely affected by salinity in the Park Valley soil extract (Table 4). When 3% T-19M was

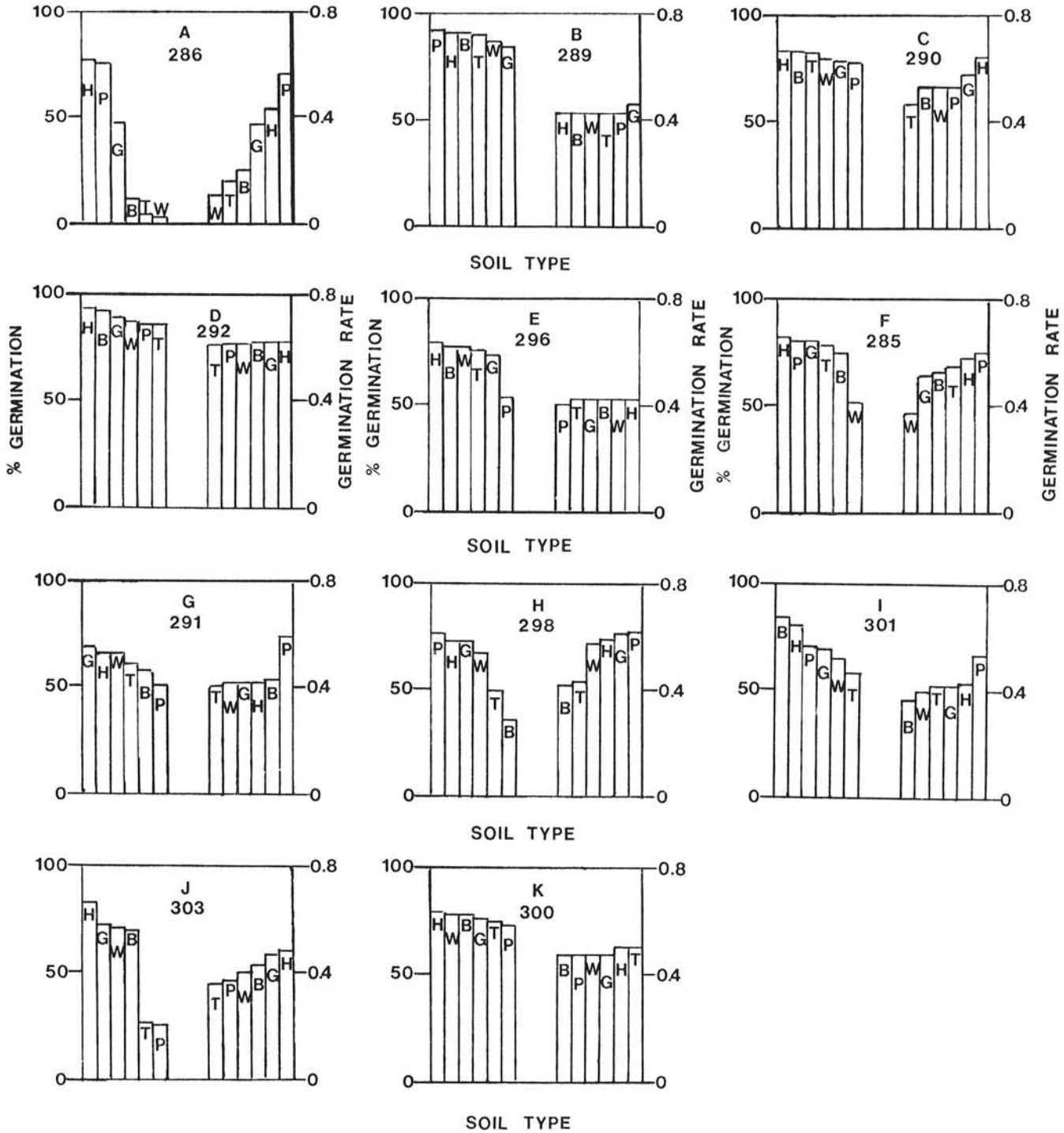

Fig. 2A–K. The effect of media made from the extracts of different soils on teliospore germination of isolates of *Tilletia fusca*. The standard deviation for germination percentage was ±10% with an LSD of ±12%. The standard deviation for germination rate was ±0.07 with an LSD of ±0.083. The values for each bar in each graph are the average of six plates. Numbers at top center of each graph correspond to isolate numbers. Letters in the bars refer to source of soil for preparing soil extract agar as follows: Hansel Valley soil (H), Green Canyon soil (G), Park Valley soil (P), Boise soil (B), Wellsville Mountains soil (W), and Togwatee Pass soil (T).

amended with NaCl, percent germination of isolate 296 was reduced to that of the Park Valley soil (Table 5, Fig. 2).

DISCUSSION

My data support the findings of Meiners and Waldher (16): Different isolates varied in cold temperature storage responses and in nutritional requirements for germination (Tables 1, 2, 4, and 5, Fig. 2). Some of this variation may be associated with different hosts, geographic locations, or microclimates. Strains of a fungal species from different locations were physiologically adapted to the particular climate from which they were isolated. Local microhabitats and substrates determined fungal distributions (25). Because conditions at a particular location and microclimate can vary among years, the environmental conditions during development might affect spore germination; if so, spore germination in a strain might differ if the strain was collected from the same location during different years. Collection date may also be an important factor. Teliospore germination might increase when spores are collected later in the growing season, and spore immaturity might explain lower germination of teliospores from samples collected early in the growing season.

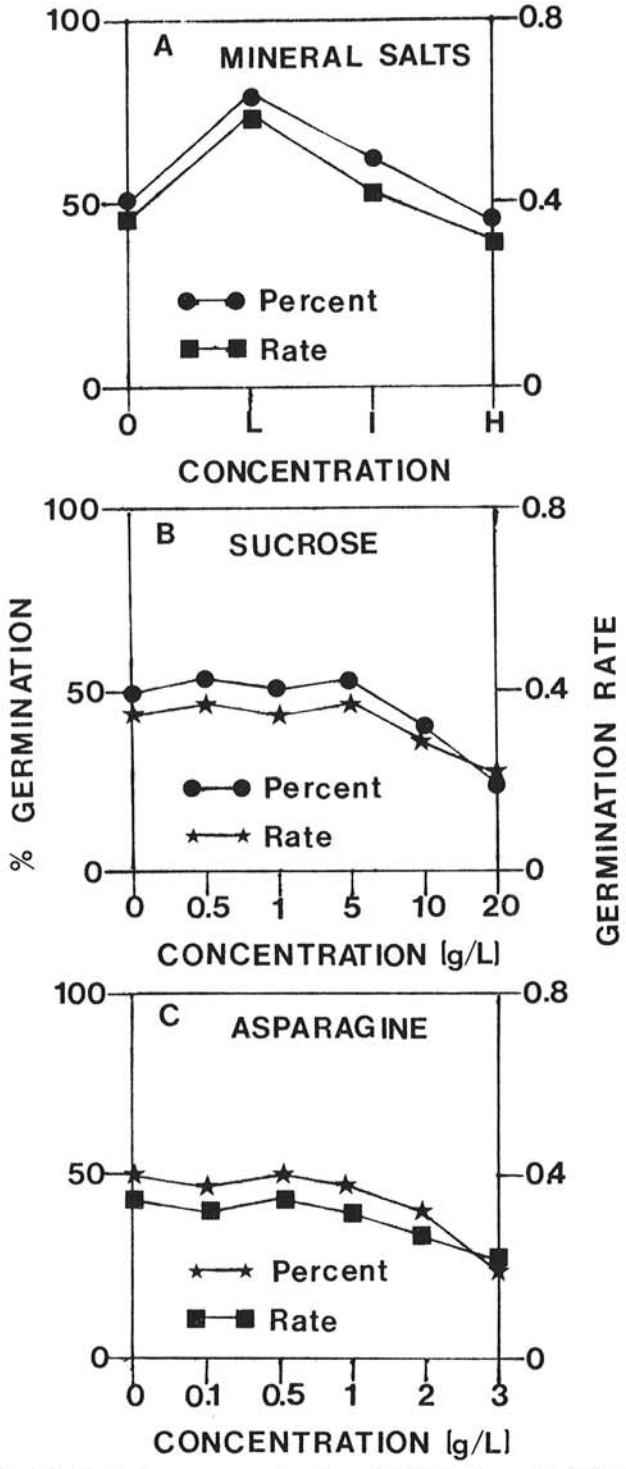

Fig. 3A–C. Teliospore germination of *Tilletia fusca* at different concentrations of mineral salts, sucrose, and asparagine. Data are the average of all isolates. Zero concentrations in all figures represent water agar controls. In A, L is low concentrations, I is intermediate concentrations, and H is high concentrations of mineral salts (see text). For percent germination, SD = ±10%; and for germination rate, SD = ±0.069.

There are several hypothesized mechanisms of constitutive dormancy in fungi, none of which is well understood in most fungi (7). There also may be a relation between a particular mechanism of dormancy and activation treatments. Although light-mediated spore germination is known to involve a photoreceptor in some fungi (21), experiments correlating the requirements for light and cold temperature activation with a specific dormancy mechanism have not been done in fungi.

In this study, light and cold temperature storage appeared to be additive on spore germination in *T. fusca*, at least initially. For example, germination at 5 C was higher in the light than in the dark at 2 mo storage at cold temperature (Fig. 1). However, after 4 mo of storage the light requirement of teliospores diminished, as indicated by the increase in germination responses at 5 C in the dark. Both light and cold temperature individually are known to influence the release of endogenous inhibitors in the seeds of higher plants. In the seeds of some plant species, both the levels of endogenous inhibitors and the requirement for light decreased with increased storage at cold temperature (13). Perhaps both light and cold temperature storage influence the release of germination inhibitors in teliospores of *T. fusca* as well, which might explain these results.

Teliospores of *T. fusca* have not been reported to possess germination inhibitors. Trimethylamine, however, which provides the characteristic odor for bunt fungi, has been reported to be involved with the dormancy of teliospores of *Tilletia*. The long incubation period required for teliospore germination of the dwarf bunt fungus appeared to be partially associated with endogenous inhibitors, of which trimethylamine was one (23). Once these substances were removed from the spores of *T. controversa*, the incubation time was substantially reduced (20). The dwarf bunt pathogen is a cool-temperature organism, and exposure to light is a requirement for maximum germination for most strains. Generally, teliospore collections with short incubation periods had less restrictive temperature and light requirements than those with long incubation periods (10). Teliospores of *T. asperifolioides* required chilling for 2–3 mo at 1–2 C for germination to occur, although the presence of endogenous inhibitors and the requirement for light was not mentioned (2). Only further experiments will determine how cold temperature and/or light activation of collections of *T. fusca* are related to a specific mechanism of dormancy.

The most common hosts of *T. fusca* are annual grasses such as *Bromus tectorum, B. japonicus, B. brizaeformis, Festuca pacifica* Piper., *F. reflexa,* and *F. octoflora* Walt. In nature, the seeds of these grass species germinate under moist conditions in early autumn (11), and the grasses survive as seedlings under the snow (15). The methods of inoculation that resulted in highest bunt incidence suggest that the grasses are infected by *T. fusca* in the fall after seedling emergence (11). Because the grass species occur in dry, open habitats (9), soil moisture appears to be critical for seed and teliospore germination and host infection. Exposure to cold temperatures and/or light in teliospores of *T. fusca* may help synchronize infection on annual hosts growing in dry habitats. Dormancy may be a mechanism by which spores remain viable until conditions are favorable for germination and subsequent infection.

This is the first detailed study concerning the effect of cold temperature storage on teliospore germination in *T. fusca*. It is not

known how extensively the requirement occurs within the organism. Several hosts, as well as the bunt, are widely distributed throughout the arid, western region of the United States; the distribution transacts several geographic locations and microclimates (9). The light and cold temperature requirement may be associated with the life cycle of certain hosts, specific environments, or a combination of the two factors. Duran and Safeeulla (3) reported that chilling was required to break dormancy in northern collections of some species of smut fungi but not in southern collections. Thus, dormancy may be selected for in certain environments and against in others.

Because germination responses of most isolates of *T. fusca* were highest on SEA and 3% T-19M (Table 2, Fig. 3), and the concentration of mineral salts in 3% T-19M and SEA were comparable, the mineral fraction in soil extracts may be the components that enhance teliospore germination. In addition, most isolates that were influenced by SEA prepared from different soils were also affected by different concentrations of mineral salts in WA and T-19M (Figs. 2 and 3, Table 2), suggesting that differential germination responses among the soil types may be correlated with concentrations of ions in the soil extracts. Results of this study indicated that inorganic ions in soil extracts and T-19M were important factors in teliospore germination of most collections of *T. fusca* (Tables 4 and 5). The same ions were not important for all isolates, one isolate could require several ions, and germination percentages and rates (both negative and positive)

TABLE 3. Chemical analysis of T-19M and extracts obtained from six different soils[a]

Element[b]	Hansel Valley	Park Valley	Boise	Wellsville Mountains	Green Canyon	Togwatee Pass	T-19M
pH	6.52	7.58	6.40	6.42	6.56	6.55	6.00
Salinity	29	125	10	14.5	25	37.5	...[c]
Basic cations							
K	3.85	4.50	0.45	1.05	1.15	1.25	199
Ca	0.95	1.60	0.85	0.65	1.05	1.05	40
Mg	0.45	0.50	0.40	0.35	0.50	0.85	24
Na	0.25	24.75	0.40	0.25	0.35	0.80	0
Nitrate-N	0.25	0.25	0.25	0.25	0.25	3.45	0
Ammonium-N	0.50	0.50	0.50	0.50	0.50	0.50	0
Phosphorus	0.36	0.05	0.17	0.14	0.06	0.04	29

[a] See text for locations and descriptions of soils tested.
[b] Listed in micrograms per milliliter (μg/ml), except for salinity and pH; salinity is measured as a unit of conductivity (EC) and expressed in μmhos/cm. Values represent the concentrations present in 1 L of SEA or T-19M.
[c] Not determined.

TABLE 4. Correlation of germination responses of isolates of *Tilletia fusca* with concentrations of ions in the soil extracts

Collection	Ion	Germination (%)				Germination rate			
		Slope	Intercept	r^2	F^2	Slope	Intercept	r^2	F^2
285	Ca	+21	+22	0.43	25.49*	+0.180	+0.342	0.47	32.00*
291	Ca	−13	+74	0.37	20.02*	+0.092	+0.382	0.67	65.67*
301	Ca	...[b]	...	...	...	+0.313	+0.115	0.69	81.67*
303	Ca	−48	+106	0.37	20.16*	...	...	...	...
286	K	+18	− 2	0.76	106.20*	+0.092	+0.126	0.75	120.00*
298	K	+ 6	+45	0.45	28.08*	+0.032	+0.469	0.41	25.00*
301	K	...	...	...	...	+0.043	+0.347	0.37	26.00*
290	P	...	...	...	...	+0.412	+0.477	0.71	90.00*
301	P	+45	+61	0.47	30.57*	...	...	...	...
303	P	+148	+37	0.52	36.49*	+0.263	+0.393	0.44	25.00*
301	Mg	−16	+74	0.39	24.74*	...	...	...	...
296	Salinity	−0.2	+79	0.89	286.42*	...	...	...	...

[a] Asterisk (*) indicates a significant F value (7.56) at $P = 0.01$.
[b] Not determined.

TABLE 5. Teliospore germination of isolates of *Tilletia fusca* on various deficient media

Collection	Nutrient[b]	Germination (%)[a]			Germination rate[a]		
		Deficient T-19M[c]	WA	3% T-19M	Deficient T-19M[c]	WA	3% T-19M
285	Ca	10	12	81	0.345	0.338	0.597
291	Ca	8	2	65	0.318	0.252	0.495
301	Ca	70	71	80	0.422	0.422	0.566
303	Ca	82	80	83	0.460	0.387	0.489
286	K	0	0	75	0.000	0.000	0.574
298	K	49	59	72	0.405	0.413	0.524
301	K	62	71	80	0.416	0.422	0.566
290	P	81	86	83	0.429	0.431	0.636
301	P	45	71	80	0.512	0.422	0.566
303	P	67	80	83	0.370	0.387	0.489
301	Mg	71	71	80	0.543	0.422	0.566
296[d]	Salinity	59	40	78	0.401	0.395	0.420

[a] Data are the average of six plates with a standard deviation of ±9% for germination percentage and 0.063 for germination rate.
[b] Refers to nutrient removed from T-19M.
[c] Refers to T-19M minus the respective nutrient.
[d] This collection was tested on 3% T-19M to which 25 μg/ml of NaCl was added.

of a single isolate could be influenced by different ions. The high concentration of sodium in the Park Valley soil extract may contribute to salinity that inhibits spore germination of isolate 296 (Tables 3 and 4, Fig. 2). However, germination of isolate 296 averaged 31% on T-19M (Table 2). Salinity is a measure of salt concentration, and calcium, magnesium, and sodium mainly contribute to salinity in soils (8). T-19M contains high concentrations of the former two ions, which might explain this result. The germination percentages of isolate 301 and 303 did not appear to correspond solely to the concentration of phosphorus in the soil extracts (Fig. 2, Table 4): An imbalance between magnesium and phosphorus might have affected germination of isolate 301, whereas an imbalance between calcium and phosphorus might have affected germination of isolate 303. This is supported by data in Table 5; percent germination of both isolates was higher on WA than on 3% T-19M minus phosphorus. Also, percent germination of 301 was not affected when magnesium was omitted, and that of 303 was not affected when calcium was omitted. High levels of magnesium and calcium are known to adversely influence the availability of phosphorus in soils (8), suggesting that magnesium and calcium inhibit spore germination of isolates 301 and 303 of *T. fusca* (respectively) when phosphorus is low or lacking. Inorganic ions (NH_4, K, Mg, PO_4) also have been reported to be important in spore germination in fungi such as *Rhizopus, Neurospora,* and *Blastocladiella* (4,5,7,22). However, some isolates of *T. fusca* did not appear to require exogenous nutrients to germinate (Table 2). Carbon and nitrogen sources did not appear to be important requirements for teliospore germination in *T. fusca*.

How the requirements of an exogenous supply of nutrients for teliospore germination pertain to host infection by *T. fusca* was not studied in depth. When available, the nutrients increased germination percentages, which could in turn increase the chances of infection. The enhancement of spore germination by substrates rich in nutrients may indicate the role of host exudates. The soilborne spores of *T. fusca* (11) may obtain the inorganic ions required for teliospore germination from the soil. The ions are probably most available when the soil is moist. Low temperature and light may be the only important factors for isolates that germinated well on water agar.

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