The Disease Cycle and Control of Geranium Rust

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ABSTRACT

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Germination of urediniospores of *Puccinia pelargonii-zonalis* required liquid water and occurred within 3 hr at the optimal temperature of 16 C. The pathogen usually formed appressoria and penetrated through the stomata 5–6 hr after inoculation. The hyphae were intercellular with intracellular haustoria. Symptom development was most rapid and extensive at 21 C. Of 35 *Pelargonium* spp. inoculated, only seven were susceptible. Cultivars of *P. hortorum* varied in susceptibility and isolates of the fungus varied in virulence. Viability of urediniospores from air-dried leaves dropped from 100 to 0.8% in 12 wk. Four fungicides (triadimefon, mancozeb, oxycarboxin, and triforine) effectively controlled *P. pelargonii-zonalis* on geraniums in greenhouse and field experiments.

In South Africa in 1926, E. M. Doidge first described *Puccinia pelargonii-zonalis* Doidge (3) and reported that the fungus produced urediniospores and teliospores on the common geranium (*Pelargonium* × *hortorum* L.H. Bailey), on *P. zonale* (L.) L'Hér. ex Ait., and on *P. inquinans* L'Hér. ex Ait. (1) the latter two of which are ancestors of the common geranium. It appears that the rust fungus also is indigenous to that part of the world.

The fungus was introduced to Europe (11) and the United States (2) during the 1960's and now occurs on geraniums in most temperate zones. Areas where geranium rust has been found have been quarantined by Canada (9), thus causing losses to geranium growers.

Published information about the disease cycle of this organism is limited. This study was designed to determine factors important in the development of the fungus and the disease, including the mode and timing of penetration by the pathogen, the effects of temperature on spore germination and symptom development, the degree of resistance to the fungus in various *Pelargonium* spp. and cultivars, and the effectiveness of chemical control measures. A preliminary report has been published (5).

MATERIALS AND METHODS

Unless otherwise stated, *P. hortorum* was used in all experiments. Greenhouse-grown plants were inoculated by spraying a urediniospore suspension with an atomizer, placing the plants in an unlighted chamber for 18 hr at 21 C, then maintaining them in a greenhouse at 21–23 C under natural light.

Tissue for scanning electron microscopy was prepared by fixation in 4% osmium tetroxide solutions made up in a 0.2 M sodium cacodylate, pH 8.0 (Ted Pella Co., P.O. Box 510, Tustin, CA 92680). Exact fixation procedure is described under appropriate headings. Tissue was rinsed in distilled water and then placed for 30 min in a saturated solution of thiocarbohydrazide (Eastman Kodak Co., Rochester, NY 14650) which had been filtered through a 0.2-μm filter (Gelman Instrument Co., 600 S. Wagner Rd., Ann Arbor, MI 41806) to form a ligand with osmium. This procedure eliminated the need for sputter coating of some tissue samples (6). Tissue was rinsed with distilled water, placed in 1% osmium tetroxide for 1 hr, rinsed again in distilled water, dehydrated in solutions of 50, 75, 95, 100, and 100% aqueous ethanol series for 15 min at each concentration. Then the tissue was run through a series of 25, 50, 100, and 100% Freon 113 in ethanol solutions for 15 min, and critical-point dried in Freon 113. After being dried, the samples either were examined directly or were coated with gold in a sputter coater (International Scientific Instruments, 1400 Stierlin Rd., Mountain View, CA 94043). Examination was by a scanning electron microscope (either a Coates and Welter Model 50, 777 N. Pastoria Ave., Sunnyvale, CA 94086 [provided by NSF Grant GB38359] or an International Scientific Instruments Super II model S11, 1400 Stierlin Rd., Mountain View, CA 94043).

Spore germination. Urediniospores were suspended in glass-distilled water (GDW) and 1 ml of this suspension (about 1,000 spores per ml) was spread over the surface of 2% agar in petri dishes. The dishes were incubated in the dark at 12, 16, 21, and 24 C.

Spores also were dusted over slides that had a drop of GDW on one end. By suspending the slides in closed containers over GDW, the spores were exposed to 0% vapor pressure deficit (VPD). These experiments were repeated once.

Penetration. Urediniospores were floated on GDW. Abaxial surfaces of detached leaves of *P. hortorum* and *P. peltatum* were dipped in the spore suspension to coat them with spores. These leaves were atomized with GDW until lightly coated and then were placed in a moist chamber in the dark at 16 C for 10 hr. The atomization was essential for good spore germination. Epidermal peels were made, placed in lactophenol mounting medium (12), and examined with a compound microscope. Tissue 0.5 cm^2 from these leaves as well as from well-developed pustules was examined with the scanning electron microscope (SEM). These tissues were fixed in 4% osmium tetroxide for 2 hr and prepared for the SEM as previously described. Either adaxial or abaxial surfaces of intact attached leaves (three each) similarly were coated with spores, encased in plastic bags, and the plants were placed in the dark at 16 C for 18 hr. The plastic bags were removed and the plants were placed in the greenhouse. Disease development was determined 2 wk later.

Infection. Plants of cultivar Sprinter with approximately 10–15 mature leaves per plant were inoculated. At 30-min intervals, two plants were removed from the moisture chamber and dried with a fan for 20 min. This procedure was followed for up to 10 hr after inoculation. After 2 wk, inoculated plants were examined for rust pustule development. This experiment was repeated twice.

Effects of temperature on symptom development. Twelve plants of cultivar Blaze were inoculated and three were incubated at 32, 27, 21, or 16 C. The plants were examined periodically for symptom development.

Internal colonization. Leaf tissue from infected plants of cultivar Blaze with well-developed rust pustules was hand-sectioned, cleared in 25% aqueous chloral hydrate, stained with 1% aqueous acid fuchsin or 1% aqueous aniline blue, and examined with a compound microscope. Tissue also was prepared for the SEM by modification of the method described by Kinden and Brown (7). Pustule areas were cut from leaves, fixed in 4% osmium tetroxide for 2 hr at 21 C and then for 18 hr at 4 C. The tissue was rinsed

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several times in distilled water, cut on two sides with a razor blade, and placed in 1% aqueous periodic acid for 2–3 min. The tissue was rinsed in distilled water several times over a period of 10 min, treated with 40% aqueous KOH at 55 C for 30 min, rinsed, exposed to 1.0% aqueous acetic acid for 5 min, and rinsed 10 times during 1 hr. It was then placed in 4% osmium tetroxide for 2 hr at 21 C, and prepared for SEM as previously described. Cut edges of the tissue were examined.

Host resistance. Thirty-five *Pelargonium* spp. and 23 *P. hortorum* cultivars were inoculated. After 2 wk in the greenhouse they were examined for rust pustule formation. Those not showing symptoms were examined again after an additional 2 wk to allow for delayed symptom expression.

Spore viability. Leaves from plants of cultivar Blaze with well-developed pustules were collected and air-dried under ambient laboratory conditions. Spores were removed at 0, 4, 10, and 12 wk and plated on 2% agar. The plates were incubated at 16 C for 18 hr, then examined for spore germination.

To determine the longevity of the spores on host tissue, cultivar Sprinter plants of approximately equal size (about 18 cm tall) were inoculated. All leaves were marked by tying paper-covered wires around the petioles and the plants were dried before a fan on a rotating table. Two plants were remoistened with water and placed in a moist chamber for 18 hr immediately and 2 and 4 wk after inoculation. Plants were examined for pustule development 2 wk after remoistening.

Control. Three replications of 10 cultivar Blaze plants each were sprayed to runoff with triforine (Cela W524 20%) at 272 ppm, oxycarboxin (Plantvax—75W 75%) at 990 ppm, mancozeb (Dithane M-45 80%) at 1,920 ppm, triadimefon (Bayleton 25%) at

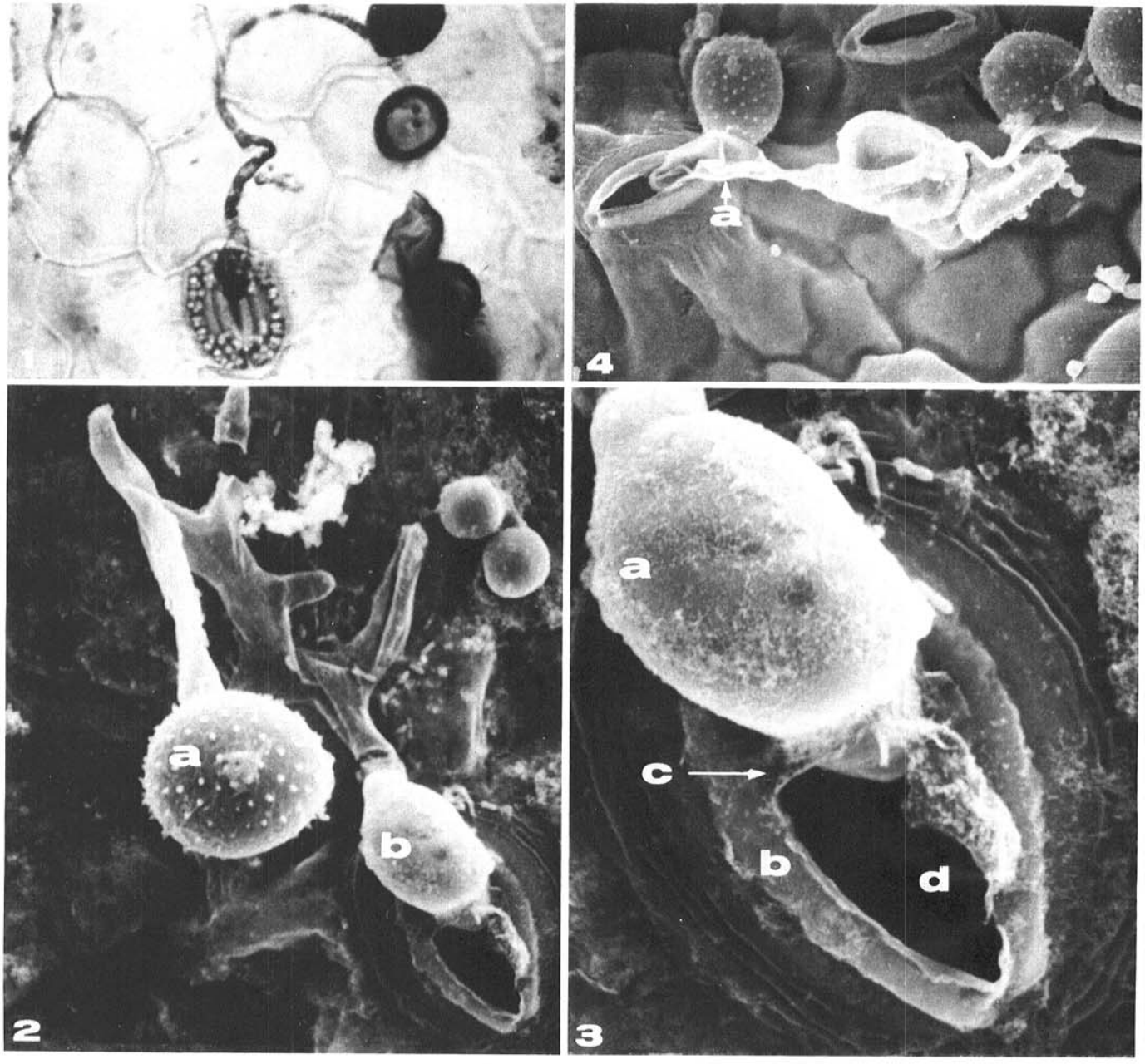

Fig. 1-4. Penetration of *Puccinia pelargonii-zonalis* through stomata of *Pelargonium* sp. showing formation of appressoria. **1,** Light micrograph of *Puccinia pelargonii-zonalis* penetrating *Pelargonium peltatum.* ×600. **2,** Scanning electronmicrograph of penetration of *Pelargonium hortorum* by *Puccinia pelargonii-zonalis* showing that the cytoplasm has moved out of the spore (a) and into the appressorium (b). ×1400. **3,** Scanning electronmicrograph of *Puccinia pelargonii-zonalis* showing an appressorium (a) apparently attached to the guard cell (b) with a mucilaginous material (c) as it enters the stomatal chamber (d). ×3300. **4,** Scanning electronmicrograph of penetration of *Pelargonium hortorum* by *Puccinia pelargonii-zonalis* showing the collapse of the appressorium (a) after the cytoplasm has moved into the stoma. ×714.

300 ppm, and a water control. The plants were dried before a fan, inoculated with a spore suspension of *Puccinia pelargonii-zonalis* and placed in the greenhouse. Rust pustules were counted 2 wk after inoculation and the procedure was repeated. The plants were rated again after 2 wk and the height from the soil level to the highest terminal bud was recorded. The fungicides also were field tested on three replications of nine cultivar Sprinter plants with established rust infection. They were applied to run off three times at 2-wk intervals at the same concentrations as in the greenhouse. Two weeks after the last application of fungicide, 10 fully expanded leaves were selected from each plant and rated for disease.

RESULTS

Spore germination. One hundred randomly selected spores exposed to 12, 16, 21, or 24 C were evaluated. Spore germination was 89% at 12 C, 93% at 16 C, 91% at 21 C, and 80% at 24 C; thus, it was optimal at 16 C. Spores incubated on agar at 16 C were evaluated hourly for germination. Germ tubes were produced 2–3 hr after exposure to water and began to branch and to develop thickened walls after 6 hr. On slides at 16 C with 0 VPD, 300 spores were randomly selected for evaluation. At 0 VPD there was no germination in the absence of liquid water. Germination was 8.3% with liquid water present.

Penetration. Germ tubes were observed forming appressoria over stomata of both *P. hortorum* and *P. peltatum*. Penetration occurred through the stomata, in association with appressoria, and through stomata surrounding developed pustules without the formation of appressoria (Fig. 1–5). Penetration and infection occurred through stomata on either side of the leaf.

Infection. Infection was established only on plants that had remained wet for at least 4.5 hr. The number of successful infections increased exponentially with time (Fig. 6).

Symptom development. At 32 C, rust symptoms did not develop but new leaves that formed were chlorotic in response to the high temperature. At 27 C, newly formed leaves were slightly chlorotic and chlorotic flecks associated with pustule formation appeared after 9 days. Small pustules formed, but spores never were released. At 21 C young leaves were not chlorotic, after 9 days chlorotic flecks also appeared, and spore release occurred after 19 days. At 16 C, small pustules first were evident after 13 days, but these remained small and released few spores.

Intercellular hyphae with bulbous, irregular, intracellular haustoria and developing pustules were observed (Fig. 7–9). Sporogenous hyphae accumulated under the host epidermis and the spores that were produced lifted and ruptured the epidermis.

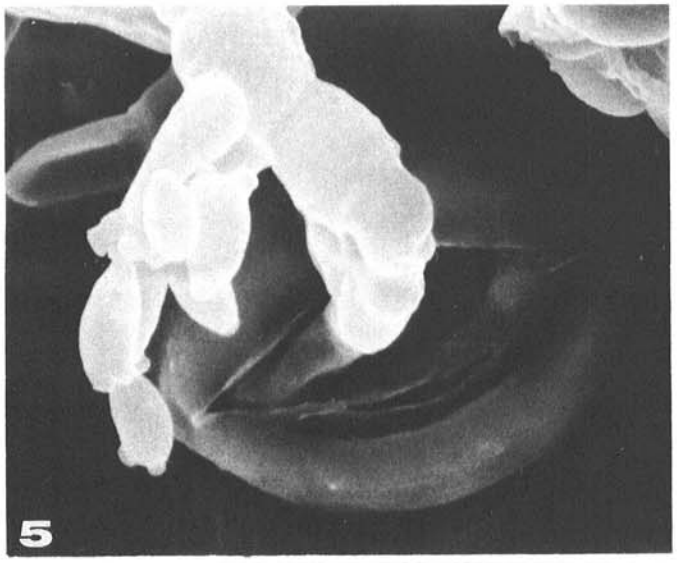

Fig. 5. Scanning electronmicrograph of penetration of *Pelargonium hortorum* by *Puccinia pelargonii-zonalis* without appressorium formation. ×3100.

Host resistance and pathogen virulence. *P. endlicherianum* Fenzl., *P. hortorum* Bailey, *P. inquinans* L'Hér. ex Ait., *P. quercifolium* L'Hér., *P. salmoneum* R. A. Dyer, *P. tabularae* L'Hér., and *P. zonale* L'Hér. were the only susceptible *Pelargonium* spp. of the 35 inoculated. The species tested which were nonsusceptible were: *P. alchemilloides* (L.) L'Hér., *P. apiifolium* Jacq. F., *P. australe* Willd., *P. capitatum* Soland, *P. citrosum* Voight ex Sprague, *P.* × *coarctatum* Sweet, *P. crispum* L'Hér. ex Ait., *A. crithmifolium* Sm., *P. dasycaule* Haw., *P. domesticum* Bailey, *P.* × *domesticum* Bailey × *P. quercifolium* L'Hér., *P. echinatum* Curtis, *P.* × *fragrans* Willd., *P. glutinosum* (Jacq.) L'Hér, *P. graveolens* L'Hér. ex Ait., *P. incrissatum* Sims, *P. lateripes* L'Hér. ex Ait., *P. luridum* Sweet, *P. odoratissimum* (L) L'Hér. ex Ait., *P. pakalunaceum*, *P. peltatum*, L'Hér. ex Ait., *P. sanquinem* Wendl., *P. tabulare* L'Hér., *P. tetragonium* (L.F.) L'Hér. ex. Ait., *P. tomentosum* Jacq., *P. tricolor* Curtis, *P. viscosissimum* Sweet, *P. vitifolium* (L.) L'Hér., and *P. xerophyton* Schlechter. Thirteen cultivars were tested with three California rust isolates (one from San Francisco, one from Piedmont, and one from Berkeley) in a factorial experiment. The isolate from San Francisco was the least virulent and the Berkeley isolate was the most virulent ($P = 0.05$) on the cultivars tested. Cultivars Penny Irene and Appleblossom were the most susceptible, whereas Springtime Irene, Dark Red Irene, and Irene were the most resis-

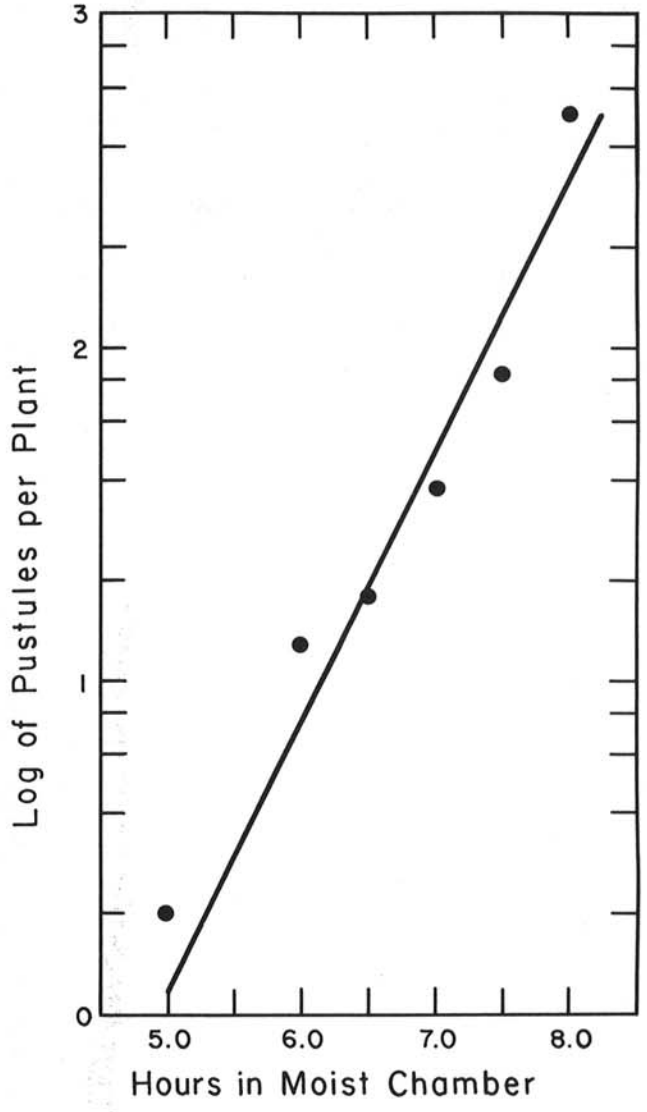

Fig. 6. Relationship of number of pustules of *Puccinia pelargonii-zonalis* to the time leaves remained moist following inoculation. The equation expressing this relationship is $Y = .82 \times - 4.06$. Correlation coefficient of linearity was .95 ($P = 0.01$).

tant ($P = 0.05$) (Table 1). Plants of 10 cultivars were tested with the Berkeley isolate only. Of these, Blaze and Snowmass were significantly more susceptible than the others (Table 2).

Spore viability. Germination of spores stored on dry leaves dropped from 100% to 0.8% durng a 12-wk period. The data were arcsin-transformed and plotted, yielding a percent germination curve with the equation, $Y_Q = 76.32 + 4.36\ X - 0.90\ X^2$ in which Y = percent germination and X = weeks since collection (Fig. 8). When spore infectivity was compared with time on plants, the numbers of pustules per two plants were 167 for plants moistened immediately after inoculation, 358 for plants moistened 2 wk after inoculation, and 0 for plants moistened 4 wk after inoculation. After 4 wk, the leaves originally inoculated had senesced, thus spores remained viable longer than the plant leaves.

Control. All fungicides gave significant ($P = 0.05$) control in both greenhouse and field experiments. In the greenhouse, where fungicides were applied prior to inoculation, triforine gave significantly less control than the others. The mean pustules per plant (based on two evaluations of 30 plants) per fungicide treatment were as follows: oxycarboxin 0.92a, triadimefon, 0.25a, mancozeb

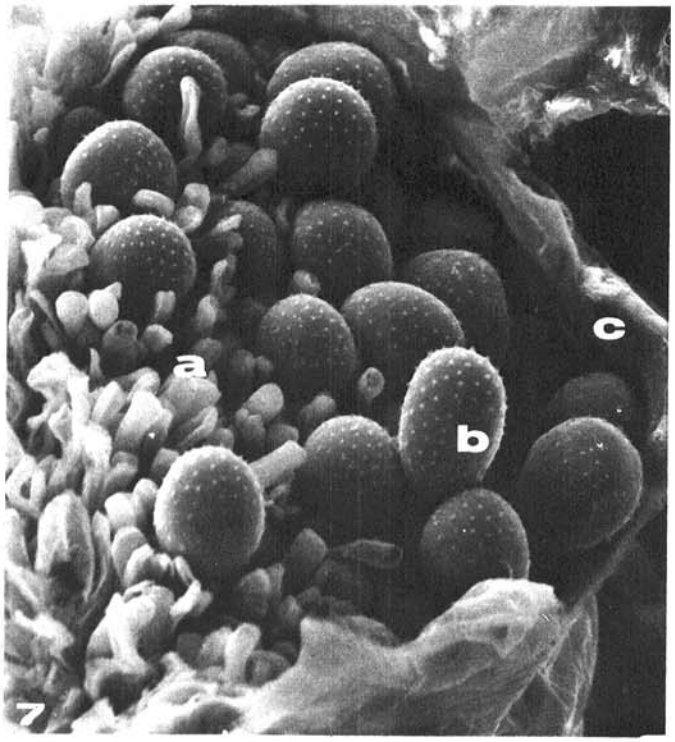

Fig. 7. Scanning electronmicrograph of *Puccinia pelargonii-zonalis* showing sporogenous hyphae (a) and spores (b) below epidermis. ×1000.

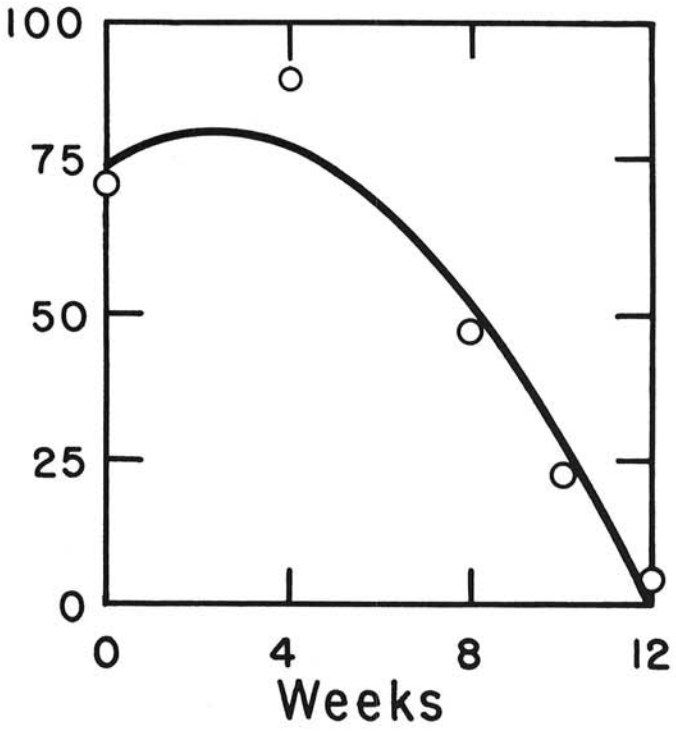

Fig. 8. The relationship between the germination percentage of urediniospores of *Puccinia pelargonii-zonalis* and the length of time stored on dry leaves.

TABLE 1. Susceptibility of *Pelargonium hortorum* cultivars to three isolates of *Puccinia pelargonii-zonalis*

Cultivar	Mean[u] pustules per leaf[v] per rust isolate			Mean[w] pustules per leaf per cultivar per all rust isolates
	San Francisco	Piedmont	Berkeley	
Springtime Irene	12.8 a[y]	25.8 abc[y]	23.2 ab[y]	27.1 A[z]
Dark Red Irene	42.4 abcdef	56.2 abcdefgh	40.8 abcde	46.4 A
Irene	97.8 efghij	24.0 ab	36.0 abcde	52.6 A
Improved Blaze	45.0 abcdefg	65.8 abcdefghi	54.0 abcdefgh	54.9 AB
Salmon Irene	31.0 abcde	50.6 abcdefg	92.2 defg	58.0 AB
Cardinal	11.0 a	27.9 abc	144.2 jk	61.0 AB
Genie	84.2 bcdefghij	30.0 abcd	74.4 abcdefghi	62.8 ABC
Snowball	88.8 cdefghij	50.8 abcdefg	72.9 bcdefghi	70.8 ABC
Better Times	74.0 abcdefghi	60.7 abcdefgh	85.4 bcdefghij	73.4 ABC
Springfield Violet	105.6 fghij	82.0 bcdefghi	45.2 abcdef	77.6 ABC
Fiat Enchantress	108.1 ghij	81.8 bcdefghi	68.2 abcdefghi	86.0 ABC
Appleblossom	56.2 abcdefgh	168.2 kl	111.9 hijk	115.2 BC
Penny Irene	29.6 abcd	127.6 ijk	209.6 l	122.2 C
Mean[x] number of pustules per leaf per isolate per all cultivars	62.3	65.5	81.8	

[u]Mean based on data from two plants.
[v]The total number of pustules per plant were counted and divided by the number of infected leaves per plant.
[w]Mean based on data from six plants.
[x]Mean based on data from 26 plants.
[y]Values in these columns followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.
[z]Values in this column followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

0.04a, and triforine 3.56b (numbers followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$). In the field where fungicides were applied to infected plants, mancozeb gave significantly less control than the others. The mean pustules per plant (based on 27 plants) per fungicide treatment were as follows: oxycarboxin 10.2a, triadimefon 6.2a, mancozeb 23.0b, and triforine 8.0a (numbers followed by the same letter are not significantly different according to Duncan's multiple range test, P = 0.05). No phytotoxicity was observed; plant heights did not differ significantly in the greenhouse experiment.

DISCUSSION

The urediniospores of *P. pelargonii-zonalis* germinated best at 16 C. This observation was in agreement with that reported by Grouet (4). The spores required liquid water to initiate germination. In the presence of liquid water at 16 C, 3 hr was sufficient time for germination. After 6 hr, branching and wall thickening occurred; therefore, it was hypothesized that penetration might occur then. The SEM was excellent for studying penetration of this pathogen. Spores on leaves germinated, formed appressoria over stomata, and penetrated the host. Appressoria were formed only over stomata. However, penetration of infected tissue surrounding established pustules did not require appressorium formation. Liquid water was required for a minimum of 5–6 hr before infection occurred. This time period probably was required for spores to germinate, reach stomata, and penetrate the host. Once established in the host tissue, the fungus was protected from drying. Thus, it was concluded that at least 5–6 hr of favorable conditions were required for successful penetration. If favorable conditions persisted, the number of spore germ tubes able to reach and penetrate stomata increased rapidly. Thus, an effective method for cultural control of *P. pelargonii-zonalis* would be to minimize the duration of foliage wetting.

Five of the *Pelargonium* spp. tested were susceptible to geranium rust; however, *P. hortorum* was the most susceptible. The other susceptible species produced fewer, smaller, pustules which appeared more slowly. It has been reported that *P. peltatum* exhibits flecking in response to the fungus (10). *Pelargonium peltatum*, therefore, was retested. Although the fungus penetrated the stomata, no flecking (small, sunken, brown areas) were observed. Some raised areas were observed on the abaxial surfaces, however, These areas were hand sectioned, cleared and stained, but no fungal tissue was detected. These raised areas also had been observed on uninoculated plants; thus, the condition did not appear to be associated with the rust infection. Some susceptibility of various *P. peltatum* cultivars was expected, since most modern cultivars are hybrids between *P. peltatum* and *P. hortorum* (8). However, lack of penetration did not account for the immunity of this species.

Spores were produced continually in fresh pustules; therefore, when spores were collected from fresh pustules, immature spores (light-colored and relatively small) always were present. Of the spores placed on water agar when the leaves were first collected, some appeared to be immature. After 4 wk, all plated spores appeared to be mature. This observation may explain the increase in percent germination noted after 4 wk. In a greenhouse situation, with rapid plant growth, leaves mature and senesce in less than 12 wk; therefore a spore landing on a leaf probably would remain viable longer than the leaf.

All fungicides tested gave good control. In the field, where infection already was established, mancozeb gave less control than the others, possibly due to its lack of systemic activity. When used as a protectant (prior to infection), in greenhouse tests, mancozeb gave excellent control.

TABLE 2. Susceptibility of *Pelargonium hortorum* cultivars to *Puccinia pelargonii-zonalis*

Cultivar	Mean[a] pustules per Leaf[b]
Crimson Fire	14.9 A
Yours Truly	25.0 A
Sincerity	25.8 A
Salmon Irene	30.3 A
Improved Ricard	49.6 A
Orange Glow	50.5 A
Quest	53.1 A
Pink Camellia	67.2 A
Snowmass	168.5 B
Blaze	196.3 B

[a]Mean is based on data from two plants.
[b]Total pustules were counted and divided by the number of infected leaves per plant, two plants per treatment.
[c]Numbers followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

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