Resistance

Effects of Growing Conditions on Wound Repair and Disease Resistance in *Pachysandra terminalis*

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ABSTRACT

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Wounds on stems of *Pachysandra terminalis* differed in susceptibility to subsequent colonization by *Volutella pachysandricola*, depending on the time of year that the wounds were inflicted and the age of the wounds when inoculated. When plants growing out of doors were wounded and inoculated in April or May, some wounds up to 11 days old were susceptible. However, plants growing in the same stand and wounded later in the growing season (June, July, September) were significantly more resistant. All but one of 210 wounds more than 4 days old were immune. Wounds on manually defoliated plants were not significantly more susceptible than nondefoliated checks. However, plants exposed to deicing salt in soil and plants growing in full sun were significantly more susceptible to colonization by *V. pachysandricola* than their counterparts growing under more favorable conditions. Histological comparisons of wound repair processes of plants growing under various conditions suggested that prolonged susceptibility was not related to the rate of lignification of parenchyma subjacent to the wound surface but may have been related to deposition of intracellular suberin in those same cells.

Additional keywords: facultative parasite, necrophylactic periderm, primary lignosuberized tissue, resistance.

When live bark of woody plants and phloem and/or cortical parenchyma in stems of non-woody dicots are injured, live cells subjacent to the wounded tissue respond with a series of biochemical and morphological changes leading to development of barrier zones (6,10,17) and, eventually, necrophylactic periderms (sensu Mullick and Jensen, 18). In previous time-course studies with stems of Japanese spurge (*Pachysandra terminalis* Sieb. & Zucc.), complete formation of primary lignosuberized tissue (PLST) around a wound was correlated with complete resistance of the wound to colonization by the facultative parasite, *Volutella pachysandricola* B. O. Dodge (14).

The rate of wound repair in bark or cortex has been measured directly via microscopic examination of cells and extraction of

lignin with thioglycolic acid, and indirectly, via permeability of the barrier zones to solutions or pathogens (9,11,13,19–22,26). In all cases, plants growing in unfavorable environmental conditions have been shown to repair wounds slower and/or be more susceptible to wound-colonizing pathogens. The objective of research presented here was to determine effects of four factors—time of year of wounding, defoliation, deicing salt in soil, and light intensity—on the rate of PLST formation in *P. terminalis* and susceptibility to *V. pachysandricola*.

MATERIALS AND METHODS

Time of year. A stand of wild *P. terminalis* growing on the floor of an eastern hemlock (*Tsuga canadensiis* (L.) Carr.)/American beech (*Fagus sylvatica* L.)/sugar maple (*Acer saccharum* Marsh) forest on the Cornell University campus, Ithaca, NY, was chosen for these experiments. On 25 April 1984, the youngest terminal shoot on each of 10 plants was wounded by gouging with a sharpened 14-gauge canula to yield a wound 1 cm long × 1–2 mm deep with the long axis of the wound parallel to the long axis of the vascular cylinder. Care was taken to ensure that wounds were limited to cortical tissue and that the vascular cylinder was not injured. At 2- to 3-day intervals for the following 11 days, 10 previously unwounded plants were wounded in a similar fashion. On day 11, stem segments containing the wounded portion of each shoot plus 0.5–1.0 cm of healthy tissue at each end of the wound were cut from the stems. The ends of the segments were immediately dipped in molten paraffin to retard drying, and the segments were suspended on modeling clay or wood supports in petri dishes containing moist filter paper. The wounds were filled with 0.02 ml of a suspension containing approximately 10^7 conidia of *V. pachysandricola* per milliliter, and the plates were covered and incubated at room temperature (20–24 C). Within 7–10 days, wounds colonized by *V. pachysandricola*, as evidenced by visible discoloration of stem tissue and development of sporodochia of the pathogen, were counted. Periodic isolations from the discolored tissue were also performed to ensure that symptoms were, in fact, due to *V. pachysandricola*.

Similar time-course studies were begun on 23 May, 6 June, 20 July, and 14 September 1984 and 22 April, 3 June, 17 June, 1 July, and 15 July 1985. For experiments conducted in June and July of both years, 12 stems were wounded on each date. Ten were harvested and inoculated as described above and the other two segments were fixed in 5% v/v gluteraldehyde in 0.5 M sodium phosphate buffer (pH 6.8). These segments were held

at 5 C for up to 6 mo until they were dehydrated in *n*-butyl alcohol, embedded in Fisher TissuePrep and cut to 10.0–15.0-μm thick sections (3) and examined with fluorescence microscopy (14) for amount of PLST formation in each age class of wound. One transverse section near the center of each wound was chosen, intact parenchyma in a layer subjacent to the wound were counted, and percentages of cells with lignin and/or suberin were determined.

Infection data were analyzed via two-way analysis of variance for the randomized complete block design with treatments being wound age and month of wounding (24).

Defoliation. Pachysandra cuttings randomly taken from a heterogeneous population of mother plants and placed in vermiculite under mist 1 mo earlier were transplanted from a propagation flat to individual 10-cm-diameter pots containing Cornell Peatlite, a soilless potting mix. The plants were grown in a greenhouse (22–25 C, 16-hr photoperiod with fluorescent lights at photon flux density of 300 μmol/sec/m^2 for an additional 2 mo before use in these experiments. On 6 June 1985, one group of plants was completely defoliated by cutting leaf laminae but not petioles with scissors. In a second group of plants, every third lamina on each plant was removed; those in a third group had two of three removed; and those in a fourth group were left intact. Two days later, the process of wounding 12 plants at 2- to 3-day intervals as described above was begun. After 11 days, the wounded portions were harvested and inoculated or fixed in gluteraldehyde for later preparation for microscopic examination. The experiment was repeated in July 1985 and April 1986.

Deicing salt. Pachysandra cuttings rooted 1 mo earlier were transplanted from a propagation flat to individual 10-cm-diameter pots containing Cornell Peatlite and grown in a greenhouse as described above for an additional month. Then, they were divided into four groups of 50 plants. Pots in one group were watered once per week for 5 wk with 50 ml of water containing 3,000 ppm sodium chloride (WinterMelt Rock Salt, Cargill, Inc., Minneapolis, MN) and twice per week with tap water. The second and third groups were watered on the same schedule with aqueous solutions of 9,000 ppm and 12,000 ppm deicing salt, respectively. A fourth group received only tap water throughout. On 6 March 1985, after the 4-wk treatment period, the plants were wounded, harvested, and inoculated as above. As shoots were harvested, xylem pressure potentials (ψ_p) of 10 shoots from each treatment were determined with a pressure bomb (Model 600, PMS Instrument Co., Corvallis, OR). Also, foliage from each treatment in that experiment was analyzed for concentrations of selected

TABLE 1. Relationship of time of year of wounding and inoculation to incidence of stem cankers on pachysandra caused by *Volutella pachsandricola*

Wound age (days)	Number of infections/10 inoculations									
	1984					1985				
	April	May	June	July	Sept.	April	May	June	July	Aug.
2	8	4	8	2	0	3	2	2	3	6
4	7	5	1	1	0	4	0	4	0	1
7	3	0	0	0	0	3	0	1	0	1
9	4	3	0	0	0	1	0	0	0	0
11	2	0	0	0	0	0	0	0	0	0
Mean	4.8	2.4	1.8	0.6	0.0	2.2	0.4	1.4	0.6	1.4

LSD (0.10) = 2.8

TABLE 2. Relationship of wound age to formation of primary lignosuberized tissue in *Pachysandra terminalis*

Wound age (days)	Percentage of cells with lignin or suberin							
	June 1984		June 1985		July 1984		July 1985	
	Lignin	Suberin	Lignin	Suberin	Lignin	Suberin	Lignin	Suberin
2	20	0	100	0	25	0	10	0
4	90	0	100	0	100	0	30	10
7	100	80	100	100	100	100	80	60
9	100	100	100	100	100	100	100	70
11	100	100	100	100	100	100	100	100

elements with an inductively coupled argon plasma atomic emission spectrometer (Model ICAP-61, Jarrell-Ash Co., Franklin, MA). The experiment was repeated with some variation in procedure twice thereafter.

On 6 June, four groups of 720 plants were selected and wounded such that for each wound date and salt concentration, three groups of 12 plants were wounded. Ten of the 12 in each group were eventually inoculated and the other two were fixed in glutaraldehyde for subsequent microscopic examination. A third experiment, similar to the first but without ψ_p measurements and foliar nutrient analyses was conducted in December 1985.

Light intensity. To determine effects of light intensity on wound repair and disease resistance, 3-mo-old pachysandras grown from cuttings were planted in late May and early June 1985 in four 1.25- × 6.0-m plots on the Cornell University campus in Ithaca, NY. The plots were situated in an open area such that there were no trees or buildings in the immediate vicinity to shade the plants except in the very early morning and late evening when all plots were shaded equally. Three of the plots were shaded by black polypropylene mesh shade cloth suspended on wood frames 40 cm above the plots. One plot was shaded by cloth allowing passage of 8% of incident sunlight, a second plot received 37% of incident sunlight, and a third received 70%. The fourth plot had no cover. Natural rainfall was the only source of water for the plants. Weeds within plots were controlled with a preplant application of Eptam 10G (Stauffer Chemical Co., Mountain View, CA) and manual cultivation thereafter. Weeds between plots were controlled with biannual applications of glyphosate (Monsanto Chemical Co., St. Louis, MO) and occasional manual cultivation.

For the first experiment in this series, beginning on 20 June 1986, 15 plants in each plot were randomly selected and wounded as described above. Wounding continued, each time on previously unwounded plants, for 7 days at 12-hr intervals. When stem segments were harvested at the end of the wounding period, 10 were inoculated and the other five were fixed for later microscopic examination. Subsequent experiments with similar protocols were started on 17 July, 18 August, and 6 October 1986.

RESULTS

Time of year. For plants wounded and inoculated in April and May, as wound age increased, the number of wounds colonized by *V. pachysandricola* decreased at a rate similar to

that observed previously (14). However, June, July, August, and September wounds became immune to colonization much more quickly after they were inflicted. Most 2-day-old wounds were resistant, and only one of a total of 210 wounds greater than 4 days old was colonized (Table 1).

Counts of cells subjacent to the wound indicated that lignin deposition, the first discernible wound repair response, began within 2 days of wounding and followed a similar course in each experiment. A continuous band of lignified cells usually was evident by 7 days postwounding (Table 2). Deposition of intracellular suberin lagged behind lignification. Suberization was usually not apparent at 4 days postwounding but was complete as early as 7 days postwounding.

Defoliation. Overall amounts of infection in each of three defoliation experiments were much lower than in other experiments. Two-day-old wounds were significantly ($P < 0.05$) more susceptible than older ones, but in none of the experiments were there significant differences in amounts of infection related to amounts of defoliation. Results of a typical experiment are shown in Table 3. Also, there was no measurable effect of defoliation on development of PLST.

Deicing salt. Pachysandras watered with water containing 0, 3,000, or 9,000 ppm of deicing salt showed no symptoms of salt injury throughout each treatment period. However, most of those watered with 12,000 ppm of deicing salt turned brown, wilted, and died in the first 2 wk of each treatment period. There were too few survivors of the 12,000-ppm salt treatment in each experiment to be used for inoculation tests.

Pressure bomb readings indicated that one effect of the deicing salt on the plants was to lower ψ_p. Plants watered with 3,000 ppm of deicing salt, though showing no evidence of salt toxicity, had ψ_p values approximately 2 times more negative than those watered only with tap water. Of those watered with 9,000 ppm of deicing salt, average values of ψ_p were almost 3 times more negative than those of plants watered only with tap water (Table 4).

Foliar nutrient analyses confirmed that despite lack of obvious symptoms, ionization products of the salt were taken up by the plants. Concentrations of Na^+ increased as exposure to salt increased, but concentrations of all major elements remained relatively constant and well within acceptable limits for normal plant growth (Table 4).

In each experiment, wounds in stems of pachysandra watered only with tap water were highly resistant to infection by *V. pachysandricola*. Infection in 2-day-old wounds averaged fewer than three of 10 wounds. However, plants periodically watered with water containing 3,000 or 9,000 ppm of deicing salt in addition to tap water were significantly ($P > 0.05$) more susceptible. Results of a typical experiment are shown in Table 5.

Microscopic evaluation of PLST development yielded results similar to those observed for the defoliation experiments. Lignification, as evidenced by autofluorescence of parenchyma cells subjacent to the wound, seemed to proceed at the same rate in all treatments. However, suberization lagged behind as concentrations of deicing salt increased (Table 5).

Light intensity. Pachysandras growing in the shadiest plot had dark green, turgid foliage. By the end of the first growing season, they had grown enough to fill in the spaces between them, resulting in a continuous, dense bed of plants. In contrast, plants in the plot with full exposure to sunlight were extremely chlorotic, and

TABLE 3. Relationship of various amounts of defoliation in April on infection of wounded pachysandra stems by *Volutella pachysandricola*

| Wound age (days) | Number of infections/10 inoculations | | | | |
| | Percent defoliation | | | | |
	0	33	67	100	Mean
2	3.0	3.0	2.0	4.0	3.0
4	0.0	0.0	2.0	3.0	1.25
7	2.0	0.0	1.0	2.0	1.25
9	0.0	0.0	0.0	1.0	0.25
11	0.0	0.0	1.0	0.0	0.25
Mean	1.0[a]	0.4	0.4	1.6	

[a] LSD (0.05) = 1.25.

TABLE 4. Effects of deicing salt in soil on xylem pressure potential and concentrations of selected elements in foliage

| NaCl concentration (ppm) | ψ^p (mPa) | Concentration of elements[a] | | | | | | |
		Na	N	P	K	Mg	Mn	Fe
0	−0.324[b]	138	1.56	0.356	2.30	0.291	44.2	57.3
3,000	−0.589	1,327	1.94	0.365	2.42	0.317	95.9	66.0
9,000	−0.922	4,349	1.43	0.337	2.66	0.390	64.8	64.5
12,000	−0.790	9,209	2.00	0.412	3.00	0.385	152.0	69.4

[a] Concentrations of N are percent dry weight. Concentrations of all other elements are in ppm.
[b] Values are means of 20 stems for each treatment.

leaves on some plants drooped on hot, midsummer days. Reduced growth of plants in full sun left the stand with a generally ragged appearance. Plots between the two extremes showed varying degrees of chlorosis positively related to the amount of sunlight they received.

Attempts to assay effects of light intensity on susceptibility of wounded pachysandra stems to *V. pachysandricola* were stymied during much of the growing season because so few of the youngest wounds were colonized by the pathogen. However, when plants were wounded in June, a gradual decline in susceptibility of wounds, similar to that seen in other experiments, was observed on plants in full sun (Table 6). Plants growing in the shadiest plot were most resistant to colonization. Similar,

but not statistically significant trends, were observed in other months.

PLST development was erratic and unpredictable during the summer months and appeared to be unrelated to shading of plants except in June where suberization was significantly less under higher light intensities (Table 7). In October, there was some indication that plants grown in the brightest light were slower than those grown in shade to form PLST.

DISCUSSION

There seems to be little doubt that variations in environmental conditions under which pachysandras were growing affected the

TABLE 5. Relationship of various concentrations of deicing salt applied to soil to infection of wounded pachysandra stems by *Volutella pachysandricola* and formation of primary lignosuberized tissue around the wound

Wound age (days)	Infection			Percent lignified cells			Percent suberized cells		
	0[a]	3,000	9,000	0	3,000	9,000	0	3,000	9,000
2	2.7[b]	8.0	10.0	58.3	60.7	42.0	0.0[c]	0.0	0.0
4	2.3	8.0	8.7	66.7	49.0	95.3	67.0	0.0	0.0
6	1.7	3.0	8.0	76.3	71.3	89.3	24.0	5.0	1.7
8	2.0	4.3	6.0	85.3	86.7	98.3	66.3	49.3	34.3
10	0.3	3.3	2.0	100.0	85.3	100.0	91.7	89.3	86.7
$\bar{x}$	1.8	5.3	6.9	77.3	70.6	85.0	49.8	28.7	24.5
		LSD (0.05) = 2.54			LSD (0.05) = 5.25			LSD (0.05) = 4.49	

[a] Salt concentrations (ppm).
[b] Each value is the mean number of infected plants from three replicates with 10 plants/replicate.
[c] Each value is the percent of cells on the perimeter of a wound that have become lignified or suberized as indicated by histochemical tests (13).

TABLE 6. Relationship of various levels of shading and time of year of wounding to infection of pachysandra stems by *Volutella pachysandricola*

Wound age (days)	Number of infections/10 inoculations															
	June				July				August				October			
	0[a]	32	63	92	0	32	63	92	0	32	63	92	0	32	63	92
2	10.0[b]	4.0	1.0	1.0	5.0	5.0	1.0	0.0	2.0	0.0	0.0	0.0	5.0	6.0	5.0	1.0
4	10.0	1.0	1.0	2.0	3.0	0.0	2.0	0.0	0.0	0.0	1.0	0.0	0.0	5.0	4.0	0.0
6	10.0	4.0	1.0	0.0	1.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	4.0	2.0	1.0
8	4.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0
10	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
$\bar{x}$	7.6	2.0	0.6	0.8	1.8	1.8	0.6	0.0	0.4	0.0	0.2	0.0	2.0	3.2	2.2	0.4
		LSD (0.05) = 1.51				NS				NS				NS		

[a] Numbers in this row refer to percent shade. 0 = full sun.
[b] Each value is the number of wounded stem segments colonized of 10 attempted.

TABLE 7. Relationship of various levels of shading and time of year of wounding to formation of primary lignosuberized tissue

Wound age (days)	June				July				August				October			
	0[a]	32	63	92	0	32	63	92	0	32	63	92	0	32	63	92
	Percent lignified cells															
2	100[b]	100	100	0	0	61	0	0	17	60	73	80	0	55	48	87
4	100	100	100	92	15	85	65	82	26	90	92	94	100	50	95	100
6	100	100	100	88	100	100	100	93	27	68	89	100	97	92	100	100
8	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
$\bar{x}$	100	100	100	76	63	89	73	75	54	83	90	94	79	79	88	97
		NS				NS				NS				NS		
	Percent suberized cells															
2	2	4	47	75	2	0	0	0	0	10	0	0	0	0	0	0
4	4	4	100	53	0	0	13	14	3	0	19	17	34	0	0	0
6	3	5	100	100	23	58	29	43	11	51	58	66	35	0	20	51
8	7	6	91	100	42	55	95	63	100	44	56	94	40	35	69	67
10	9	7	96	100	100	82	78	100	57	86	64	89	27	44	76	73
$\bar{x}$	5	5	86	85	33	39	43	44	34	38	39	53	27	15	33	38
		LSD (0.05) = 23.3				NS				NS				NS		

[a] Numbers in this row refer to percent shade. 0 = full sun.
[b] Each value is the percentage of cells around the perimeter of a wound showing presence of lignin and suberin as indicated by histochemical tests (13).

susceptibility of the plants to a common facultative parasite. For the most part, results of experiments reported here were consistent with those of other investigators who have reported effects of stress on other perennial plant-pathogen systems (1,2,5,22,23). However, some additional insight into the role of wound repair processes, environment, and disease resistance was also gained.

The time of year that pachysandras were wounded and inoculated with *V. pachysandricola* was a significant factor in determining whether or not infection would occur. Experiments specifically designed to test this were initiated because preliminary greenhouse experiments, not reported in detail here, indicated such to be the case. In those preliminary experiments, groups of plants growing in a greenhouse and wounded and inoculated between November and April became predictably more resistant to infection over the course of about 8 days. However, plants growing in the same greenhouse and wounded and inoculated between May and October were virtually immune to infection within, in most cases, 24 hr. In addition, plants used in experiments to test effects of other parameters (shade, salt, defoliation) were more susceptible when those experiments were conducted between May and October. It was for that reason that all subsequent greenhouse experiments reported here were done during winter and early spring.

Increased susceptibility of perennial plants to facultative parasites during host dormancy has been demonstrated on several other occasions (4,19,27) and is certainly not unique to *P. terminalis*. Marshall (15) recognized this long ago and cautioned against wounding trees during dormancy for fear of providing infection courts for pathogens when hosts were most susceptible.

Results reported here failed to identify statistically significant differences in susceptibility to *V. pachysandricola* associated with defoliation. However, examination of the raw data suggests a trend towards higher susceptibility with increased levels of defoliation. Such would be consistent with observations of Wargo (25) and Schoenweiss (22) regarding increased susceptibility of other species of perennial plants to facultative parasites following defoliation.

Increased susceptibility of plants watered with deicing salt was expected because excess salt in soil decreases ψ_p, and water stress has repeatedly been associated with increased susceptibility of trees and shrubs to facultative parasites (1,2,5,22,23). A certain level of salt was tolerated, inasmuch as there were no significant differences in disease susceptibility between plants watered with tap water and those periodically receiving 50 ml of a solution containing 3,000 ppm of NaCl. However, periodic applications of solutions containing 9,000 ppm of NaCl were clearly beyond some as yet undefined predisposing threshold.

Of particular interest was that only plants periodically receiving 12,000 ppm of salt showed symptoms of salt injury, and those died early in the course of the experiments. Plants receiving 9,000 ppm of salt showed virtually no symptoms of salt injury but were significantly more susceptible to disease. It may be necessary to expand testing of salt tolerance in plants in general to include factors such as disease susceptibility in addition to the current practice of only evaluating visual symptoms.

Inasmuch as the normal habitat for *P. terminalis* is the forest floor, that plants growing in bright sunlight were chlorotic and more susceptible to disease was not unexpected. As with the deicing salt experiments, there apparently is a threshold beyond which disease resistance mechanisms in the plants become demonstrably less effective. In the case of light intensity, this threshold appeared to be somewhere between 0 and 32% shade.

In addition to learning about the relative effects of environmental factors on disease occurrence, we also hoped that experiments described herein would provide added insight into mechanisms responsible for resistance. Earlier work with *P. terminalis* (14) and with other species of plants (6,7,10,12,16,17,19) suggested that the rate of barrier zone formation (PLST and/or impervious tissue followed by necrophylactic periderm) was a critical factor. However, when rates of barrier zone formation on plants growing under various levels of stress were actually measured, differences were not nearly as distinct as differences

in disease susceptibility. Lignification of parenchyma subjacent to wounds occurred at approximately the same rate within the limits of stress imposed by any one series of experiments. Deposition of intracellular suberin, suspected to result in formation of an impervious boundary zone, appeared to be slowed by the same conditions that resulted in increased disease susceptibility. Biggs (8) found no clear effect of water stress on development of PLST but did note that the rate of necrophylactic periderm formation was slowed in water stressed trees.

That plants became virtually immune to colonization by *V. pachysandricola* so soon after they were wounded in midsummer with no discernible correlation with wound repair processes suggests that another resistance mechanism must occur at that time. Perhaps there is rapid synthesis and transport of a phytoalexin or other compound to the wound site. This and the overall issue of wound responses and disease resistance warrant continued investigation.

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