Wound-Healing Process in Geranium Cuttings in Relationship to Basal Stem Rot Caused by *Pythium ultimum*

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ABSTRACT

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Cuttings of *Pelargonium* × *hortorum* Bailey cultivars Blaze, Yours Truly, and Salmon Irene were inoculated with an isolate of *Pythium ultimum* at various stages in the wound-healing process. Disease severity and incidence decreased as the period of time between wounding and inoculation increased, particularly with Yours Truly. Extensive basal stem rot occurred on all freshly broken cuttings but was restricted on cuttings that had been healing 1 day before inoculation. Basal stem rot rarely occurred on cuttings that had been healing for 2 days or longer before inoculation. Disease severity decreased in all three cultivars when Rootone F or Hormodin No. 1 was used before inoculation. Severity ratings generally increased as the time of air-drying before inoculation increased.

Geraniums are largely propagated by cuttings even though new seed strains are available. Unrooted, callused, or rooted cuttings can be purchased commercially and represent various stages in the wound-healing process.

Blackleg (3), a basal stem rot disease caused by several *Pythium* spp., is a problem in geranium production only when the pathogen is introduced by poor sanitation practices into sterile soil stockpiles, plant beds, and potted plants. Blackleg is a continual problem for Illinois growers, particularly those who root cuttings from their own stock rather than purchase rooted cuttings from specialist propagators.

Losses in commercial geranium production have been reported to be due to infection by *P. debaryanum* Hesse (2,3), *P. splendens* Braun (2,14), *P. vexans* de Bary (1), *P. ultimum* Trow (6), and *P. mamillatum* Meurs (6). Miller and Sauve (14) determined the relative susceptibility of geraniums to 17 species of *Pythium* and found that 10 caused varying degrees of basal stem rot. Their work showed the lack of specificity of many species of *Pythium* to geraniums.

In the horticultural literature, claims are made that callused geranium cuttings are less susceptible than fresh cuttings to diseases such as blackleg (12) and that drying the basal end of a geranium cutting for several hours before inserting it into the rooting medium will help prevent disease (7,12). This investigation was initiated to study the disease susceptibility of geranium cuttings at various stages in the wound-healing process, using *P. ultimum* as the pathogen. The effects of various cultural practices, such as the use of rooting hormones and the practice of air-drying cuttings, on the wound-healing process and disease susceptibility were also determined.

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MATERIALS AND METHODS

The geranium cultivars Blaze, Yours Truly, and Salmon Irene (fast-, intermediate-, and slow-rooting, respectively) were used. Stock plants from which cuttings were taken were originally derived from culture-virus-indexed cuttings supplied by Oglevee Associates, Inc., Connellsville, PA 15425. Stock plants were not allowed to flower; all buds were regularly removed to promote vegetative growth. Greenhouse temperatures were maintained at about 21–23 C during the day and 16–18 C during the night.

Terminal cuttings 8–15 cm long with at least three leaves were taken from stock plant stems just above a node, and the broken end was cut with a sterile razor blade. Cuttings were then inserted directly into raised benches containing a steam-pasteurized soil:sand:peat (1:1:1, v/v) mixture. (A preliminary study comparing five medium types showed no effect of media on disease incidence and severity.) After the prescribed time period, the cuttings were transferred from the benches to Com-Paks (Florist Products, Inc., Des Plaines, IL 60018) containing 12 individual compartments measuring 4.5 × 4.5 × 6.0 cm deep that were filled with a steam-pasteurized soil:sand:peat:perlite (1:1:1:1, v/v) mixture infested with *P. ultimum*. Each compartment held 50 g of the rooting medium. Cuttings in either the benches or the Com-Paks were held under intermittent mist for 7.5 sec every 5 min for 9–12 daylight hours.

Eleven *Pythium* isolates obtained from naturally infected geranium cuttings and from other hosts were tested on the three geranium cultivars. *P. ultimum*, originally isolated from snap bean in New York, was the most pathogenic strain or isolate and was selected for use in all experiments. Stock cultures of *P. ultimum* were maintained on a modified V-8 juice agar (10), transferred weekly, and incubated at 21 C.

Inoculum was produced by washing 7- to 10-day-old cultures on V-8 juice agar with 10 ml of sterile distilled water and fragmenting the suspension in a Waring Blendor for 10 sec. The inoculum mixture, consisting of oospores, sporangia, and mycelial fragments, was added to the rooting medium in the Com-Paks at the rate of 200 propagules per gram of soil by drenching each compartment with 10 ml of the inoculum mixture. Com-Paks filled with the infested rooting medium were irrigated and maintained at ambient temperatures in the greenhouse 24 hr before the cuttings were inserted. Blackleg symptoms occurred over a range of 18–28 C and were equally severe at all temperatures, since infected cuttings failed to root.

Because no standard rating scale for stem rot of cuttings exists and because the rating scales used for foliage disease, such as the Horsfall-Barratt scale (11), are not appropriate, a new rating system was devised. The rating scale for disease severity ranged from 0 to 5, with 0 = no symptoms; 1 = only the surface cells of the basal end are infected and black, rot does not progress up the stem; 2 = basal end is infected and rot progresses up the stem (lesion length > 0 and < 15 mm); 3 = same as 2 but lesion length is > 15 and < 30 mm; 4 = rot progresses up the stem but has not reached meristem (lesion length > 30 mm); and 5 = plant is dead, entire length of stem is infected, and rot has progressed up the petioles and into the leaves. Cuttings rated 0 always rooted; 1, generally rooted; 2, some rooted; 3 and 4, generally did not root; and 5, never rooted. Disease incidence was also recorded.

The increase in basal stem diameter was measured and expressed as the difference between the diameter at the base of the cutting and that 5 mm above the base. The formation of adventitious roots was recorded as the percentage of cuttings in some stage of rooting: 1) adventitious roots breaking through the basal end of the cutting; 2) partially rooted (roots have emerged, but not from around the entire circumference of the basal end, and have begun to elongate); and 3) fully rooted (roots have emerged from around the entire circumference of the basal end and are dense and elongated).

Freshly broken (untreated) cuttings, cuttings that had been healing for 1, 2, or 3 days in the rooting medium, callused cuttings (7–14 days), and fully rooted Yours Truly cuttings (21 days) were inserted into the rooting medium infested with *P. ultimum* in the Com-Paks. The fully rooted cuttings were planted in 15-cm pots and were not placed under mist. Cuttings in uninfested rooting medium served as controls. Four replications with 24 cuttings each were carried out 0, 1, 2, 3, 7, and 14 days after wounding. Two replications with 12 cuttings each were made on fully rooted cuttings. Two replications with 24 cuttings each were made 0, 1, 2, and 3 days after wounding using Blaze and Salmon Irene. Cuttings were rated for basal stem rot severity after 10 days except for the fully rooted cuttings, which were evaluated after 3 mo.

Cuttings were treated with a combination of naphthylacetamide and thiram (Rootone F) or indolebutyric acid (Hormodin No. 1) before being inserted into infested soil. The basal end of the cutting was dipped into the hormone powder, and the excess powder was removed by tapping. As controls, untreated cuttings and treated cuttings were placed in uninfested soil and untreated cuttings were placed in infested soil. Two replications with 24 cuttings each per rooting hormone were made for Yours Truly, and 12 cuttings per rooting hormone per replication were made for Blaze and Salmon Irene. Disease development was evaluated after 14 days.

Cuttings were allowed to air-dry for 0, 2, 4, 6, 8, and 12 hr at ambient greenhouse temperatures but not in direct sunlight before they were inserted into infested soil. Controls included cuttings subjected to 12 hr of air-drying and untreated cuttings, both inserted into uninfested soil. For each time period, two replications with 24 cuttings were made for Yours Truly. One replication with 12 cuttings per time period was made for Blaze and Salmon Irene. Disease development was evaluated after 10 days.

RESULTS

Symptoms of blackleg developed on all freshly broken, untreated cuttings (Table 1). In the surviving cuttings, the basal portion of the stem always increased in diameter with the formation of adventitious roots. Disease incidence and severity decreased as the time between wounding and inoculation increased. Disease incidence in Yours Truly cuttings that had been healing for 1 day was 99% compared with 16% in cuttings that had been healing for 2 days before inoculation. At the end of the 10-day incubation period, there was an inverse correlation between disease severity ratings and 1) the percentage of cuttings with an increase in basal stem diameter and 2) the percentage of cuttings in some stage of rooting. Disease severity in Yours Truly, the intermediate-rooting cultivar, decreased rapidly over time for healing, and most plants were resistant within 2 days. Disease was severe in Salmon Irene, the slow-rooting cultivar, even after 3 days of healing before inoculation.

In addition to the cutting rot, roots that formed in infested media occasionally showed necrosis of the root tip. *P. ultimum* was reisolated from all cuttings with basal stem or root tip symptoms and occasionally from symptomless cuttings.

Inoculation of fully rooted cuttings (21 days) resulted in no basal stem rot or root rot, and these plants were deemed commercially acceptable. *P. ultimum*, however, was recovered from symptomless root segments of inoculated plants.

Disease severity decreased in the three cultivars when either Rootone F or Hormodin No. 1 was used before

Table 1. Effect of length of time between wounding and inoculation on severity of blackleg caused by *Pythium ultimum* in geranium cuttings

Time (days)	Disease rating[a] Severity (%) YT[d]	B	SI	Incidence (%) YT	B	SI	Stems with diameter increase[b] (%) YT	B	SI	Cuttings with roots[c] (%) YT	B	SI
0	3.6	3.5	5.0	100	100	100	0	0	0	0	0	0
1	2.1	2.0	3.8	99	71	100	38	29	0	12	4	0
2	0.3	1.5	3.4	16	67	100	90	44	0	41	8	0
3	0.2	1.1	1.7	9	48	93	94	48	23	45	8	0
7	0.1	...[e]	...	4	...	...	95	...	...	61	...	...
14	0.1	...	...	0	...	...	100	...	...	71	...	...
0 (uninoculated)	0.0	0.0	0.0	0	0.0	0.0	100	100	83	29	38	4
FLSD[f] ($P = 0.05$)	0.7	1.2	1.3	10	13	5	27	12	20	...	...	...

[a] Severity rating based on a scale of 0–5, with 0 = no symptoms, 1 = only surface cells of basal end infected, 2 = lesion length > 0 and < 15 mm, 3 = lesion length > 15 and < 30 mm, 4 = lesion length > 30 mm, and 5 = dead plant.
[b] Based on percentage of cuttings with an increase in basal stem diameter.
[c] Based on percentage of cuttings in some stage of root development.
[d] YT = Yours Truly, B = Blaze, SI = Salmon Irene.
[e] Not determined.
[f] Mean separation in columns by Fisher's least significant difference test (FLSD).

Table 2. Effects of rooting hormones on severity of blackleg caused by *Pythium ultimum* and on subsequent root development in cuttings of three geranium cultivars after 14 days

Treatment	Disease rating[a] Severity (%) YT[d]	B	SI	Incidence (%) YT	B	SI	Stems with diameter increase[b] (%) YT	B	SI	Cuttings with roots[c] (%) YT	B	SI
Inoculated												
Rootone F	1.2	1.2	1.9	100	100	83	90	83	17	81	96	58
Hormodin No. 1	2.3	2.6	3.1	94	88	95	33	42	11	58	75	37
Freshly cut	3.0	3.5	3.6	100	100	100	0	0	0	0	0	0
Uninoculated												
Rootone F	0.0	0.0	0.0	0	0	0	100	100	100	100	100	96
Hormodin No. 1	0.0	0.0	0.0	0	0	0	100	100	100	100	100	83
Freshly cut	0.0	0.0	0.0	0	0	0	100	100	100	74	100	53
FLSD[e] ($P = 0.05$)	1.7	1.6	1.1	NS	NS	NS	34	31	20	15	6	33

[a] Severity rating based on a scale of 0–5, with 0 = no symptoms, 1 = only surface cells of basal end infected, 2 = lesion length > 0 and < 15 mm, 3 = lesion length > 15 and < 30 mm, 4 = lesion length > 30 mm, and 5 = dead plant.
[b] Based on percentage of cuttings with an increase in basal stem diameter.
[c] Based on percentage of cuttings in some stage of root development.
[d] YT = Yours Truly, B = Blaze, SI = Salmon Irene.
[e] Mean separation in columns by Fisher's least significant difference test (FLSD).

inoculation (Table 2). Rootone F, a rooting hormone combined with a fungicide, was more effective in reducing disease severity than was Hormodin No. 1, a rooting hormone without a fungicide. The hormone-treated inoculated cuttings of all three cultivars showed adventitious root formation, whereas the untreated inoculated controls were infected to the point where the rooting process was inhibited.

The disease interaction of these three cultivars with the rooting hormones was distinct. In Salmon Irene, cuttings treated with Rootone F rotted for 1–3 mm at the basal end and then rooted above the rot. The cuttings increased in stem diameter above the rotted portion. In Blaze and Yours Truly, the basal end of the cutting was black, but rot did not progress up the side of the stem. Callus proliferations were not observed. Cuttings of all three cultivars treated with Hormodin No. 1 before inoculation generally rotted between 6 and 20 mm and then rooted above the rotted portion.

Disease severity ratings generally increased as the time of air-drying before inoculation increased. Some of the cuttings showed an increase in basal stem diameter, but roots did not form after any inoculated, air-drying treatment. All air-dried inoculated cuttings became infected and showed extensive basal stem rot.

Macroscopic observations of air-dried cuttings before inoculation revealed that the cortex and vascular tissues collapsed to form a ring around the pith. The pith cells also collapsed, and the entire basal end appeared slightly shriveled. The leaves of the cuttings lost turgidity. When placed in the rooting medium after being under mist for several hours, the cuttings regained turgidity, the basal end lost its shriveled appearance, and the pith cells bulged as they took up water.

DISCUSSION

As time for wound healing increased, disease susceptibility decreased, as evidenced by a decrease in disease incidence and severity. Yours Truly had the greatest response. The differences among the geranium cultivars was not surprising, since the rate of wound healing has also been reported to vary in potato cultivars (16).

The relationship between wound healing and disease susceptibility to both fungi and bacteria has been shown in tuber crops, such as potato (5), gladiolus corms (13), and woody species (8). These researchers also observed a decrease in disease susceptibility as time after wounding increased. The observed disease resistance has been attributed to events in the wound-healing process, namely, suberization of the cells at the wounded surface and formation of a suberized wound periderm (17).

The observations in this study led us to investigate the histochemistry of the wound-healing process in geranium cuttings (4). We concluded that formation of suberinlike deposits on the cell walls and in the intercellular spaces and subsequent suberization of the wound periderm are the processes that bring about a decrease in blackleg incidence and severity in geranium cuttings. Suberized callus tissue, if present, may provide added protection, since *Pythium* has been reported to be unable to penetrate suberized tissues (1,4,9); not all investigators agree (14), however. This fact indicates that differences in disease susceptibility exist in specific geranium cultivar-pathogen interactions.

Rooting hormones are frequently used to facilitate rooting. Rooting hormones stimulated the wound-healing process despite the presence of the pathogen (Table 2). Rosenstock and Kahl (15) also reported that hormones enhanced the wound-healing rate in various plant tissues.

Air-drying of cuttings did not result in any decrease in disease severity, since suberized deposits failed to occur on the cell walls or in the intercellular spaces of air-dried cuttings (4). Wigginton (16) also reported the inhibition of suberization during wound healing when potato tissues were allowed to dry. Therefore, the recommendation to air-dry cuttings before inserting them into the rooting medium as a means of disease prevention (7,12) seems impractical.

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