Studies on Biology and Control of Daylily Rust in Canada

TOM HSIANG, SANDRA COOK AND YONG ZHAO
Department of Environmental Biology
University of Guelph, Guelph, Ontario
Canada N1G 2W1

Prior to 2000, daylily rust, caused by *Puccinia hemerocallidis* was not found in North America, but restricted to the native range of daylilies in east Asia (Bergeron, 2003). This disease was first confirmed in the southern U.S. during fall 2000 (Williams-Woodward et al. 2001), and had rapidly spread to over 20 states within a year (Buck and Williams-Woodward 2002). The infection and spread of this disease in nurseries has been extremely rapid, and in some cases has rendered plants unsaleable. During the 2001 growing season, daylily rust was detected on some daylily collector or breeding gardens in Ontario and Quebec. Unlike the situation in the U.S. where many products are available, Canada has only chlorothalonil labeled for use against diseases on lilies (and daylilies aren't even true lilies).

The purpose of this work was to develop a method for studying this rust under laboratory conditions since at the start of this study, the distribution of this rust in Canada was limited and we did not want to enhance its spread. We first wanted to see if the rust could be grown on leaves in petri plates so that we could propagate the rust for other studies. After developing a rust propagation method, the first series of tests involved control of the rust using fungicides under greenhouse and laboratory conditions, as well examining the sensitivity of various daylily cultivars to rust. Then we attempted to address important questions as to how long the rust spores remain viable, and the conditions that favor rust spore production.

Rust Production Methods

Some previous studies with rusts of other plants have used detached leaves as a method to both propagate the rust and to study the rust under controlled laboratory conditions (e.g. Hsiang and van der Kamp, 1985). We used the following method to prepare daylily leaves for inoculation. Full length leaves were used that were mature but not senescent (no yellowing). These leaves were cut into 4 to 5 cm

segments, and washed gently in tap water. This was to remove autolytic cells contents at the cut ends (to prevent browning at the ends), and as well, the water wet the leaf surface so it did not repel the inoculum suspension as much. Three to five leaf segments were placed in each petri plate with upper leaf surface (adaxial) down on filter paper (Figure 1). In preliminary trials, we found that more uredia (spore producing structures) formed on the abaxial surface than the adaxial surface. The filter paper had been previously watered with 1 ml of 100 ppm (0.1 g/L) gibberellic acid (kept in fridge), which helped to delay leaf senescence in the petri plates.

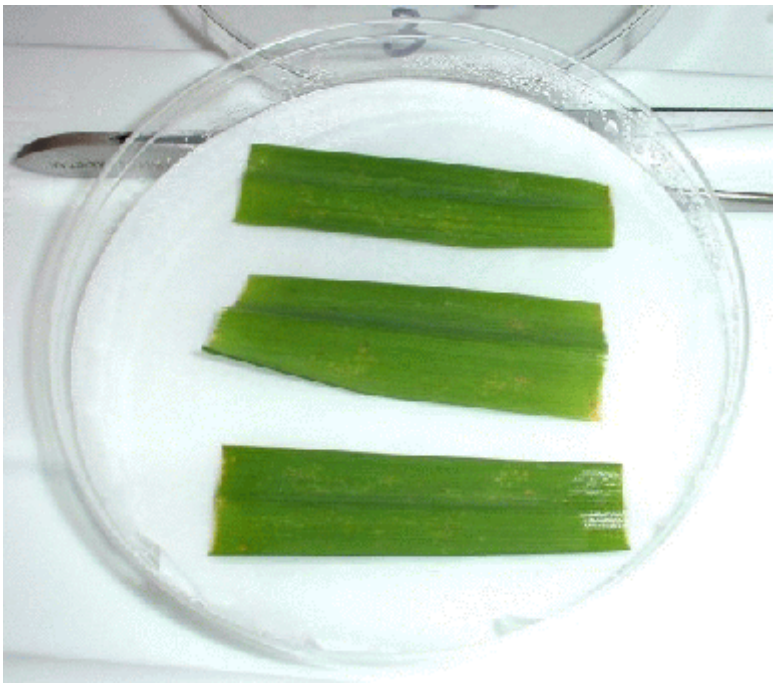


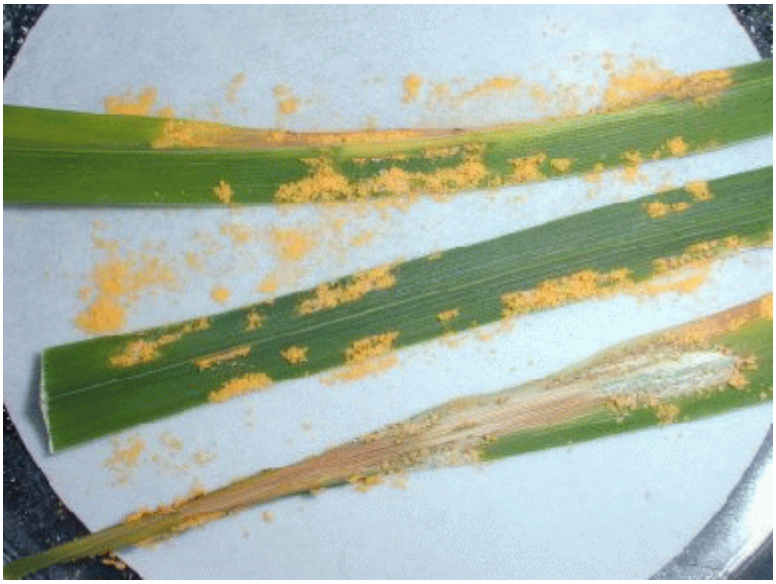
FIGURE 1.
Daylily leaf segments placed in a 9 cm (3.5") diameter Petri dish with a wet piece of filter paper underneath.

Rust Inoculation Method

We used the following method to gather and prepare the rust for inoculation. Rust spores were collected from previously inoculated leaf sections (Figure 2) by gentle scraping with a sterilized scalpel. The spores were placed into a 15 mL vial, and 5 to 10 mL of 0.05% Tween-20 (a detergent) were added to disperse the spores uniformly. The spore concentration was measured with a hemocytometer and adjusted to 10^4 - 10^5 spores/mL.

For inoculation, we tried three different methods: spore suspension droplets, dry spore transfer and spore swabs. For spore suspension droplets, we applied 100 uL onto each leaf segment by dribbling over the whole surface. For dry spore transfer, a leaf segment heavily infected with daylily rust and with many sporulating

FIGURE 2.
On the daylily leaf segments, abudant spores of the rust could be produced within three weeks. Freshly harvested spores were used for subsequent inoculation trials.



uredia was rubbed onto uninfected daylily leaf tissue. For inoculating whole plants, two to five infected leaf segments were used, while for leaf segments, one infected leaf segment was used to provide inoculum for up to 10 uninfected leaf segments. For spore swabs, a clean Q-tip swab was dipped into a spore suspension and the solution gently smeared over the surface of the daylily leaf segment, to leave a film of water on the surface. For each new leaf segment, the swab was dipped again into the solution before inoculation. When petri plates were used with leaf segments, they were sealed with parafilm and placed under continuous fluorescent lights (35 umol m^{-2} s^{-1}, equivalent to 2700 lux) and kept at 20 C (68 F). Water was added to plates when necessary.

The first uredia generally appeared within 10 days. Infected leaf segments could last up to 2 months but in some cases, they became senescent after 2 weeks. This might be an age-related effect, since we chose full length leaves from a variety of plants which probably differed in age. The spore swab method gave the most consistent and uniform results in terms of infection and subsequent uredial production. However, quantification of inoculum dose is problematic with this method since it is not certain how much of the spore suspension stays on the leaf surface or is retained by the swab. For the cultivar resistance tests and the greenhouse fungicide efficacy test with whole plants, we used the droplet/spray method for better quantification of inoculum. For the fungicide efficacy tests with leaf segments, we used the spore swab method for more uniformity of infection and better assurance of disease pressure.

Cultivar Resistance

Leaf segments were inoculated in petri plates using the spore suspension droplet method. After 2 weeks, the number of mature uredia (with spores, Figure 3) were assessed for each leaf. The results are presented in Table 1.

Table 1: Resistance of inoculated leaf segments of daylily cultivars (*Hemerocallis* sp.) to rust caused by *Puccinia hemerocallidis*

Daylily cultivar	Spots/3-cm leaf segment[1]		Rating by Mueller et al. 2002
Catherine Woodbery (Childs-F.67)	11.4	A	
Cherry Cheeks (Peck 68)	8.7	AB	moderately susceptible
Pardon Me (Apps 82)	8.5	AB	susceptible
Hemerocallis fulva	6.9	ABC	
Cleopatra (Thomas 64)	4.6	BCD	
Rosy Returns (Apps 99)	3.6	BCD	
Summer Wine (Wild 73)	3.5	BCD	
Happy Returns (Apps 86)	2.6	CD	
Pandora's Box (Talbot 80)	1.7	CD	
Stella De Oro (Jablonski 75)	0.5	D	moderately resistant

[1] Based on an average of 40 leaf segments per cultivar, the means followed by a letter in common do not differ significantly at the 95% confidence level.

These results show that there is a wide range in resistance of daylily cultivars to daylily rust. This is a favorable result from the point of view of disease resistance selection. However, several factors need to be considered. First, this was an laboratory test with daylily leaf segments and may not fully reflect the results with whole plants in a field or greenhouse test. Secondly, we only had a single isolate of daylily rust for this test, and the results might differ with other isolates. The results show general agreement with those of Mueller et al. (2002) who conducted cultivar resistance trials with 42 cultivars of intact daylily plants.

Fungicide Efficacy Trial with Whole Plants in the Greenhouse

Fifty plants of 1-month-old "Pardon Me" were placed in a greenhouse at the Vineland Research Station, Vineland, Ontario in July 2002. These fifty plants were divided into sets of five for seven fungicide treatments, one water control treatment, and two extra sets of untreated plants. Fungicide treatments were made twice, first on 17 July 2002, and again on 31 July 2002 by spraying each plant with 50 mL of fungicide solution (Table 2).

Table 2: Treatments in fungicide efficacy trials

Trade name	Chemical name	Product/L water	Product /100 gal
1. Banner 130EC	14.3% propiconazole	0.67 mL	8 fl oz
2. Daconil 2787F	40% chlorothalonil	2.5 mL	30 fl oz
3. Daconil Ultrex	82.5% chlorothalonil	1.7 g	1.4 lb
4. Fore 80WP	80% mancozeb	0.83 g	1.5 lb
5. Heritage 50WG	50% azoxystrobin	0.3 g	2 - 4 oz
6. Nova 40W	40% myclobutanil	0.3 g	4 oz
7. Dishwashing liquid	Ultra Dawn	1%	1%
8. Inoculated check	Spore suspension	--	--
9. Untreated control	Water	--	--

Except for the untreated control and the two extra sets of five, all plants were inoculated with rust four times (18 July, 7 August, 14 August, 21 August). The first two times, the plants were sprayed with rust spore suspensions (15 mL per plant at 100,000 spores/mL). The last two times, the plants were inoculated by dry spore transfer directly from infected leaf segments. After each round of rust inoculation, the plants were misted under just before runoff and placed in sealed plastic bags overnight for at least 15 hours. This incubation period with wet leaves is necessary for spore germination (Figure 4) and penetration into plants. Mueller and Buck (2003) found that at least 5 hours of leaf wetness were required to allow daylily rust infection to occur.

Observations of Rust on Inoculated Plants

The plants were checked weekly for the appearance of uredia, but no rust was observed on any of the plants. Daytime temperatures in this greenhouse ranged up to 40 C (104 F). The plants were moved on 28 August to the University of Guelph, and placed just outside the Bovey Building greenhouses. The plants were monitored weekly for rust, and uredia were first seen 6 October on inoculated plants, which was 7 weeks after the last round of rust inoculations. For the uninoculated control plants, no rust was seen. We speculate that rust infections (spore germination and penetration into plants) occurred after the rust inoculations, but rust symptoms were not seen because the temperatures were too high in the greenhouse for rust growth. (Note that in spite of frequent watering, these high termpatures also curtailed plant growth in the greenhouse during July and August.) Alternatively, spores may have survived on the leaf surfaces and then germinated and penetrated when temperatures were more favorable. More research is needed to evaluate these alternatives.

Suppression of Rust on Test Plants

All treatments were able to significantly suppress rust disease on the daylily plants (Table 3). Surprisingly, the dishwashing solution showed the fewest number of uredia per plant. Although the two treatments (17 & 31 July 2002) were not observed to cause phytoxicity on these plants, we do not know the effects of longer term (e.g. season long) multiple applications of diluted dishwashing liquid on daylily plants. This remains to be tested.

Table 3: Effect of various fungicide treatments for daylily rust control on whole daylily plants.

Treatment	Uredia per plant on 8 Oct 2002	Uredia per plant on 18 Oct 2002
Inoculated check	97.6 A [1]	148.4 A
Daconil 2787F	21.4 B	46.0 B
Fore 80WP	11.8 B	30.4 B
Banner 130EC	4.6 B	26.8 B
Daconil-Ultrex	2.0 B	25.4 B
Nova 40W	3.4 B	8.0 B
Heritage	1.4 B	5.4 B
Dishwashing liquid	0.6 B	3.4 B
Untreated control	0 B	0.6 B

[1] Based on 5 plants per treatment, the means in each column followed by the same letter do not differ significantly at the 95% confidence level.

A few rust uredia per plant were found on uninoculated plants set next to the inoculated plants outside the greenhouse. However, for plants a bit further away, and around the corner on another side of the greenhouse, no rust was observed on these plants. This indicates that the rust was able to spread from plant to plant for very short distances (within 2 m, 6.5'), but did not spread to plants 10 m (33') away, during the time the sporulation was first observed (6 October) until the plants were removed (31 October) due to cold weather.

Fungicide Efficacy Trials on Detached Leaves in Petri Dishes

We had initially intended to repeat the greenhouse tests, but because the weather was not favorable to disease development, we were only able to perform the greenhouse trial once. To obtain replicated and supplementary data that would be needed for fungicide minor use registration, we started fungicide tests with leaf segments in place of intact plants.

FIGURE 3.
Spores of daylily (ciruclar shapes) with hyphal germination tubes (rope-like growths) which can penetrate into daylily leaves to cause infections (microscopically magnified)

FIGURE 4.
Close-up of an uredium (spore-producing strucutre) of daylily rust on a leaf surface. Each small oval body is a spore capable of causing another infection.

Table 4. Efficacy of various fungicide treatments for daylily rust control on daylily leaf segments with fungicide applied at various intervals before or after rust inoculation.

Treatment	Number of rust spot by day of observation after fungicide treatment											
	Fungicide1 day before inoculation			Fungicide1 day after inoculation			Fungicide 3 days after inoculation			Fungicide 7 days after inoculation		
	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Inoculated check	0.4	40.9	43.8	0.0	26.1	26.3	0.0	54.9	65.1	6.2	46.7	53.0
Daconil Ultrex	0.3	1.3	1.1	0.0	0.0	0.0	0.0	125.3	121.8	0.0	77.5	80.8
Daconil 2787F	0.0	0.1	0.1	0.0	0.0	0.0	0.0	114.3	111.8	0.0	48.1	58.8
Fore 80WP	0.0	1.3	1.7	0.0	0.0	0.1	0.0	94.4	95.3	0.0	37.3	57.8
Dishwashing liquid	0.0	0.3	0.3	0.0	0.4	0.5	0.0	32.0	24.8	0.0	15.8	51.6
Nova 40W	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.3	2.2
Banner 130EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.3	2.2
Heritage 50WG	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	1.2
Untreated control	0.0	0.5	0.5	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
LSD[1]	0.3	23	24	ns	12	12	ns	58	57	ns	29	25

[1] The LSD (test of Least Significant Difference) represents the minimum difference between any two means within a column which would allow the two means to be considered significantly different at the 95% confidence level. For example, in the last data column, the inoculated check (53.0) differs significantly from any value greater than 53 + 25 = 78 or less than 53 - 25 = 28.

Healthy daylily leaves were cut into 5-cm-long sections and rinsed in water. For each fungicide treatment, there were three replicate petri plates with four daylily leaf pieces in each plate. Each 5-cm-long leaf piece was dipped into a fungicide solution (Table 2) for 5-10 sec. Fungicides were applied at different times before (1 day) or after (1, 3, or 7 days) rust inoculation. The leaf segments were rated at weekly intervals after fungicide application for the number of uredia (both sporulating and nonsporulating), and the results are shown in Figure 5.

These results imply that all fungicide treatments were able to suppress rust development on daylily leaves if applied within one day after rust inoculation. However, by three days after rust inoculation, full rust suppression was provided only by the systemic chemicals (Heritage containing azoxystrobin, Banner containing propiconazole, and Nova containing myclobutanil). Among the contact fungicides applied three days after inoculation, the dishwashing liquid was able to reduce rust development to a level not significantly different from that of the systemic fungicides, but this value was also not significantly different from the inoculated check. The remaining contact fungicides (Daconil containing chlorothalonil and Fore containing mancozeb) did not suppress disease compared to the inoculated control, and may have even enhanced disease although not to a statistically significant level from the inoculated control for treatments three days after inoculation. For fungicide treatments seven days after rust inoculation, there was distinct separation of systemic and contact treatments. All three systemic fungicides were able to fully suppress disease development, while the contact fungicides resulted in uredial counts equal or higher than that of the inoculated control. In this final test, the Daconil-Ultrex treatment seemed to enhance rust development since its uredial counts were significantly greater than that of the inoculated control. One mechanism by which a fungicide may seem to enhance disease levels is through the suppression of natural antagonists to the disease-causing organism. However, more research is needed to assess the effect.

Rust Incubation Period

What are the conditions that are favorable for daylily rust development on host tissue? To address this question, daylily leaves were cut into 4-cm-long segments and washed in running water for 20 min. Three leaf segments were placed into each petri dishes on filter paper containing 2 ml of 100 ppm gibberellic acid. Leaf segments were inoculated using the swab method. Four incubation temperatures of 4, 12.5, 20 or 26C (39F, 54.5F, 68F and 79F) and two continuous light levels (dark or 15 to 20 umol m^{-2} s^{-1}) were used in this study resulting in a total of 32 plates (4 replicate plates per treatment). The dark treatments were obtained by wrapping plates in aluminum foil. The plates were observed daily for uredial production, and the physiological state of leaf segments were also evaluated. The results are presented in Table 4.

Table 4: Latent period and uredial production under different temperature and light conditions using the detached leaf assay for daylily rust

Temperature (C)	Light conditions	Latent period[1] (days)	Uredia per leaf segment	Time of rust rating (DAI[2])
26	light	7	32	14
	dark	6	n/a [3]	14
20	light	8	54	14
	dark	7	90	14
12.5	light	14	55	19
	dark	15	7	19
4	light	>35 [4]	0	35
	dark	>35	0	35

[1] Latent period is the time between inoculation and when the first uredia appeared.
[2] DAI = days after rust inoculation
[3] n/a means not available, because more than 80% of leaf segments were senescent.
[4] No uredia were visible by 35 days after inoculation at 4 C (39 F)for either light or dark conditions; however, when these plates were transferred to 20 C (68 F) after day 35, uredia were visible within 7 days indicating that the rust was still viable and infective on or within these leaf segments.

These results demonstrate that temperature is a major constraint on daylily rust development Furthermore, light also influenced rust development by slightly lengthening the latent period. In our system with leaf segments, light also influenced the rate of senescence since leaves in the dark senescenced (turned yellow) more quickly than leaves under light. Mueller and Buck (2003) looked at the effect of temperature on daylily rust development on whole plants. They found that 22 C (72 F) was the optimal incubation temperature for disease development compared to 4, 10, 30 or 36 C (39, 50, 86 or 97 F, respectively), which agrees with our results.

Rust Spore Survival

In our greenhouse test mentioned earlier, it seemed as if rust spores might have survived on the surface of plants, going dormant under hot conditions, and then causing infections when the weather became cooler and wetter. To address the issue of spore survival, rust spores were collected from infected leaf segments in petri dishes. The spores were placed in 15 mL vials, sealed with screw caps, and kept on the bench at 20 C. After set intervals (0,1,9,10,19,24,34 days), spores were taken from these vials and a spore suspension (10^4 to 10^5 spores/mL) was made for swab inoculation onto "Pardon Me" daylily leaf segments. These leaf segments (3 per plate, with 4 replicate plates) were sealed with parafilm and incubated under continuous fluorescent light (35 umol m^{-2} s^{-1}). After 14 days, the number of uredia per leaf segment was counted. The results are in Table 5.

Table 5: Viability and infectivity of daylily rust spores after storage at 20C

Days of Storage	Uredia per plant at 14 days after inoculation [1]
0	125 B
1	129 B
3	178 A
10	129 B
19	51 C
26	41 C
34	80 C

[1] Based on 10-15 leaf segments per treatment, the means followed by a different letter differ significantly at the 95% confidence level.

When rust spores were stored at 20C, over half were still viable and infective after 34 days of storage. More research is needed on the survival of spores under different storage conditions (e.g. lower temperatures) and for longer periods (several months) to address the issue of whether spores can survive through a winter.

References

Bergeron, S. 2003. Daylily rust information page. http://www.ncf.ca/~ah748/rust.html. Last update Aug 5, 2003.

Buck, J.W., and Williams-Woodward, J.L. 2002. Efficacy of fungicide treatments for control of daylily rust. The Daylily Journal 57:53-59.

Hsiang, T. and B.J. van der Kamp. 1985. Variation in rust virulence and host resistance of *Melampsora occidentalis* on black cottonwood. Canadian Journal of Plant Pathology 7:247-252.

Mueller, D.S. and Buck, J.W. 2003. Effects of light, temperature, and leaf wetness duration on daylily rust. Plant Dis. 87:442-445.

Mueller, D.S., Williams-Woodward, J.L. and Buck, J.W. 2002. Resistance of daylily cultivars to the daylily rust pathogen (*Puccinia hemerocallidis*). SNA Research Conference 47:219-222.

Williams-Woodward, J.L., Hennen, J.F., Parda, K.W., and Fowler, J.M. 2001. First report of daylily rust in the United States. Plant Dis. 85:1101.

Acknowledgments

Funding for this research was provided by Syngenta, Landscape Ontario, and the American Hemerocallis Society. Other contributors to this project include research assistants Sarah Bowerman, Moh Afunian, and Michelle MacDonald, as well Ontario Ministry of Agriculture and Food Technical Specialists Jennifer Llewellyn and Wayne Brown. A copy of a presentation given to the Landscape Ontario Growers' Group on February 12, 2003 with many color images can be found at: http://www.uoguelph.ca/~thsiang/rust/dayrust/