EFFECT OF FUNGICIDE TIMING AND ENVIRONMENTAL CONDITIONS ON PRODUCTION AND VIABILITY OF UREDINIOSPORES OF *PUCCINIA HEMEROCALLIDIS*

by

KIERSTEN A. WISE

(Under the Direction of James W. Buck)

ABSTRACT

Daylilies infected with *Puccinia hemerocallidis* were placed under continuous light or alternating light-dark and dark-light treatments for 24 h. A separate experiment tested the effects on urediniospore germination and production of three temperatures (15, 20, and 30ºC) and low vs. high relative humidites. Light, temperature, and humidity did not significantly affect urediniospore germination, but urediniospore production per lesion was significantly higher with continuous light exposure, 30ºC and low relative humidity. Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon were tested to determine fungicide efficacy on three post-inoculation stages of disease development. All fungicides reduced urediniospore germination at all stages of disease development to < 45% of control one day after treatment application. Only azoxystrobin and chlorothalonil were able to reduce urediniospore germination at all stages of disease development to < 5% seven days after treatment. Fungicides did not have a significant effect on urediniospore production per lesion.

INDEX WORDS: *Hemerocallis*, *Puccinia hemerocallidis*, Daylily, Daylily rust, Light, Temperature, Humidity, Fungicide timing, Urediniospore germination, Urediniospore production

EFFECT OF FUNGICIDE TIMING AND ENVIRONMENTAL CONDITIONS ON PRODUCTION AND VIABILITY OF UREDINIOSPORES OF *PUCCINIA HEMEROCALLIDIS*

by

KIERSTEN A. WISE

B.S., Iowa State University, 2002

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

EFFECT OF FUNGICIDE TIMING AND ENVIRONMENTAL CONDITIONS ON PRODUCTION AND VIABILITY OF UREDINIOSPORES OF *PUCCINIA HEMEROCALLIDIS*

by

KIERSTEN A. WISE

Major Professor: James W. Buck

Committee: Harald Scherm
Katherine Stevenson

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2004

DEDICATION

To my parents, for their unwavering love and support.

ACKNOWLEDGEMENTS

Special thanks to Dr. James Buck for his patience and guidance through this degree. Thanks to Dr. Harald Scherm for sharing his wisdom in refining my project, and thanks to Dr. Katherine Stevenson for her involvement along the way.

Thanks also to Dr. Daren Mueller for the training, ideas and support through this experience, and Melissa Case who helped with the dirty work of the project. Also, thanks to all faculty, staff and student members of the Department of Plant Pathology who assisted and guided me through my experience at the University of Georgia.

Finally, I want to acknowledge my family and friends, especially Lacie and Robert, for their constant encouragement and support.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

QUARANTINES AND ORNAMENTAL RUSTS[1]

[1] Wise, K.A., Mueller, D.S. and Buck, J.W. 2004. APSnet February feature article.
http:www.apsnet.org/online/feature/quarantine/
Reprinted here with permission of publisher.

CHAPTER 1

QUARANTINES AND ORNAMENTAL RUSTS

The production of ornamental plants, including both nursery and floriculture crops, is a thriving and quickly expanding industry. Over a six-year span, the value of this industry in the United States increased over 23% to $14.3 billion in 2002 (12, 33). Ornamental plant production is also a major industry in Australia, Canada, Europe, and South America. The U.S. Department of Agriculture (USDA) recognizes deciduous and evergreen trees, woody ornamental plants, and shrubs as nursery crops, while floriculture crops include foliage plants, cut flowers, flowering potted plants, and bedding plants (30, 31). The wholesale value of the entire floriculture crop in 2002 was estimated to be worth $4.8 billion. California and Florida lead the nation in floriculture crop production ($1.8 billion in 2002), and combined these two states produce almost 40% of total U.S. wholesale floriculture sales (32).

Many floriculture crops are produced in the U.S. including geranium (*Pelargonium x hortorum*), chrysanthemum (*Dendranthema morifolium*), gladiolus (*Gladiolus* spp.) and daylily (*Hemerocallis* spp.). The value of the U.S. wholesale potted geranium crop from cuttings and seeds was $150 million in 2002 (32). Geraniums also have a substantial market as flowering hanging baskets and potted flowering plants. The U.S. wholesale crop of chrysanthemum was valued at $103 million for potted flowering bedding plants and $77 million for potted flowering foliage plants in 2001 (32). Gladiolus production for cut flowers in the U.S. in 2001 totaled

$24.2 million (32). Daylilies are popular landscape plants that along with other herbaceous perennials were valued at $571 million in 2002 (32).

One serious disease that can negatively affect production of many ornamental crops is a fungal infection called rust. Infected plants develop lesions (pustules) on the lower surfaces of plant leaves, which increase in size and eventually rupture the epidermis and release spores. These spores are typically brightly colored and are characteristic in diagnosing rust infections (Fig. 1.1). Pustules also can be present on the upper surface of leaves and can coalesce to form large necrotic areas (Fig. 1.2). Severe infections can result in premature leaf drop. Rust pustules also can form on stems and scapes, if present (Fig. 1.3). Rust spores are carried easily on wind currents and also can be disseminated by water splash, but long-distance dispersal of rusts on ornamental plants is mainly attributed to the movement of infected plants. Rust fungi typically have complex lifecycles involving one (autoecious) or two (heteroecious) hosts. Over 125 species of fungi that cause rust have been reported on 56 different ornamental crops (4). Examples of some of these rusts are presented in Table 1.1.

Integrated management practices, including scouting, proper sanitation, use of resistant varieties (if available), and preventative fungicide applications, are used to manage rust outbreaks in floriculture crops and minimize potential disease losses (4, 13). Several chemical classes of fungicides are registered for and have efficacy against rusts on ornamental crops. These include the strobilurins (e.g., azoxystrobin), sterol biosynthesis inhibitors (e.g., myclobutanil, propiconazole), and broad-spectrum protectants (e.g., chlorothalonil, mancozeb) (11).

Rusts have the potential to dramatically affect floriculture production because these pathogens cannot be adequately detected on symptomless but contaminated or infested

propagation material entering the U.S. or moving state-to-state. For example, quiescent rust spores can easily lodge in the crown of plants that have had foliage removed for shipping purposes (Fig. 1.4). Symptomless plants then can be moved long distances through international or interstate trade, dispersing the pathogen and introducing it into areas that were previously pathogen-free (28). Rust fungi are obligate parasites that do not usually kill infected plants. However, infection by rusts will reduce plant health and vigor, reduce flower production, and decrease the aesthetic value of ornamental crops due to the presence of pustules. Also, quarantine restrictions and eradication efforts can be costly and have a significant economic impact on floriculture production.

Plant Quarantines

Plant quarantines can be used to restrict the movement of plants into the U.S. and to limit their state-to-state movement. Regulatory control of ornamental plants was recently reviewed by Stebbins and Johnson (28). The first federal regulatory act designed to control the introduction of foreign pests into the U.S. was passed into law in 1912. This law, called the Plant Quarantine Act, and ensuing regulations help prevent or delay the introduction of foreign pathogens, including rusts, into the U.S. Rust pathogens of ornamental crops that are currently on the Plant Protection and Quarantine (PPQ) Regulated Pest List are found in Table 1.2. Quarantines have been used to limit movement of rust pathogens of geranium, chrysanthemum, daylily, and gladiolus into the U.S. A complete list of plant pathogens regulated by the U.S. Animal and Plant Health Inspection Service (APHIS) can be found at the USDA APHIS web page.

Examples of quarantines proven effective in the U.S.

Chrysanthemum white rust. Chrysanthemum white rust, caused by the fungus *Puccinia horiana*, is presently classified as a quarantine significant pathogen in the U.S. (Table 1.2) and Australia. This rust has been described as the most serious disease of greenhouse- produced chrysanthemums because infected plants are unmarketable resulting in large economic losses (15, 21, 22, 25). *Puccinia horiana* is an autoecious rust pathogen that is native to Asia. Infections are characterized by yellow lesions on the upper leaf surface that become necrotic. White pustules produce basidiospores on teliospores on the lower leaf surface under favorable environmental conditions (1).

Chrysanthemum white rust was introduced into England from Japan in 1963 (36). For more than twenty years an eradication campaign and quarantine measures were in place to prevent movement of the pathogen. These measures were ultimately unsuccessful and in 1989 the quarantine was lifted. Chrysanthemum white rust is now endemic in England (36). The rust has also become endemic in the Netherlands, which exports almost half of their chrysanthemum cuttings and flowers (25). Colombia, which is the second largest flower exporter behind the Netherlands, sends 97% of its total chrysanthemum exports to the U.S. (22). White rust has been present in Colombia since the late 1980s and eradication efforts have been in place to remove the pathogen from export-producing areas. If white rust were to be detected on imported plant material from Colombia, all U.S. imports would be stopped, resulting in enormous financial losses for Colombian producers and U.S. distributors. A strict eradication and control campaign has been implemented in Colombia to keep all chrysanthemum exports free of *P. horiana* (22). This campaign has been funded by emergency funds obtained from Colombian growers and financial backing from the flower industry (22).

Isolated outbreaks of white rust have occurred in the 1990s in New Jersey, Pennsylvania, Washington, and Oregon (1, 3). The discovery of white rust on chrysanthemum plants in production areas of California in 1992 prompted a reevaluation of the eradication program in place for control of the disease (3). Weekly sprays of triazole or strobilurin fungicides such as azoxystrobin, hexaconazole, myclobutanil, and propiconazole were found to be suitable regulatory treatments for exclusion and eradication of this pathogen (3, 15, 19). However, in 2001 isolates of white rust insensitive to both the triazole and strobilurin classes of chemicals were found in England (5). Outbreaks of white rust in the U.S. have been limited, and the quarantine is still deemed effective (1, 3).

Gladiolus rust. Six gladiolus rust pathogens (*Puccinia gladioli, P. mccleanii, Uredo gladioli-buettneri, Uromyces gladioli, Uromyces nyikensis, Uromyces transversalis*) are listed as quarantine significant pathogens by the PPQ (Table 1.2). Transverse leaf rust (*Uromyces transversalis*) is an autoecious rust pathogen native to South Africa. The fungus spread into production areas of Europe and South America in the late 1960s (2) and into Australia in the 1990s (2). The rust is characterized by orange pustules that form on the leaf surface. Pustules can also form on the inflorescence and flower spike of the plant. The disease has resulted in 100% losses and has made production of gladiolus for cut flowers almost impossible without fungicide use in parts of Africa (6).

Examples of quarantines that have proven ineffective in the U.S.

Daylily rust. Daylily rust caused by *Puccinia hemerocallidis* is a heteroecious rust native to Southeast Asia. The alternate host is the herbaceous perennial *Patrinia* (20). Yellow pustules form on leaf surfaces (Fig. 1.1). These pustules produce urediniospores that can continually re-

infect the host and spread to other daylilies. The pathogen was first detected in Florida and Georgia production areas in 2000 (38) and later identified as *P. hemerocallidis* (9). Although it is suspected that the infected plants came from Central America, the original source of the inoculum has not been pinpointed (37, 38). By fall of 2001, the rust was present in over 24 states within the U.S. and in Costa Rica (9, 35). The pathogen was officially quarantined, and plant movement was regulated in the U.S. in 2001 (35). In 2002, daylily rust was recognized as endemic in the southeastern U.S. Containment of the pathogen in the U.S. was deemed unrealistic due to widespread movement of plants by hobbyists and nurseries, and the USDA PPQ lifted the federal quarantine in January 2002.

Geranium rust. Geranium rust caused by *Puccinia pelargonii-zonalis* infects the zonal geraniums (*Pelargonium* x *hortorum*) (26). *Puccinia pelargonii-zonalis* is an autoecious rust pathogen that produces dark brown urediniospores on the lower surfaces of the leaves and chlorotic halos on the upper leaf surface (8). As lesions age, concentric rings of urediniospores are produced (Fig. 1.5). The pathogen was introduced into Europe from South Africa in the early 1960s and by 1967 it had been introduced into greenhouses in California, New York, and Canada (18). Despite quarantine restrictions and the destruction of infected plants, the rust became endemic in Europe and California by the 1970s (27). The constant re-introductions of the pathogen into production areas led to the lifting of most quarantines in the early 1980s (29). In 1997, an epidemic of this rust negatively impacted commercial geranium production in the southeastern U.S. (11).

Why do quarantines fail?

Many factors can contribute to the introduction of rust-infected stock into commercial production areas. International trade of ornamental crops has made the exclusion of rust

pathogens difficult because contaminated plant parts may be symptomless and inadvertently allowed to enter quarantined areas. With repeated introductions, pathogens may become endemic causing the quarantine to fail. The inability to adequately detect rust pathogens on contaminated or infected propagation materials severely hinders quarantine efforts. While rusts can be easily diagnosed when sporulating lesions are present, young non-sporulating lesions are often small and may remain undetected if only a few pustules are present in a shipment of tens of thousands of plants. Improved detection methods are needed to more accurately diagnose infections. New diagnostic methods and keys are being developed to more quickly and accurately identify quarantined pathogens (34). The effective implementation of these techniques must be the next step in quarantine enforcement.

Quarantines may also fail when rust-infected crops are unregulated. For example, daylily hobbyists and hybridizers can trade and sell plants in federally unregulated markets, such as farmers' markets and trade shows. This compromises the effectiveness of quarantines and was one of the reasons for lifting the daylily rust quarantine in 2002 (35). Some of the isolated outbreaks of chrysanthemum white rust in North America also were attributed to hobbyists bypassing inspectors when transporting cuttings (1). To ensure the effectiveness of quarantines, the information exchange between federal agencies and hobbyists should be improved to better inform growers of the potential implications of moving infected plants.

New fungicidal developments in the 1980s and 1990s led to fungicides such as myclobutanil and azoxystrobin that have eradicant activity for some rust fungi (3, 5). This technology can ease the pressure on quarantine restrictions, because the fungus can theoretically be eradicated from diseased shipments, allowing trade to continue. The chrysanthemum white rust quarantine and eradication campaign in England was ended in 1989, after propiconazole was

proven to be effective at eradicating the pathogen (36). However, the reliance on chemical controls as the sole means of managing white rust contributed to the development of fungicide resistance in *P. horiana* (5). Additional research is needed to determine if different fungicides display eradicant activity against a variety of rust pathogens, and to develop treatments that kill quiescent spores on plant foliage. Adopting sound disease management practices whether rusts are endemic or not will help prevent future outbreaks and minimize existing problems.

Literature cited

1. Anonymous. 1988. Chrysanthemum white rust. Yoder Brothers Inc. Barberton, OH.

2. Beilharz, V., Parbery, D. G., and Pascoe, I. G. 2001. Gladiolus rust (caused by *Uromyces transversalis*) in eastern Australia. Aust. Plant Pathol. 30:267-270.

3. Bonde, M. R., and Rizvi, S. A. 1995. Myclobutanil as a curative agent for chrysanthemum white rust. Plant Dis. 79:500-505.

4. Chase, A. 2002. Controlling rust diseases. Chase News 1:1-6.

5. Cook, R. T. A. 2001. First report in England of changes in the susceptibility of *Puccinia horiana*, the cause of chrysanthemum white rust, to triazole and strobilurin fungicides. Plant Pathol. 50:792.

6. Ferreira, J. F., and Nevill, W. G. 1989. Evaluation of bitertanol and triadimefon for the control of gladiolus rust caused by *Uromyces transversalis*. Plant Dis. 73:987-990.

7. Ferrin, D. M., and Rohde, R. G. 1991. Tests compare fungicides for control of rust on green-house carnations. Cal. Ag. 45:16-17.

8. Harwood C. A., and Raabe, R. D. 1978. The disease cycle and control of geranium rust. Phytopathology 69:923-927

9. Hernandez, J. R., Palm, M. E., and Castlebury, L. A. 2002. *Puccinia hemerocallidis*, cause of daylily rust, a newly introduced disease in the Americas. Plant Dis. 86:1194-1198.

10. Jeffers, S. N., Miller, R. W., and Powell, C. C. 2001. Fungicides for ornamental crops in the nursery. Pages 409-416 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R. K., and Benson, D. M. (Eds.). APS Press, St. Paul, MN.

11. Jeffers, S. N. 1998. Geranium rust: A potentially serious threat to the zonal geranium industry. Greenhouse Product News 8:20-23.

12. Jerardo, A. 2003. Floriculture and Nursery Crops Outlook. USDA Economic Research Service. FLO-02 http://www.ers.usda.gov/publications/flo/sep03/fl02.pdf

13. Jones, R. K., Simone, G. W., von Broembson, S. L., and Dutky, E. 2001. Integrated Disease Management. Pages 376-383 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R. K., and Benson, D. M. (Eds.). APS Press, St. Paul, MN.

14. Koike, S. T., and Scholler, M. 2001. First occurrence of a rust fungus on English daisy (*Bellis Perennis*) in North America. Plant Dis. 85:562.

15. Lam, C. H., and Lim, T. K. 1993. Efficacy of hexaconazole for the control of white rust on chrysanthemum and powdery mildew on roses. Int. J. Pest Management 39:156-160.

16. Lambe, R. C. 1970. Chrysanthemum rust. Virginia Polytechnic Institute Plant Disease Control Notes Extension Division. Control Series 108.

17. Littlejohn, G. M., and Blomerus, L. M. 1997. Studies on Gladiolus resistance to transverse rust. Acta Hort. 430:509-516.

18. Nichols, L. P., and Forer, L. B. 1972. Geranium rust discovered in Pennsylvania. Plant Dis. 56:759.

19. O'Neill T. M., and Pye, D. 1997. Evaluation of fungicides for control of chrysanthemum white rust. Ann. Appl. Biol. (Suppl.) 130:8-9.

20. Ono, Y. 2003. Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan? J. Gen. Plant Pathol. 69:240-243.

21. Orlikowski, L. B. and Wojdyla, A. 1982. Chemical control of chrysanthemum white rust *Puccinia horiana*. Acta Hort. 125:201-206.

22. Ortega, L. 1999. Prevention and eradication of white rust (*Puccinia horiana*) in Colombia. Acta Hort. 482:187-195.

23. Preece, T. F., Weber, R. W. S., and Webster, J. 2000. Origin and spread of the daisy rust epidemic in Britain caused by *Puccinia distincta*. Mycol. Res. 104:576-580.

24. Raabe, R. D., and Pyeatt, L. 1990. Control of rust of china aster and comments on the name of the pathogen. J. Environ. Hort. 8:89-92.

25. Rattink, H., Zamorski, Cz. and Dil, M. C. 1985. Spread and control of chrysanthemum white rust (*Puccinia horiana*) on chrysanthemums on artificial substrate. Med. Fac. Landbouww. Rijksuniv. Gent 50:1243-1249.

26. Rytter, J. L. 1993. Rust. Pages 230-232 in: Geraniums IV. White, J. W. (Ed.) Ball Publishing, Batavia, IL.

27. Rytter, J. L., Lukezic, F. L., Craig, R. and Moorman, G. W. 1989. Biological control of geranium rust by *Bacillus subtilis*. Phytopathology 79:367-370.

28. Stebbins, T. and Johnson, D. 2001. Regulatory control. Pages 457-458 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R. K., and Benson, D. M. (Eds.). APS Press, St. Paul, MN.

29. Stephens, C. T. 1981. Geranium rust. University of Missouri Extension Bulletin E-1493.

30. United States Department of Agriculture National Agricultural Statistics Service. 2001. Nursery crops 2000 summary. http://usda.mannlib.cornell.edu/reports/nassr/other/nursery/nurser01.pdf.

31. United States Department of Agriculture Agricultural Statistics. 2003. Fruits, Trees, Nuts and Horticultural Specialties. http://www.usda.gov/nass/pubs/agr03/03_ch5.pdf.

32. United States Department of Agriculture National Agricultural Statistics Service. 2003. Floriculture Crops 2002 summary. http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/floran03.pdf.

33. United States Department of Agriculture Economics and Statistics System. 1997. Floriculture and Environmental Horticulture Yearbook. FLO-1997. http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/flor0497.txt

34. United States Department of Agriculture 1999.Animal and Plant Health Inspection Service Plant Protection and Quarantine Risk Assessment Safeguarding Study. http://www.safeguarding.org/

35. United States National Plant Board. 2001. Daylily Rust Pest Alert. U. S. Animal and Plant Health Inspection Services. http://www.aphis.usda.gov/npb/daylily.html.

36. Whipps J. M. 1993. A review of white rust (*Puccinia horiana* Henn.) disease on chrysanthemum and the potential for its biological control with *Verticillium lecanii* (Zimm.)Viegas. Ann. Appl. Biol. 122:173-187.

37. Williams-Woodward, J. L., and Buck, J. W. 2001. Daylily rust caused by *Puccinia hemerocallidis*: A new disease on daylily in the U.S. Proc. South. Nursery Assoc. Res.Conf. 46:234-236.

38. 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. 85:1121.

Table 1.1. Selected ornamental rusts directly or indirectly affecting U.S. ornamental production

Host Plant	Fungus	Geographical distribution	Reference
Carnation (Dianthus caryophyllus)	Uromyces dianthi	Worldwide	7
China Aster (Callistephus chinensis)	Coleosporium asterum	Canada, England, and Northwest U.S.	24
Chrysanthemum (Dendranthema xmorifolium)	Puccinia chrysanthemi P. horiana	Australia, England, and U.S.	3, 16, 19
Daisy (Bellis perennis)	P. obscura P. laegenophora P. distincta	California, England	14, 23
Daylily (Hemerocallis spp.)	P. hemerocallidis	Costa Rica, U.S.	37
Geranium (Pelargonia xhortorum)	P. pelargonii-zonalis	England, South Africa and U.S.	8
Gladiolus (Gladiolus spp.)	U. transversalis	Australia, Europe, South Africa,	2, 6
Snapdragon (Antirrhinum spp.)	P. antirrhini	England, U.S.	4

Table 1.2. Causal agents and common disease names of ornamental rusts regulated by USDA APHIS PPQ in 2004[a]

Scientific name	Common name
Chrysomyxa ledi	Rhododendron-spruce needle rust[b]
Puccinia gladioli	Gladiolus rust
Puccinia horiana	Chrysanthemum white rust
Puccinia mccleanii	Gladiolus rust
Uredo gladioli-buettneri	Graminicolous rust
Uromyces gladioli	Gladiolus rust
Uromyces nyikensis	Gladiolus rust
Uromyces transversalis	Gladiolus rust

a. Obtained from PPQ Regulated Plant List. http://www.aphis.usda.gov/ppq/regpestlist/
b. Listed as Chrysomyxa leaf rust on the American Phytopathological Society list of common names of plant diseases. http://www.apsnet.org/online/common/names/rhododen.asp

Figure 1.1. Sporulating lesions (pustules) of daylily rust. These pustules are characteristic signs used to diagnose rust infections. (Photo by D.S. Mueller)

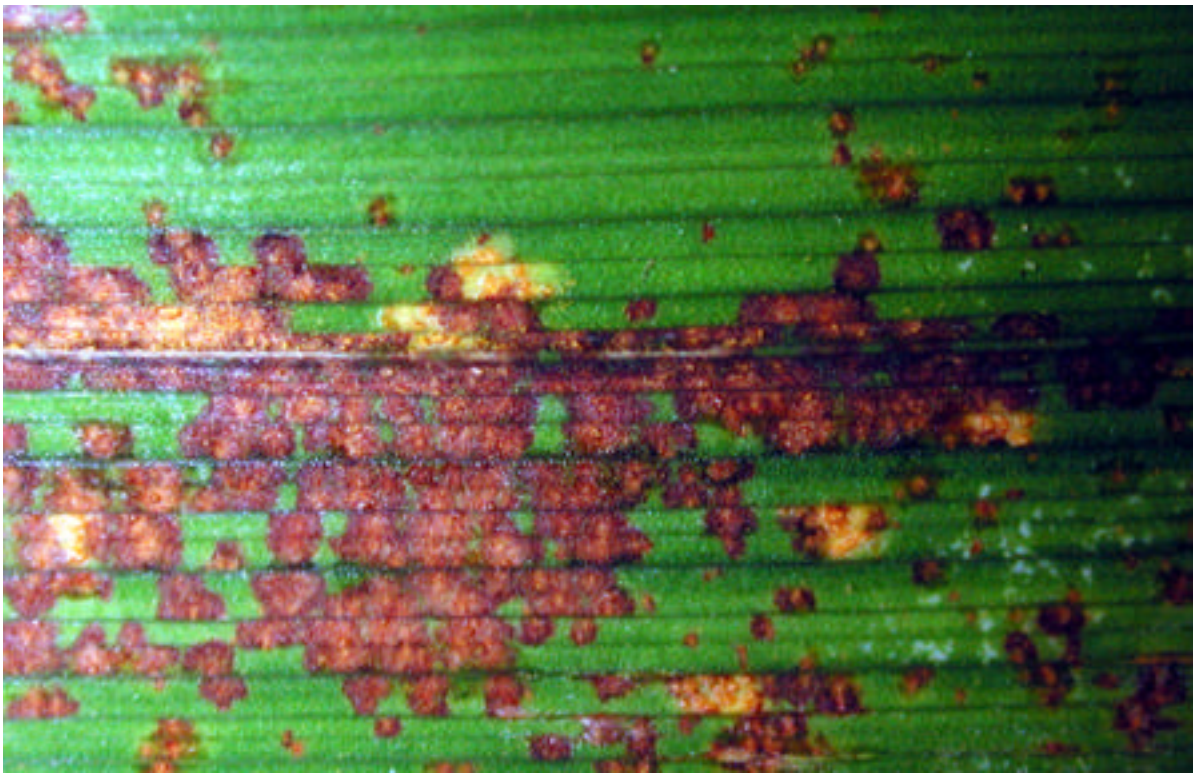

Figure 1.2. Coalescing lesions of daylily rust. (Photo by D.S. Mueller)

Figure 1.3. Daylily rust pustules on the scape of the plant. (Photo by D.S. Mueller)

Figure 1.4. Rust spores resting between leaves in the crown of a plant that has been cut back. (Photo by D.S. Mueller)

Figure 1.5. Sporulating lesions of geranium rust. As the infection progresses, concentric rings of pustules erupt around the initial infection point. (Photo by D.S. Mueller)

CHAPTER 2

INTRODUCTION

Introduction to culture of *Hemerocallis*

Daylily (*Hemerocallis* spp.) is an important ornamental crop that combined with other herbaceous perennials was valued over $571 million in 2002 (41). A member of the Hemerocallidaceae family, the daylily is native to China, Japan, Korea, and Siberia (14). The daylily has been propagated for thousands of years, with its earliest recorded history beginning in China in 2697 B.C. By the 1890s it was widespread in Europe and America (29). To date, there are at least 25 recognized species (14, 29) and over 45,000 registered cultivars, with numbers increasing each year due to hybridizations (40).

Daylilies have a fibrous root system and sword-shaped leaves that emerge from the crown of the plant in a fan shape. Flowers are borne on scapes and have three sepals and three petals (33). Propagation is by division of fans in the spring or fall (2). Daylilies are arranged in three broad categories that refer to their foliage survival in winter: dormant, semi-evergreen, and evergreen (2, 33). Dormant cultivars are truly deciduous and die back to the crown each winter in landscape settings. Semi-evergreen plants retain some foliage and have new growth each spring, while the foliage of evergreen cultivars does not die back during the winter months in the southeastern U.S. (14, 33). Daylilies can be further grouped into categories pertaining to flower form and color, ploidy, texture, size, habit, and hardiness (14, 29, 33). For example, daylily plants may be classified by bloom habit (cascading, continuous blooming, reblooming), flower shape (circular, ruffled, trumpet), and color (bitone, reverse bitone, throat), among others (1, 33).

Daylily breeding is a lucrative field that consists of hobbyists and enthusiasts who breed new varieties each year. The value of new varieties can be as high as $100 to $200 or more per fan (8).

The daylily prefers full sun or partial shade and well-drained soil, but it can tolerate a wide range of environmental conditions (2,14). It has been classified as a relatively disease-free plant (2), with most reported disease problems previously being attributed to leaf streak (*Aureobasidium microstictum*) and spring sickness (unknown etiology) (14, 45). As discussed in Chapter 1, the recent introduction of daylily rust (*Puccinia hemerocallidis*) into the U.S. has negatively impacted daylily production (44). This disease has become the focus of most disease management programs for daylily.

Biology of *P. hemerocallidis*

Puccinia hemerocallidis Thüm is the fungus that causes daylily rust. It was first identified in Russia in 1878 and is native to the Orient (13). Daylily rust has been observed in China, Japan, Korea, and Siberia (15). The first recorded incidence of daylily rust in the United States was in 2000 (41) and the identity of the rust was confirmed by ITS DNA sequence as *P. hemerocallidis* soon after (13).

Puccinia hemerocallidis is a heteroecious rust. The complete life cycle occurs on daylily and the perennial *Patrinia* spp. of the Valerinaceae family (32). The infection cycle can start when aeciospores land on daylily leaves, infect the host, and form discrete yellow pustules (uredia) on the underside of the leaf. These uredia produce many yellow or orange urediniospores, which can re-infect the host continually throughout the season, and wind-disperse to infect other daylilies (32). Under optimum conditions the pustules will erupt,

releasing urediniospores 7 to 9 days after infection (24). As winter approaches, telia are formed in response to reduced temperatures, which produce one or two-celled teliospores on the daylily leaves. These spores undergo karyogamy and meiosis and germinate in the spring, forming a basidium with haploid basidiospores. The basidiospores are wind-dispersed to *Patrinia* spp. where they infect the leaf tissue and produce spermagonia on the upper surface of the leaf. Two compatible spermagonia hyphae undergo plasmogamy and a dikaryotic mycelium develops. From this mycelium, aecia are formed on the lower side of the leaf, and aeciospores are discharged and wind-dispersed onto daylily (13, 16, 32). Species of *Patrinia* have not been successfully inoculated with basidiospores in the U.S.

To date, only urediniospores and teliospores of *P. hemerocallidis* have been observed on daylily foliage in the U.S. (46). The fungal mycelium of the rust is unable to overwinter in dormant plant tissue of infected plants (32), and urediniospores are not capable of overwintering in the absence of a host (17). However, urediniospores of the fungus are able to overwinter on infected foliage in southern areas of the U.S. (e.g., Georgia, South Carolina, Mississippi), and reinfect host plants in the spring (6, 21). However, urediniospores do not remain viable on plant tissues in climates with extended periods of temperatures below freezing (32).

In vitro germination of uredinospores of *P. hemerocallidis* is also affected by temperature (27). Urediniospores are able to germinate from 7 to 34°C, with optimal germination around 24°C. Temperature also influences daylily rust development. Disease development, measured as lesions cm^{-1} leaf length, was highest when plants were kept at 22 to 30°C after inoculation. No disease was observed on plants incubated at 34°C. Once plants were infected, temperature did not have as great an impact on disease development as during the infection process (27). Similar observations are reported for other *Puccinia* species. *In vitro* germination of

urediniospores of *Puccinia recondita* and *P. graminis*, the causal agents of wheat rust, is optimum over a range of 15 to 20°C, with germination occurring from 6 to 28°C (15). Over 90% of urediniospores of *P. substriata* var. *indica* (pearl millet rust) germinate *in vitro* at temperatures between 19 and 22°C (38).

Environmental conditions such as light quality and quantity, and relative humidity also can interact with temperature to significantly affect rust disease development. Light and temperature significantly effect urediniospore germination of *P. substriata* (38). Germination of urediniospores was inhibited by lower temperatures (10°C) under dark conditions. Urediniospore germination was delayed after 2-h of continuous light exposure (cool-white fluorescent lamps at 1650 lux), but 1 h of light followed by 1 h of dark stimulated urediniospore germination (38). Urediniospore germination of *P. graminis* was initially inhibited by 2 h of exposure to light (warm-white fluorescent lamps, 400 ft-c), but no difference was observed between urediniospore germination after 6 to 8 h of light exposure and urediniospores incubated in darkness. Inhibition of urediniospore germination by light treatment was reversed by following a light period with a 1-h period of darkness (11).

Germination of urediniospores of *P. graminis* and *P. recondita* was inhibited by 100% relative humidity at temperatures above 26°C (37). This study also showed that light positively affects germination in water-saturated air, but inhibits germination when humidity is reduced. Similarly, studies on stripe rust of wheat (*P. striiformis*) indicated that urediniospore germination increased with light exposure (fluorescent and incandescent bulbs, 2000 ft-c) at temperatures of 15°C and relative humidity between 65 and 80%. However, germination was significantly reduced when exposed to light or dark at a lower temperature of 6°C (39). Studies on *P. xanthii*, a fungus used as a biological control agent on cocklebur, have shown that teliospore and

basidiospore production is significantly impacted by interactions between humidity, light, and temperature (23). Basidiospore production increased with time when exposed to 100% relative humidity at temperatures between 20 and 25°C. Basidiospore production was inhibited by dark periods followed by light (warm-white fluorescent tubes, 28 W/m^2), but teliospore germination increased when exposed to light after a 12-h period of darkness (23). Direct comparisons of the effect of light on urediniospore germination are difficult because of variations in light intensity and quality associated with different light sources.

Effects of environmental conditions on production and viability of urediniospores of *P. hemerocallidis* are unknown. Urediniospore production by other *Puccinia* species is influenced by abiotic factors (e.g., light, temperature, humidity), rust genotype, host cultivar, lesion age, and lesion density (22, 35, 36). For example, rust lesions of *P. recondita* have been classified into three age groups: young, mature, and old lesions (36). Young lesions have a high growth phase, while mature lesions have reduced vegetative growth and increased sporulation. Old lesions are essentially stagnant, with little sporulation or vegetative growth (36). Mature lesions (14 to 17 days after inoculation) produced the highest numbers of urediniospores, which had the highest infection efficiency of any age group (36).

Increasing the density of lesions on leaves reduces urediniospore production per lesion by *P. triticina* (brown rust of wheat) and *P. recondita* (wheat leaf rust) (35, 36). No significant effect of lesion density on urediniospore production was observed up to three days after lesions began, sporulating and there was a weak effect of density on urediniospore production after 5 days. However, 11 days after sporulation began, urediniospore production decreased as lesion density increased until lesions stopped sporulating (36). Light intensity had no effect on colonization rate or pustule size of *P. striiformis* on wheat, but urediniospore production per

pustule significantly increased with increasing light intensity (22). An increase in temperature from 10 to 20 C reduced the duration of sporulation and maximum rate of sporulation on most wheat cultivars tested (22).

Management of Daylily Rust

A multi-faceted approach to disease management is key in keeping rust inoculum to a minimum in plant production settings. Scouting for disease, good sanitation, use of resistant cultivars when possible, and judicious use of fungicides are all important components in a disease management plan (14). For example, daylily cultivars vary in their susceptibility to the rust pathogen. Thirty-two percent of 84 commercial cultivars tested were resistant or moderately resistant to daylily rust (28). Susceptible cultivars had lesion numbers at least as high as those of 'Pardon Me'; the cultivar daylily rust was first observed on in the U.S. in 2000 (28, 46). However, susceptibility to *P. hemerocallidis* is unknown for the vast majority of daylily cultivars (>40,000). New varieties are usually susceptible to rust infection, and some breeding programs are now dictated by cultivar susceptibility to rust (8).

In 2001, USDA APHIS advised daylily growers to use fungicides labeled for other herbaceous perennial rusts (42). Suggested fungicides included: azoxystrobin, chlorothalonil, flutolanil, mancozeb, myclobutanil, propiconazole, and triadimefon (17, 42). Producers are advised to alternate between fungicides with different modes of action (e.g., systemic vs. protectant activity) to reduce the potential for resistance to develop in target populations. Fungicides recommended for use on ornamental rusts and their chemistries are described in Table 2.1. These chemicals vary in their ability to manage ornamental rust pathogens. For example, in trials where China aster rust (*Coleosporium asterum*) was present in production fields, triadimefon was most effective in managing the disease (34). Daisy rust (*P. distincta*) was

effectively managed by mancozeb when sprayed prior to infection, and myclobutanil provided good control when applied pre-infection and displayed eradicative properties when applied post-infection (43). Myclobutanil reduced both disease incidence and severity of carnation rust (*Uromyces dianthii*) in infected field plots (10). A combination of chlorothalonil and mancozeb reduced disease severity of chrysanthemum white rust (*P. horiana*) by 81% (31). Propiconazole was curative against young infections of *P. horiana* in chrysanthemum (9) and myclobutanil and azoxystrobin displayed curative properties against this rust as well (4, 47). Azoxystrobin, myclobutanil, and propiconazole all effectively controlled sweet william rust (*P. arenariae*) (30), while snapdragon rust (*P. antirrhinum*) was reduced with applications of azoxystrobin, fenarimol, myclobutanil, and triadimefon (7).

Azoxystrobin, chlorothalonil, mancozeb, and triadimefon effectively reduced daylily rust development (pustules cm^{-1} leaf length) when applied prior to inoculation. However, myclobutanil and propiconazole were not as effective in reducing pustule development (5). Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon were also effective at controlling disease development (measured as total lesion number per plant) of *P. hemerocallidis* when applied up to 10 days prior to inoculation (24). Azoxystrobin, propiconazole, and triadimefon significantly reduced disease development when applied up to 5 days post-inoculation, and myclobutanil effectively at reduced lesion number when applied up to 3 days after inoculation. However, chlorothalonil was not effective in reducing disease development when applied after inoculation (24).

In vitro studies on *P. hemerocallidis* have shown that azoxystrobin, chlorothalonil, copper sulfate, mancozeb, and trifloxystrobin are all fungicidal to urediniospores, i.e., no germination was observed after treatment with these fungicides (25). Myclobutanil,

propiconazole, and triadimefon prevented urediniospore germination during exposure to fungicides, but urediniospores that were treated with these fungicides and then washed to remove fungicide residue were still able to germinate at levels of 40 to 60% (25). Symptoms of rust epidemics often represent a continuum of lesion ages for which the effect of fungicide timing on spore production and viability is currently unknown.

Research Objectives

To assist in the management of daylily rust, caused by *P. hemerocallidis*, research has been conducted on host resistance (28), management with fungicides (5, 24, 25), and the effects of various environmental variables on disease (27). Very little information is available on the effects of environmental variables or fungicide applications on urediniospore production and viability of *P. hemerocallidis*. Thus, the specific objectives of this research were to:

1. Determine effects of light, temperature, and relative humidity on urediniospore production and germination of *P. hemerocallidis*. Different environmental variables (e.g., light, humidity, temperature) can have a significant effect on germination and production of urediniospores of various *Puccinia* species (12, 16, 23, 27, 37, 38, 39). *In vitro* studies on the effect of light intensity and temperature have been conducted on *P. hemerocallidis* (27). Observing the effects and interactions of light, temperature, and humidity on urediniospore germination and production *in vivo* will provide a more complete understanding of how abiotic factors affect disease development on daylily.

2. Determine the effects of fungicide sprays applied post-infection on production and germination of *P. hemerocallidis* urediniospores. There is a need for specific fungicide application recommendations to achieve effective disease management at the lowest cost to the

producer. Previous research on *P. hemerocallidis* indicated that fungicides differ in their preventative and curative properties (24). However, results from these studies indicate that the timing of fungicide applications with respect to lesion age affects disease development. Chemicals that were most efficacious at reducing urediniospore germination *in vitro* will be tested *in vivo* under greenhouse conditions at three post-inoculation stages of disease development to determine the efficacy of chemical formulations on urediniospore production and germination.

Literature Cited

1. American Hemerocallis Society. 2002. Daylily Dictionary. http://daylilies.org/ahs_dictionary/.

2. Armitage, A. 1997. Herbaceous Perennial Plants, 2nd ed. Stipes Publishing, Champaign, IL.

3. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Manage. Sci. 58:649-662.

4. Bonde, M.R., and Rizvi, S.A. 1995. Myclobutanil as a curative agent for chrysanthemum white rust. Plant Dis. 79:500-505.

5. Buck, J.W., and Williams-Woodward, J.L. 2003. The effect of fungicides on urediniospore germination and disease development of daylily rust. Crop Prot. 22:135-140.

6. Buck, J.W., and Williams-Woodward, J.L. 2001. In vitro fungicide sensitivity and optimum germination temperature of the daylily rust pathogen, *Puccinia hemerocallidis*. Proc. Southern Nursery Assoc. Res. Conf. 46:237-241.

7. Chase, A. 2002. Controlling rust diseases. Chase News 1:1-6.

8. Davis, T. 2003. Rust rocks the daylily sector. Nursery Management Pro 5:60-63.

9. Dickens, J.S.W. 1990. Studies on the chemical control of chrysanthemum white rust caused by *Puccinia horiana.* Plant Pathol. 39:434-442.

10. Ferrin, D.M., and Rohde, R.G. 1991. Tests compare fungicides for control of rust on greenhouse carnations. Cal. Ag. 45:16-17.

11. Fungicide Resistance Action Committee Fungicide List. 2003. FRAC. http://www.frac.info/publicaitons/frac_list02.html.

12. Givan, C. V., and Bromfield, K. R. 1964. Light inhibition of uredospore germination in *Puccinia graminis* var. *tritici*. Phytopathology 54:382-384.

13. Hernandez, J.R., Palm, M.E., and Castlebury, L.A. 2002. *Puccinia hemerocallidis*, cause of daylily rust, a newly introduced disease in the Americas. Plant Dis. 86:1194-1198.

14. Hill, L., and Hill, N. 1991. Daylilies: The perfect perennial. Storey Communications. Pownal, VT.

15. Hiratsuka, N., and Hasebe, S. 1978. A taxonomic revision of the species of *Puccinia* parasitic on the Liliales (Liliaceae, Amaryllidaceae, Dioscoreaceae and Iridaceae) in the Japanese Archipelago. Rep. Tott. Mycol. Inst. 16:1-36.

16. Hollier, C. A., and King, S. B. 1985. Effect of dew period and temperature on infection of seedling maize plants by *Puccinia polysora*. Plant Dis. 69:219-220

17. Jeffers, S.N., Miller, R.W., and Powell, C.C. 2001. Fungicides for ornamental crops in the nursery. Pages 409-416 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R.K., and Benson, D.M. (Eds.). APS Press, St. Paul, MN.

18. Jones, R. K., Simone, G. W., von Broembsen, S. L., and Dutky, E. 2001. Integrated Disease Management. Pages 376-383 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R. K., Benson, D. M. (Eds.). APS Press, St. Paul, MN.

19. Kramer, C.L., and Eversmeyer, M.G. 1992. Effect of temperature on germination and germ-tube development of *Puccinia recondita* and *P. graminis* urediniospores. Mycol. Res. 96:689-693.

20. Littlefield, L. 1981. Biology of the Plant Rusts. Iowa State University Press. Ames, IA.

21. Martinez, A., Mueller, D., Wise, K., and Buck, J. 2003. Daylily rust survival in homeowner and landscape situations. Georgia Green Industry Assoc. 14:20.

22. McGregor, A.J., and Manners, J.G. 1985. The effect of temperature and light intensity on growth and sporulation of *Puccinia striiformis* on wheat. Plant Pathol. 34:263-271.

23. Morin, L., Brown, J.F., and Auld, B.A. 1992. Effects of environmental factors on teliospore germination, basidiospore production, and infection of *Xanthium occidentale* by *Puccinia xanthii*. Phytopathology 82:1443-1447.

24. Mueller, D.S., Jeffers, S.N., and Buck, J.W. 2004. Effect of timing of fungicide applications on development of rusts on daylily, geranium and sunflower with fungicides. Plant Dis. 88:657-661.

25. Mueller, D.S., Jeffers, S.N., and Buck, J.W. 2004. Toxicity of fungicides to urediniospores of six rust fungi that occur on ornamental crops. Plant Dis. Submitted.

26. Mueller. D.S., and Buck, J.W. Effect of fluorescent light on rusts on daylily, geranium, iris, native azalea, spearmint, and sweet corn. (manuscript in preparation for submission).

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

28. Mueller, D.S., Williams-Woodward, J.L., and Buck, J.W. 2002. Resistance of daylily cultivars to the daylily rust pathogen, *Puccinia hemerocallidis*. HortScience 38:1137-1140.

29. Munson, R.W. Jr. 1989. *Hemerocallis*, The Daylily. Timber Press. Portland, OR.

30. O'Neill, T.M., and Pye, D. 1996. Evaluation of fungicides for control of sweet william rust. Ann. Appl. Biol. (Suppl.) 128:18-19.

31. O'Neill T.M., and Pye, D. 1997. Evaluation of fungicides for control of chrysanthemum white rust. Ann. Appl. Biol. (Suppl.) 130:8-9.

32. Ono, Y. 2003. Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan? J. Gen. Plant Pathol. 69:240-243.

33. Petit, T.L., and Peat, J.P. 2000. The Color Encyclopedia of Daylilies. Timber Press. Portland, OR.

34. Raabe, R.D., and Pyeatt, L. 1990. Control of rust of china aster and comments on the name of the pathogen. J. Environ. Hort. 8:89-92.

35. Robert, C., Bancal, M.-O., and Lannou, C. 2002. Wheat leaf rust uredospore production and carbon and nitrogen export in relation to lesion size and density. Phytopathology 92:762-768.

36. Sache, I. 1997. Effect of density and age of lesions on sporulation capacity and infection efficiency in wheat leaf rust (*Puccinia recondita* f.sp. *tritici*). Plant Pathol. 46:581-589.

37. Sood, P.N., and Wiese, M.V. 1974. Effects of pregermination environments on the germinability of uredospores of two wheat rust fungi. Phytopathology 64:1244-1248.

38. Tapsoba, H., and Wilson, J.P. 1997. Effects of temperature and light on germination of urediniospores of the pearl millet rust pathogen, *Puccinia substriata* var. *indica*. Plant Dis. 81:1049-1052.

39. Tollenaar, H., and Houston, B.R. 1965. Effect of temperature during uredospore production and of light on *in vitro* germination of uredospores from *Puccinia striiformis*. Phytopathology 56:787-790.

40. Tomkins, J.P., Wood, T.C., Barnes, L.S., Westman, A. and Wing, R.A. 2001. Evaluation of genetic variation in the daylily (*Hemerocallis* spp.) using AFLP markers. Theor. Appl. Genet. 102:489-496.

41. United States Department of Agriculture National Agricultural Statistics Service. 2003. Floriculture Crops 2002 Summary. http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/floran03.pdf.

42. United States National Plant Board. 2001. Daylily Rust Pest Alert. U. S. Animal and Plant Health Inspection Services. http://www.aphis.usda.gov/npb/daylily.html.

43. Weber, R.W.S., and Tilston, E.L. 1999. Evaluation of three rust-controlling fungicides for control of daisy rust (*Puccinia distincta*) under greenhouse conditions. Ann. Appl. Biol. (Suppl.) 134:16-17.

44. Williams-Woodward, J.L., and Buck, J.W. 2001. Daylily rust caused by *Puccinia hemerocallidis*: A new disease on daylily in the U.S. Proc. Southern Nursery Assoc. Res. Conf. 46:234-236.

45. Williams-Woodward, J. L., and Buck, J.W. 2002. Diseases and pests of daylily. Pages 222-239 in: New Daylily Handbook. F. Gatlin and J. Brennan, eds. American Hemerocallis Society. Kansas City, MO.

46. 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. 85:1121.

47. Wojdyyla, A.T., and Orlikowski, L.B. 1999. Strobilurin compounds in control of rust, powdery mildew and black spot on some ornamental plants. Mededelingen Faculteit Landbouwkundige En Toegepaste Biologische Wetenschappen Universiteit Gent 64:539-545.

Table 2.1. Common fungicidal chemistries used on ornamental rusts[a]

Fungicide chemical name	Physical mode of action	Category	Chemical mode of action
Azoxystrobin	Protectant +Eradicative	QoI[b]	Inhibits electron transfer complex in mitochondrial ETC halting ATP production
Benodanil	Protectant +Eradicative	Benzanilide	Repiratory enzyme inhibitor
Chlorothalonil	Protectant	Unclassified	Multi-site MOA[e]
Fenarimol	Protectant +Eradicative	SBI-DMI[c]	Binds to C-14 demethylase in sterol biosynthesis
Flutolanil	Protectant +Eradicative	Benzanilide	Respiratory enzyme inhibitor
Maneb	Contact/ Protectant	EBDC[d]	Multi-site MOA
Myclobutanil	Protectant +Eradicative	SBI-DMI	Binds to C-14 demethylase in sterol biosynthesis
Propiconazole	Protectant +Eradicative	SBI-DMI	Binds to C-14 demethylase in sterol biosynthesis
Triadimefon	Protectant +Eradicative	SBI-DMI	Binds to C-14 demethylase in sterol biosynthesis
Zineb	Protectant	EBDC	Multi-site MOA

[a] Information collected from references 3, 11 and 17.
[b] QoI = quinone outside inhibitors.
[c] SBI-DMI = sterol biosynthesis inhibitors—demethylation inhibitor.
[d] EBDC = ethylene bis-dithiocarbamate.
[e] MOA = mode of action.

CHAPTER 3

EFFECTS OF LIGHT, TEMPERATURE, AND HUMIDITY ON PRODUCTION AND VIABILITY OF UREDINIOSPORES OF *PUCCINIA HEMEROCALLIDIS*[2]

[2] Wise, K.A. and Buck, J.W. To be submitted to Plant Disease.

CHAPTER 3

EFFECTS OF LIGHT, TEMPERATURE, AND HUMIDITY ON PRODUCTION AND VIABILITY OF UREDINIOSPORES OF *PUCCINIA HEMEROCALLIDIS*

Daylily (*Hemerocallis* spp.) is a popular herbaceous perennial that is common in landscape settings. In 2002, daylily, along with other herbaceous perennials, was valued at $571 million (14), part of an overall ornamental industry worth over $14.3 billion in the United States (4). The introduction of the rust pathogen *Puccinia hemerocallidis* into the U.S. in 2000 has had a negative impact on the daylily industry (16). Rust is a serious concern on a plant that was previously considered to be relatively pest and disease-free (15). The pathogen was quarantined by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) until January of 2002 (14). Once the USDA deemed the rust endemic in the U.S., focus shifted from eradication to control. Currently the recommended control procedures include integrated management practices that rely heavily on fungicide applications. Fungicides add a considerable cost to production of daylilies, a commodity that was initially very low-input. Also, while resistance to *P. hemerocallidis* has been observed in a small number of daylily cultivars (8), growers and hybridizers are still struggling to find cultivars that are both resistant to disease and commercially popular (1).

Little is known about the biology and epidemiology of *P. hemerocallidis*. The fungus is a heteroecious rust whose alternate host is herbaceous perennials in the genus *Patrinia*. The fungus alternates between hosts in Japan, but there is no documentation of *P. hemerocallidis*

infecting *Patrinia* spp. in the U.S. (9, 15). The fungus is an obligate parasite that does not usually kill the host, but it does diminish plant health and produces yellow/orange pustules that reduce the plant's aesthetic value. In addition to reducing plant vigor, these pustules produce large numbers of urediniospores that are easily wind or splash-dispersed and can cause repeated infection cycles on the infected host or on surrounding daylilies.

Urediniospore germination of other species of *Puccinia* is impacted by relative humidity in conjunction with temperature (3, 11). Light intensity and temperature have been shown to impact urediniospore production in other *Puccinia* species (5, 10). Previous *in vitro* studies on *P. hemerocallidis* indicated that urediniospore germination ranged from 60 to 87% at 16 to 30°C, and germination dropped sharply at temperatures > 30°C. Light was shown to affect *in vitro* urediniospore germination negatively; as light intensity increased, urediniospore germination decreased significantly (7). Percentage germination of urediniospores of *P. hemerocallidis* exposed to high light intensity (608 $\mu mol \cdot s^{-1} \cdot m^{-2}$) from cool-white fluorescent lights for 18 h was significantly lower than that of urediniospores incubated in the dark (6). These studies provide information on the basic biology of the fungus, but the relationship between temperature and light on *P. hemerocallidis* spore production and germination *in vivo* is unknown. Our working hypothesis is that high light intensity (400 to 500 $\mu mol \cdot s^{-1} \cdot m^{-2}$) will reduce urediniospore germination and affect urediniospore production *in vivo*. To test our hypothesis, studies were conducted to determine the effect of: i) light, and ii) temperature and humidity on *in vivo* urediniospore production and viability.

Materials and Methods

Pathogen and plant maintenance. *Puccinia hemerocallidis* was maintained on susceptible daylily stock plants (cv. Pardon Me) in a greenhouse with mean day/night temperatures of

approximately 26/22ºC. Urediniospore inoculum was collected bi-weekly by vacuum and stored dry at 4ºC. Experimental plants of the susceptible cultivar LeeBea Orange Crush (8) were grown in 3.8-L pots in Metro Mix 360 (The Scotts Company, Marysville, OH) and kept in a rust-free greenhouse with mean day/night temperatures of approximately 28/24ºC. Plants were watered as needed and fertilized weekly with Peter's 20-20-20 liquid fertilizer (The Scotts Company).

Plant inoculation. Urediniospores were suspended in a sterile 0.05% Tween 20 solution (J.T. Baker, Phillipsburg, NJ) and filtered through five layers of sterile cheesecloth. Urediniospores were enumerated using a hemacytometer, and the concentration was adjusted to 3 to 4 x 10^5 spores per ml^{-1}. Test plants were watered immediately prior to inoculation without wetting foliage. Plants were sprayed to the point of runoff with the urediniospore suspension. Inoculated plants were immediately placed in plastic bags; the bags were sealed, and placed in the dark at 23°C. Relative humidity was typically $\geq$ 95% in each bag. Plants were removed from bags after 24 h and placed in a greenhouse with mean day/night temperatures of approximately 26/22°C. Plants with actively sporulating lesions (12-14 days after inoculation) were used in all experiments.

Effects of light on spore production and spore germination. Inoculated plants with actively sporulating lesions were placed into Conviron E15 growth chambers (Controlled Environments Inc., Pembina, ND) set at one of the following three light treatments: 24 h of continuous light, 12 h of dark followed by 12 h of light, and 12 h of light followed by 12 h of dark. Growth chambers were set to maintain 22°C. Watchdog dataloggers (Model 150, Spectrum Technologies, Plainfield, IL) were placed at plant level to monitor temperature and humidity levels. Relative humidity ranged from 70 to 80%. A combination of 15 160-W cool-white fluorescent (Osram Sylvania, Danvers, MA) and 13 60-W incandescent (Philips Lighting

Company, Somerset, NJ) light bulbs was used in each growth chamber 80 cm above the growth chamber floor. Photosynthetically active radiation (PAR) was measured using a LI-189 Quantum/Radiometer Photometer with a LI-190SA Quantum sensor (LI-COR, Lincoln, NE). Light intensity at plant level was measured each experiment and ranged from 450 to 500 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Five plants with 6 to 8 fully expanded leaves were used for each treatment. Urediniospores were removed from all leaves in each treatment by vacuum immediately prior to placement in growth chambers. Urediniospores were collected by vacuum from each plant 12 and 24 h after treatments began for the first two experimental runs. In the third run, urediniospores were collected from each plant at 24 h after treatment. Different plants were used for each experimental run.

Urediniospores from each plant surface were collected into separate vials and suspended in 0.5 to 10 ml of 0.05% Tween 20 solution and enumerated with a hemacytometer to determine total urediniospore production per plant. Total numbers of lesions per plant were recorded and urediniospore concentration was divided by lesion number to obtain urediniospore production per lesion. Fifty-microliter aliquots of each urediniospore suspension were also placed onto the surface of potato dextrose agar (PDA) amended with 100 µg of chloramphenicol ml^{-1} (Sigma, St. Louis, MO). Each petri dish was placed into an incubator at 22°C in the dark for 24 h. Plates were then removed and observed microscopically (200 X magnification) for urediniospore germination. Germination was assessed for a minimum of 200 urediniospores per dish. Urediniospores were considered germinated if the germ tube length was at least one-half the diameter of a urediniospore. Percent germination was determined by dividing the number of germinated urediniospores by the total number of urediniospores observed per dish and multiplied by 100. Data for the 12 and 24-h collection periods in the first two experimental runs

were added together to obtain total urediniospore production and percent germination per 24-h period. The experiment was repeated three times, and the three runs were considered replicates in the data analysis.

Effect of temperature and relative humidity on urediniospore production and germination. The experimental design was a split-plot with temperature designated as the main plot and humidity as the sub-plot. Inoculated plants were placed in separate growth chambers set at temperatures of 15, 20, and 30°C. For each temperature, two relative humidity treatments were designated high (90-100%) and low (55-70%). The high relative humidity treatment was achieved by placing five plants with six to eight fully expanded leaves in 34 x 16 x 24 plastic containers (Sterlite, Townsend, MA) to which 800 ml of water were added. Lids were placed tightly on containers. For the low relative humidity treatment, five plants were placed in similar plastic containers with no water added, and lids were placed on container tops, but not sealed tightly. This allowed airflow through the plants, reducing relative humidity. Relative humidity inside the containers was measured with Watchdog dataloggers placed at plant level. Light followed a diurnal cycle, with 12 h of light and 12 h of darkness. Light was of the same quality and intensity as in the previous experiment. Urediniospores were collected as previously described at the same collection times for all plants in each treatment in each trial. Urediniospore concentration and germination as well as, urediniospore production per lesion were assessed as previously described. The experiment was repeated three times, and the three runs were considered replicates in the analysis.

Data analysis. Data from the light experiment were analyzed using a one-way analysis of variance (ANOVA) for a randomized complete block design in the general linear model (GLM) of SAS (SAS Institute, Cary, NC). Means were separated using Fisher's protected least

significant difference test (LSD) with $P = 0.05$. Data from the temperature and humidity experiments were analyzed in PROC GLM using a two-way analysis of variance (ANOVA) for a split-plot design. Means for temperature were separated using Fisher's protected least significant difference test (LSD), and means for humidity were separated by a student's t-test ($P = 0.05$).

Results

Light. Light intensity significantly affected average urediniospore production per lesion (Table 3.1). Significantly more urediniospores were produced in lesions exposed to a continuous light treatment than in lesions exposed to light followed by dark (Fig. 3.1a). However, there were no statistical differences between urediniospore production per lesion in the light followed by dark or dark followed by light treatments. Also, there were no statistical differences between urediniospore production per lesion in the continuous light and dark followed by light treatments. There was no significant effect of light treatments on mean percent germination of urediniospores (Table 3.2, Fig. 3.2b).

Temperature and humidity. Temperature had a significant effect on mean urediniospore production per lesion (Table 3.3). There were no differences in urediniospore production per lesion between 15 and 20°C, but at 30°C, urediniospore production per lesion increased significantly (Fig. 3.2a). Urediniospore production per lesion was significantly higher at the treatment of low relative humidity (55 to 70%) compared with high relative humidity (90 to 100%) (Fig. 3.3a). There was no significant interaction between temperature and humidity on uredinospore production per lesion (Table 3.3).

Temperature and humidity did not have significant effects on mean urediniospore germination (Figs. 3.2b, 3.3b). The interaction between temperature and humidity on mean urediniospore germination was also non-significant (Table 3.4).

Discussion

Abiotic factors such as temperature, light and humidity influence the growth and development of rust fungi (2, 6, 7, 12, 13). In the present study, no effect was observed on germination of urediniospores of *P. hemerocallidis* collected from plants exposed to different light, temperature, and humidity treatments. However, urediniospore production on daylily was affected by these treatments. Continuous light exposure (24 h) significantly increased the number of urediniospores produced per lesion when compared to 12 h of light followed by 12 h of dark. These results are similar to studies on *P. striiformis* where increasing light intensity increased sporulation while lower light levels reduced urediniospore production per pustule and pustules per unit area (5).

A previous in vitro study on *P. hemerocallidis* indicated that exposure of urediniospores to increasing light intensity over an 18-h period reduced urediniospore germination (7). Cool-white fluorescent light at 400 to 600 $\mu mol \cdot s^{-1} \cdot m^{-2}$ had been shown previously to reduce *in vitro* germination of *P. hemerocallidis* urediniospores significantly (6). In comparison, outside readings of PAR for Griffin, GA, on 31 March, and 13 July 2004 taken on clear, sunny days were 599 and 1525 $\mu mol \cdot s^{-1} \cdot m^{-2}$, respectively. In the present study, mean urediniospore germination was not significantly different regardless of light treatment over a 24-h period. This was unexpected, since treatment with similar light intensities (400 to 600 $\mu mol \cdot s^{-1} \cdot m^{-2}$) significantly reduced subsequent *P. hemerocallidis* urediniospore germination in vitro (6). Other

studies on *Puccinia* have shown that light does not negatively affect urediniospore germination over time (2, 12, 13). In fact, high light intensities promote uredinospore germination of *P. striiformis* at optimal temperatures (13). In some cases, urediniospore germination after exposure to continuous light may be initially inhibited, but this inhibition is reversible as length of exposure at a favorable temperature increases (2, 12). Studies on *P. substriata* var. *indica* and *P. striiformis* showed that a 1-h light treatment followed by 1-h of darkness stimulates urediniospore germination (2, 12). In the present study, the lack of inhibition of urediniospore germination by light may reflect the differences in exposure of urediniospores on plant surfaces to high light intensity compared to *in vitro* studies on agar. Shading of lower leaf surfaces by younger leaves and shading of urediniospores within a pustule by other urediniospores could result in differences in light exposure for all urediniospores on the plant. However, this is a more representative picture of how urediniospore germination would be affected by light intensity in a plant production setting.

All temperature treatments included in the present study were within the range of temperatures at which *P. hemerocallidis* has been shown to germinate *in vitro* at levels > 50% (7). *Puccinia hemerocallidis* also has a high infection efficiency at high humidity (7). A 10°C increase in temperature from 20 to 30°C in the present study increased urediniospore production per lesion. However, average germination levels for urediniospores produced at higher temperatures were not statistically different than average germination levels for urediniospores produced at lower temperatures, indicating that after 24 h spore viability at 30°C was equal to spore viability at 20 and 15°C. Temperature and humidity did not interact to influence spore production per lesion, however, lower relative humidity significantly increased urediniospore production per lesion.

The fact that temperature and humidity did not have an impact on urediniospore germination individually or collectively indicates that in future experiments a wider range of temperatures and/or humidities should be tested to determine if there is a more distinct range at which urediniospore germination is affected on live plants. Also, further research should be conducted to determine if urediniospores produced at certain temperatures (e.g., 20°C) are more or less likely to germinate within a range of temperatures (e.g., 15 to 30°C).

An increase in urediniospore production per lesion with temperature was also observed for *P. striiformis* (5). When temperatures were raised to 15° C from the optimum temperature (10° C) for urediniospore germination, spore production increased along with colonization rate (5). It was noted that as temperature increases, leaves senesce faster. The spores produced on senescing leaves may have reduced viability, therefore offsetting the increase in spore production (5). Over time, increased plant transpiration rates at lower humidities and higher temperatures could influence urediniospore production and germination because of leaf senescence. These interactions should be studied to determine how time plays a role in urediniospore production and viability on *P. hemerocallidis*.

Literature Cited

1. Davis, T. 2003. Rust rocks the daylily sector. Nursery Management Pro. 5:60-63.

2. Givan, C.V., and Bromfield, K.R. 1964. Light inhibition of uredospore germination in *Puccinia graminis* var. *tritici*. Phytopathology 54:382-384.

3. Hollier, C.A., and King, S.B. 1985. Effects of temperature and relative humidity on germinability and infectivity of *Puccinia polysora* uredospores. Plant Dis. 69:937-939.

4. Jerardo, A. 2003. Floriculture and Nursery Crops Outlook. USDA Economic Research Service. FLO-02 http://www.ers.usda.gov/publications/flo/sep03/fl02.pdf.

5. McGregor, A.J., and Manners, J.G. 1985. The effect of temperature and light intensity on growth and sporulation of *Puccinia striiformis* on wheat. Plant Pathol. 34:263-271.

6. Mueller, D.S., and Buck, J.W. Effect of fluorescent light on rusts on daylily, geranium, iris, native azalea, spearmint, and sweet corn. (manuscript in preparation for submission).

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

8. Mueller, D.S., Williams-Woodward, J.L., and Buck, J.W. 2002. Resistance of daylily cultivars to the daylily rust pathogen, *Puccinia hemerocallidis*. HortScience 38:1137-1140.

9. Ono, Y. 2003. Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan? J. Gen. Plant. Pathol. 69:240-243.

10. Sache, I., and de Vallavieille-Pope, C. 1993. Comparison of the wheat brown and yellow rusts for monocyclic sporulation and infection processes, and their polycyclic consequences. J. Phytopathology 138:55-65.

11. Sood, P.N., and Wiese, M.V. 1974. Effects of pregermination environments on the germinability of uredospores of two wheat rust fungi. Phytopathol. 64:1244-1248.

12. Tapsoba, H., and Wilson, J.P. 1997. Effects of temperature and light on germination of urediniospores of the pearl millet rust pathogen, *Puccinia substriata* var. *indica*. Plant Dis. 81:1049-1052.

13. Tollenaar, H., and Houston, B.R. 1966. Effect of temperature during uredospore production and of light on in vitro germination of uredospores from *Puccinia striiformis*. Phytopathology 56:787-790.

14. United States Department of Agriculture National Agricultural Statistics Service. 2003. Floriculture Crops 2002 Summary. http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/floran03.pdf.

15. Williams-Woodward, J.L., and Buck, J. W. 2001. Daylily rust caused by *Puccinia hemerocallidis*: A new disease on daylily in the U.S. Proc. Southern Nursery Assoc. Res. Conf. 46:234-236.

16. 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. 85:1121.

Table 3.1. ANOVA table for comparison of light treatments on mean urediniospore production per lesion by *Puccinia hemerocallidis* on daylily

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	589411.02	15.8	0.0126
Treatment[a]	2	303190.1	8.13	0.0390
Error	4	37305.81		

[a] 24 h light, 12 h light followed by 12 h darkness, or 12 h darkness followed by 12 h light.

Table 3.2. ANOVA table for comparison of light treatments on mean urediniospore germination by *Puccinia hemerocallidis*

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	100.34	1.92	0.2602
Treatment[a]	2	68.44	1.31	0.3650
Error	4	52.23		

[a] 24 h light, 12 h light followed by 12 h darkness, or 12 h darkness followed by 12 h light.

Table 3.3. ANOVA table for comparison of temperature and humidity treatments on mean urediniospore production per lesion by *Puccinia hemerocallidis* on daylily

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	50,3501	8.57	0.0175
Temperature[a]	2	30,069	5.12	0.0505
Main-plot error	4	76,138		
Humidity[b] (H)	1	54,736	9.31	0.0225
T*H	2	1,678	0.29	0.7613
Sub-plot error	6	5,878		

[a] Temperature treatments were 15, 20, and 25°C.
[b] Humidity treatments were high (90 to 100%) and low (55 to 70%) relative humidity.

Table 3.4. ANOVA table for comparison of temperature and humidity treatments on mean urediniospore germination by *Puccinia hemerocallidis*

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	197.4	3.59	0.0942
Temperature[a] (T)	2	29.4	0.54	0.6108
Main-plot error	4	105.9		
Humidity[b] (H)	1	108.7	1.98	0.2092
T*H	2	27.3	0.50	0.6317
Sub-plot error	6	54.9		

[a] Temperature treatments were 15, 20, and 25°C.
[b] Humidity treatments were high (90 to 100%) and low (55 to 70%) relative humidity.

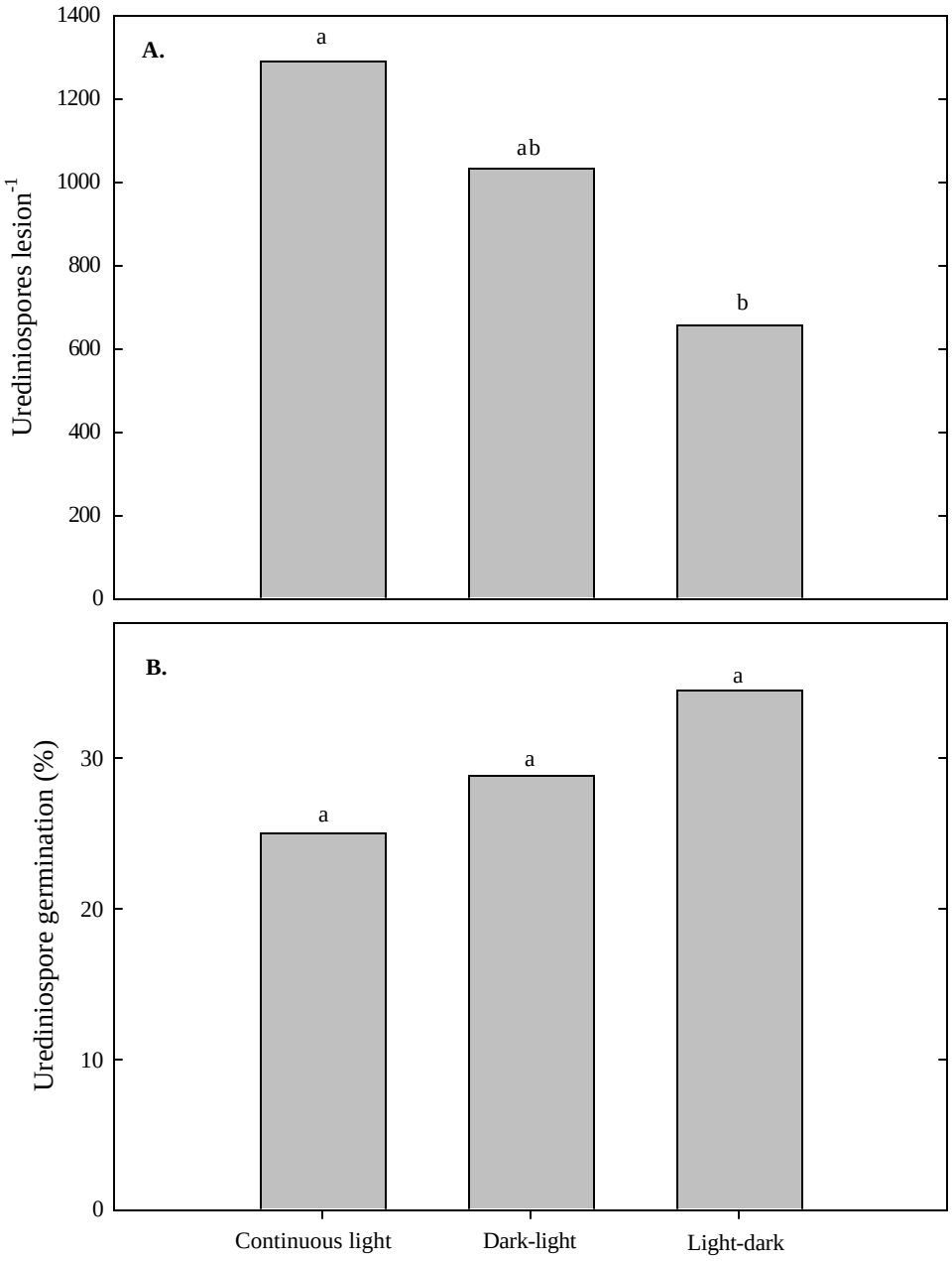

Figure 3.1. Effect of light on mean urediniospore production per lesion (A) and urediniospore germination (B) by *Puccinia hemerocallidis*. Means are based on data averaged across three replications, each with five plants per treatment. Values with the same letter are not significantly different based on Fisher's Protected LSD test ($P = 0.05$).

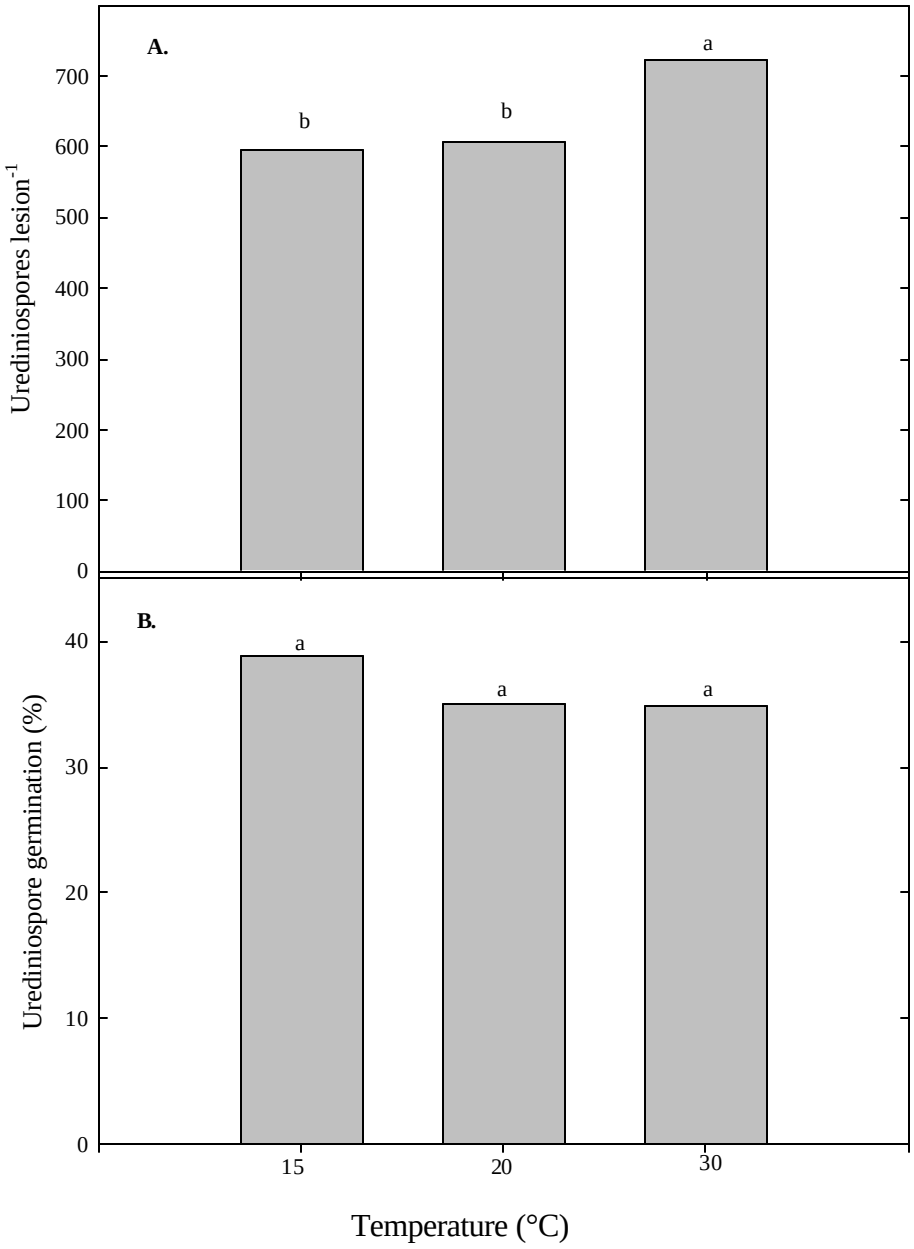

Figure 3.2. Effect of temperature on mean urediniospore production per lesion (A) and urediniospore germination (B) by *Puccinia hemerocallidis*. Means are based on data averaged across three replications, each with five plants per treatment. Values with the same letter are not significantly different based on Fisher's Protected LSD test ($P = 0.05$).

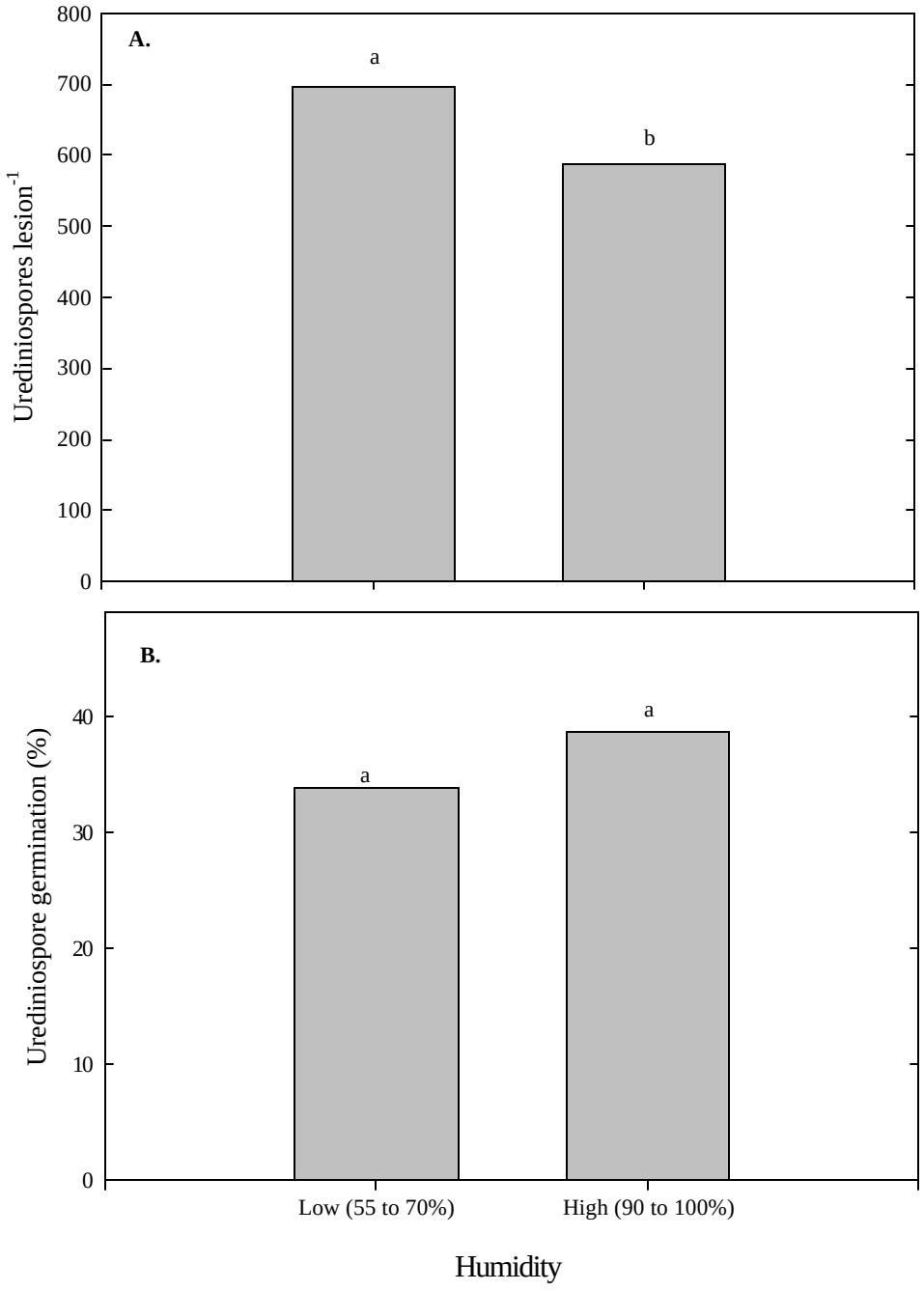

Figure 3.3. Effect of humidity on mean urediniospore production per lesion (A) and urediniospore germination (B) by *Puccinia hemerocallidis*. Means are based on data averaged across three replications, each with five plants per treatment. Values with the same letter are not significantly different based on a student's t-test (P = 0.05).

CHAPTER 4

EFFECT OF FUNGICIDE TIMING ON UREDINIOSPORE PRODUCTION AND VIABILITY OF *PUCCINIA HEMEROCALLIDIS*[3]

[3] Wise, K.A., Scherm, H., and Buck, J.W. To be submitted to Plant Disease.

CHAPTER 4

EFFECT OF FUNGICIDE TIMING ON UREDINIOSPORE PRODUCTION AND VIABILITY OF *PUCCINIA HEMEROCALLIDIS*

Ornamental plant production is a thriving and rapidly expanding industry in the United States. Composed of both nursery and floriculture crops, the value of the ornamental industry in the U.S. has increased in recent years to over $14.3 billion in 2002 (7). Included in ornamental production is daylily (*Hemerocallis* spp.), a popular and low-maintenance plant that along with other herbaceous perennials was valued at over $571 million in 2002 (13).

Daylily and many other ornamental plants are affected negatively by a fungal disease known as rust. Rust has serious implications in the ornamental industry because the primary form of long-distance dispersal of the pathogen is through movement of contaminated or infected plants, and it is difficult to detect rust pathogens on symptomless but infected propagation material entering the country or moving state-to-state (10, 13, 16). Establishment of rust pathogens in ornamental production areas can decrease the aesthetic value of plants due to the presence of visible lesions, and increase production costs through quarantine restrictions and eradication programs (16).

Daylily rust, caused by *Puccinia hemerocallidis* is a fungal disease that has been problematic in ornamental production since its introduction into the U.S. in 2000 (18). The pathogen was initially quarantined in 2001 by the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) (14). The quarantine was lifted in 2002 when

the USDA recognized the pathogen as endemic in the southeastern U.S. Management of rust diseases requires an integrated approach employing sanitation practices, using resistant cultivars when available, and applying fungicide rotations (8). The added expense of chemical applications to a previously low-input ornamental crop has created a need to find efficacious chemicals and accurate fungicide timing intervals.

Initial fungicide recommendations for daylily rust (14) included products efficacious against other rust pathogens (6). Recommended active ingredients include azoxystrobin, chlorothalonil, flutolanil, mancozeb, myclobutanil, propiconazole and triadimefon (14). However, fungicides vary in their efficacy against different ornamental rust pathogens. For example, myclobutanil has eradicative properties when applied post-infection to daisy rust (*P. distincta*), and azoxystrobin, myclobutanil and propiconazole displayed curative properties against chrysanthemum white rust (*P. horiana*) (1, 5, 15, 17). However, myclobutanil and propiconazole, reduced disease but were not as effective as other products against *P. hemerocallidis* in reducing disease development or *in vitro* urediniospore germination (3). Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon are effective at controlling disease development by *P. hemerocallidis* on daylily (measured as total lesion number per plant) when applied up to 10 days prior to inoculation, and azoxystrobin, propiconazole, and tradimefon significantly reduced disease development when applied up to 5 days post-inoculation (10). Chlorothalonil was not effective in reducing disease development when applied after inoculation, and myclobutanil was only effective when applied up to 3 days after inoculation (10).

Azoxystrobin, chlorothalonil, copper sulfate, mancozeb, and trifloxystrobin are all fungicidal to urediniospores of *P. hemerocallidis* (9). Urediniospores did not germinate while

exposed to myclobutanil, propiconazole, and triadimefon, but 40 to 60% of urediniospores germinated after these fungicides were washed off the spores (9). These results suggest that many fungicides are toxic to urediniospores, but *in vivo* testing is necessary to observe fungicide efficacy under natural conditions.

Mueller et al. (10) suggested that the timing of fungicide applications with respect to the infection process of *P. hemerocallidis* will influence fungicide efficacy. During a daylily rust epidemic a continuum of lesion developmental stages (young, non-erumpent to older senescing lesions) will be present at the same time. It is unknown what effect a fungicide application will have on subsequent urediniospore production and viability. The objective of this study was to determine the effect of fungicide applications to lesions at different developmental stages on subsequent urediniospore production and germination of *P. hemerocallidis* on daylily.

Materials and Methods

Pathogen and plant maintenance. *P. hemerocallidis* was maintained on susceptible daylily stock plants (cv. Pardon Me) in a greenhouse with mean day/night temperatures of approximately 26/22°C. Inoculum was collected twice weekly with a vacuum and stored dry at 4°C. Experimental plants of the susceptible daylily cultivar LeeBea Orange Crush were grown in 3.8-liter pots in Metro Mix 360 (The Scotts Company, Marysville, OH), and kept in a rust-free greenhouse with mean day/night temperatures of approximately 28/24°C. Plants were watered as needed and fertilized weekly with Peter's 20-20-20 liquid fertilizer (The Scotts Company).

Plant inoculation. Urediniospores were suspended in a sterile solution of 0.05% Tween 20 (J.T. Baker, Phillipsburg, NJ) and filtered through five layers of sterile cheesecloth. Urediniospores were enumerated using a hemacytometer, and the concentration was adjusted to 3 to 4 x 10^5

spores ml^{-1}. Plants were watered immediately prior to inoculation without wetting foliage. Plants were sprayed to the point of runoff with the urediniospore suspension. Inoculated plants were immediately placed in plastic bags, the bags were sealed, and plants were placed in the dark at 23°C. Relative humidity was typically above 95% in each bag. Plants were removed from bags after 24 h and placed in a greenhouse with mean day/night temperatures of approximately 26/22°C.

Three different stages of lesion development were examined in this experiment. The first stage of lesion development was characterized by presence of yellow lesions on the lower leaf surface, but lesions were not erumpent or sporulating (approximately 5 days after inoculation) (Fig. 4.1). The second stage consisted of yellow/orange erumpent lesions that were just beginning to sporulate (approximately 8 days after inoculation) (Fig. 4.2). The third stage of lesion development involved lesions that were fully erumpent and sporulating profusely (approximately 11 days after inoculation) (Fig. 4.3). To obtain the three stages concurrently prior to fungicide applications, inoculation times were staggered. The first set of plants was inoculated 11 days prior to fungicide application to achieve stage 3 of lesion development at time of treatment. The second set of plants was inoculated 8 days prior to application to obtain plants with stage 2 of lesion development at treatment application, and the third set was inoculated 5 days prior to application for stage 1 lesion development. At each inoculation time a new inoculum suspension was freshly prepared.

Fungicide application. The experiment was designed as a split-plot with three replications. Fungicide active ingredient served as the main plot and lesion development stage as the sub-plot. Each infection stage had five fungicide active ingredients (azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon) along with an unsprayed control was also present

for each of the three infection stages. Fungicides were prepared using formulated commercial products and applied at labeled rates (Table 4.1) with a hand-held sprayer to the point of runoff . A replication was considered to be an infected daylily plant with rust symptoms on six to eight fully expanded leaves. Three separate suspensions of each fungicide were prepared, and one plant of each infection stage was treated with the corresponding fungicide suspension.

At 1, 3 and 7 days after treatment, urediniospores were collected by vacuum from each plant into separate vials using a hand-held vacuum collector. Urediniospores were suspended in 0.5 to 10 ml of 0.05% Tween 20 and enumerated with a hemacytometer to determine total spore production per plant. Fifty-microliters of each spore suspension was placed onto the surface of potato dextrose agar (PDA) amended with 100 µg of chloramphenicol ml^{-1} (Sigma, St. Louis, MO). Urediniospores were incubated at 22°C in the dark for 24 h. Spores were observed microscopically (200 X magnification) and germination was assessed for a minimum of 200 urediniospores per dish. Urediniospores were considered germinated if the germ tube was at least one-half the diameter of the spore. Percent germination was determined by dividing the number of germinated urediniospores by the total number of urediniospores observed per dish and multiplied by 100. Total numbers of lesions per plant were recorded and urediniospore concentration was divided by lesion number to obtain urediniospore production per lesion.

Data analysis. To determine if there were significant differences between the control and fungicide treatments, an initial analysis of variance of urediniospore production per lesion and percent germination with single-degree-of-freedom contrasts was performed using the general linear models procedure (PROC GLM) of SAS (SAS Institute Inc., Cary, NC). In subsequent analyses the data were expressed as a percent of the untreated control for the corresponding lesion development stage and analyzed in PROC GLM using analysis of variance (ANOVA) for

a split-plot design. The analysis was done separately for each assessment date. Treatment means were separated using Fisher's protected least significant difference test (LSD) with P = 0.05.

Results

Urediniospore production per lesion was not significantly different than the control for any collection time (P = 0.09, 0.47, 0.84, respectively). However, germination of urediniospores collected from non-treated control plants was significantly different from all fungicide treatments for the three collection times ($P < 0.0001$; $P = 0.0023$ and; $P < 0.0001$, respectively). Significant interactions between fungicide and lesion development on percent germination were observed 1 and 3 days after fungicide applications (Tables 4.2 and 4.3). The fungicide by infection stage interaction was not significant 7 days after fungicides were applied, although fungicide still had a significant effect on percent germination (Table 4.4). Treatment means for each collection time and infection stage are shown in Figures 4.4 and 4.5. Plants treated at the earliest stage of disease development did not have any sporulating lesions present 1 day after fungicide treatment, therefore no data are shown for percent germination or urediniospores per lesion for this collection time.

All fungicide treatments on lesions in the second stage of disease development resulted in urediniospore germination that was < 45% of the non-treated control 1 day after treatment (Fig.4.4). No germination was observed for urediniospores collected from lesions treated with azoxystrobin and chlorothalonil in stage 2 or stage 3 of lesion development. Triadimefon, myclobutanil, and propiconazole did not negatively affect germination of urediniospores from stage 3 lesions 1 day after treatment application. Three days after fungicide treatment, plants in

the first stage of disease development had developed sporulating lesions, and percent germination of urediniospores collected from all treatments was < 42% of control (Fig. 4.5a). Germination of urediniospores collected from plants treated with chlorothalonil or azoxystrobin was < 2% of the control. Significantly higher germination was observed for urediniospores collected 3 days after fungicide was applied from lesions in the second stage of disease development when treated with myclobutanil (373% of control), propiconazole (369% of control), and triadimefon (100% of control). No germination was observed for urediniospores collected from lesions treated with azoxystrobin or chlorothalonil (Fig. 4.5a). Applications of myclobutanil, propiconazole and triadimefon to actively sporulating lesions in the third stage of disease development did not reduce urediniospore germination below 100% of the control 3 days after application (Fig. 4.5a).

Seven days after treatment, only percent germination of urediniospores collected from azoxystrobin and chlorothalonil treated plants was < 5% of control for all infection stages (Fig. 4.5b). All other treatments with the exception of triadimefon on stage 1 lesions had urediniospore germination that ranged from 60 to 140% of control.

There was a significant treatment by infection stage interaction for urediniospore production per lesion 1 day after fungicide treatment (Table 4.5). A significant fungicide effect on urediniospore production per lesion 1 day after treatment was present at the second stage of disease development (lesions erumpent and just sporulating) (Fig. 4.6a). However, as stated above, urediniospore production per lesion was not significantly different from the control for all treatments. There was no effect of treatment on urediniospores produced per lesion at the third lesion development stage 1 day after treatment (Fig. 4.6b). At 3 and 7 days after treatment, there was no significant interaction between fungicide and lesion development stage (Tables 4.6 and

4.7), and fungicide also had no significant effect on urediniospore production per lesion 3 and 7 days after fungicide application (Fig. 4.7). One day after treatment, no urediniospores were produced from lesions on plants at the earliest stage of infection at the time of fungicide application.

Discussion

The efficacy of fungicide applications on symptomatic plants and sporulating disease lesions has been referred to as postsymptom activity (11). The goal of these postsymptom fungicide applications is usually to reduce inoculum and prevent further crop damage from disease (11). This reduction of inoculum can occur by reducing spore viability or amount of inoculum produced. In this study, efficacy of myclobutanil, propiconazole, and triadimefon on urediniospore germination was significantly affected by postsymptom developmental stage of lesions of *P. hemerocallidis* on daylily. Fungicides applied to non-erumpent lesions reduced urediniospore germination up to 7 days after treatment, with the exception of myclobutanil, which reduced urediniospore germination only until 3 days after application. Myclobutanil, propiconazole, and triadimefon applications to lesions just beginning to sporulate did not affect urediniospore germination negatively. In fact, these fungicides promoted urediniospore germination, and germination percentages were close to or greater than 100% of the control throughout the study. This could possibly be a stress-induced response of the urediniospores to a fungicide application. However, infection efficiency of fungicide-treated urediniospores was not examined in this experiment. Although urediniospores treated with DMI fungicides were able to germinate, how well these urediniospores were able to infect a host subsequently was not

observed. Efficacy of azoxystrobin and chlorothalonil was not affected by stage of lesion development.

One factor that has the potential for reducing disease development is fungicidal inhibition of urediniospore production. Reducing or eliminating urediniospore production at a time when pustules are producing large numbers of urediniospores that are highly infective could prevent rust epidemics. However, five of the fungicides that have been shown to reduce lesion development on *P. hemerocallidis* on daylily (10) did not significantly reduce urediniospore production at any of the three stages of disease development investigated in this study.

Chlorothalonil is a protectant fungicide and had no activity against *P. hemerocallidis* on daylily when applied post-inoculation (10). Propiconazole and triadimefon along with azoxystrobin, were able to reduce lesion development significantly up to 5 days after inoculation, but this is typically before lesions begin sporulating (10). In the present study, chlorothalonil was toxic to urediniospores produced in lesions up to 7 days after fungicide application. Our data suggest that chlorothalonil could slow disease spread by reducing viability of urediniospores produced after fungicide treatment.

Azoxystrobin reduced lesion development of *P. hemerocallidis* on daylily significantly up to 7 days after inoculation (10). In the present study, propiconazole and triadimefon had some initial effect on reducing urediniospore germination of newly sporulating lesions, but no effect on reducing urediniospore germination once plants were actively sporulating. Therefore, azoxystrobin was the only systemic fungicide tested in the current study able to reduce or prevent urediniospore germination on actively sporulating plants.

Although azoxystrobin is very effective against *P. hemerocallidis*, and there have been no reports of fungicide resistance in this pathogen, there are other species of *Puccinia* that have

developed resistance to QoI fungicides, including *P. horiana* (chrysanthemum white rust). Resistance to both QoI (strobilurin) and triazole compounds has been present in populations of *P. horiana* since 2001 (4). Conserving fungicide chemistries that are prone to resistance is the best way to maintain fungicide efficacy against rust pathogens. Fungicide applications to actively sporulating fungi greatly increase the chance of developing fungicide resistance by selecting for resistant genotypes already present in the population (2, 7). A preventative chemical application before disease is visible on new shipments of plants could prevent or delay epidemics in production settings and conserve fungicide chemistries. Chemicals should be applied to actively sporulating plants if absolutely necessary, and disease management should rely on integrated pest management practices to prevent epidemics.

Literature Cited

1. Bonde, M.R., and Rizvi, S.A. 1995. Myclobutanil as a curative agent for chrysanthemum white rust. Plant Dis. 79:500-505.

2. Brent, K.J. 1995. Fungicide resistance in crop pathogens: how can it be managed? Global Crop Protection Federation, Brussels, Belgium.

3. Buck, J.W. and Williams-Woodward, J.L. 2001. In vitro fungicide sensitivity and optimum germination temperature of the daylily rust pathogen, *Puccinia hemerocallidis*. Proc. Southern Nursery Assoc. Res. Conf. 46:237-241.

4. Cook, R.T.A. 2001. First report in England of changes in the susceptibility of *Puccinia horiana*, the cause of chrysanthemum white rust, to triazole and strobilurin fungicides. Plant Pathol. 50:792.

5. Dickens, J.S.W. 1990. Studies on the chemical control of chrysanthemum white rust caused by *Puccinia horiana.* Plant Pathol. 39:434-442.

6. Jeffers, S.N., Miller, R.W., and Powell, C.C. 2001. Fungicides for ornamental crops in the nursery. Pages 409-416 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R.K., and Benson, D.M. (Eds.). APS Press, St. Paul, MN.

7. Jerardo, A. 2003. Floriculture and Nursery Crops Outlook. USDA Economic Research Service. FLO-02 http://www.ers.usda.gov/publications/flo/sep03/fl02.pdf.

8. Jones, R.K., Simone, G.W., von Broembson, S.L., and Dutky, E. 2001. Integrated disease management. Pages 376-383 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R.K., Benson, D.M. (Eds.). APS Press, St. Paul, MN.

9. Mueller, D.S., Jeffers, S.N., Buck, J.W. Toxicity of fungicides to urediniospores of six rust fungi that occur on ornamental crops. (in preparation for submission to Plant Dis.).

10. Mueller, D.S., Jeffers, S.N., Buck, J.W. 2004. Effect of timing of fungicide applications on development of rusts on daylily, geranium, and sunflower. Plant Dis. 88:657-661.

11. Szkolnik, M. 1981. Physical modes of action of sterol-inhibiting fungicides against apple diseases. Plant Dis. 65:981-985.

12. Stebbins, T., and Johnson, D. 2001. Regulatory control. Pages 457-458 in: Diseases of Woody Ornamentals and Trees in Nurseries. Jones, R. K., Benson, D. M. (Eds.). APS Press, St. Paul, MN.

13. United States Department of Agriculture National Agricultural Statistics Service. 2003. Floriculture Crops 2002 Summary. http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/floran03.pdf.

14. United States National Plant Board. 2001. Daylily rust pest alert. U. S. Animal and Plant Health Inspection Services. http://www.aphis.usda.gov/npb/daylily.html.

15. Weber, R.W.S., and Tilston, E.L. 1999. Evaluation of three rust-controlling fungicides for control of daisy rust (*Puccinia distincta*) under greenhouse conditions. Ann. Appl. Biol. (Suppl.) 134:16-17.

16. Wise, K.A., Mueller, D.S., and Buck, J.W. 2004. Quarantines and ornamental rusts. APSnet February feature article. http://www.apsnet.org/online/feature/.

17. Wojdyyla, A.T., and Orlikowski, L.B. 1999. Strobilurin compounds in control of rust, powdery mildew and black spot on some ornamental plants. Mededelingen Faculteit Landbouwkundige En Toegepaste Biologische Wetenschappen Universiteit Gent 64:539-545.

18. Williams-Woodward, J.L., and Buck, J.W. 2001. Daylily rust caused by *Puccinia hemerocallidis*: A new disease on daylily in the U.S. Proc. Southern Nursery Assoc.Res. Conf. 46:234-236.

Table 4.1. Sources of fungicide active ingredients and rates used in experiments on daylily plants inoculated with *Puccinia hemerocallidis*[a]

Active ingredient	Trade name	Source	Formulation[b]	Rate of product/liter	Category
Azoxystrobin	Heritage	Syngenta Crop Protection, Inc., Greensboro, NC	50 WDG	300 mg	QoI[c]
Chlorothalonil	Daconil Ultrex	Syngenta Crop Protection, Inc., Greensboro, NC	82.5 WDG	1700 mg	Broad-spectrum protectant
Myclobutanil	Systhane	Dow AgroSciences LLC, Indianapolis, IN	40 WSP	300 mg	SBI-DMI[d]
Propiconazole	Banner Maxx	Syngenta Crop Protection, Inc., Greensboro, NC	1.3 EC	400µl	SBI-DMI
Triadimefon	Strike	Olympic Horticultural Products Co., Mainland, PA	50 WDG	300 mg	SBI-DMI

[a] Based on Jeffers et al. (5).
[b] Percentages of active ingredients in commercial products formulated as water dispersible granules (WDG), wettable powders (WSP), or emulsifiable concentrate (EC).
[c] QoI = quinone outside inhibitor.
[d] SBI-DMI = sterol biosynthesis inhibitors—demethylation inhibitor.

Table 4.2. ANOVA table for comparison of fungicide treatments on urediniospore germination by *Puccinia hemerocallidis* 1 day after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	624	1.49	0.2502
Fungicide[a] (F)	4	6,787	16.15	< 0.0001
Main-plot error	8	130.8		
Stage[b] (S)	2	20,859	49.64	< 0.0001
F*S	8	3,570	8.50	< 0.0001
Sub-plot error	20	420.2		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Table 4.3. ANOVA table for comparison of fungicide treatments on urediniospore germination by *Puccinia hemerocallidis* 3 days after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	17775.55	4.98	0.0183
Fungicide[a] (F)	4	73914.39	20.70	< 0.0001
Main-plot error	8	5214.39	1.46	0.2360
Stage[b] (S)	2	89047.99	22.16	< 0.0001
F*S	8	79134.77	7.04	0.0002
Sub-plot error	19	25142.64		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Table 4.4. ANOVA table for comparison of fungicide treatments on urediniospore germination by *Puccinia hemerocallidis* 7 days after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	851.85	1.23	0.3140
Fungicide[a] (F)	4	23639.41	34.08	< 0.0001
Main-plot error	8	278.08	0.40	0.9069
Stage[b] (S)	2	2897.10	4.18	0.0305
F*S	8	1058.92	1.53	0.2100
Sub-plot error	20	693.55		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Table 4.5. ANOVA table for comparison of fungicide treatments on urediniospore production by *Puccinia hemerocallidis* per lesion 1 day after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	568.75	0.30	0.7438
Fungicide[a] (F)	4	6170.79	3.26	0.0327
Main-plot error	8	1535.66	0.81	0.6010
Stage[b] (S)	2	25514.23	13.48	0.0002
F*S	8	6786.45	3.59	0.0097
Sub-plot error	20	1892.88		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Table 4.6. ANOVA table for comparison of fungicide treatments on urediniospore production per lesion by *Puccinia hemerocallidis* 3 days after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	76881.19	0.94	0.4069
Fungicide[a] (F)	4	25403.82	0.31	0.8666
Main-plot error	8	58702.23	0.72	0.6721
Stage[b] (S)	2	202213.49	2.48	0.1104
F*S	8	55673.19	0.68	0.7014
Sub-plot error	19	81527.81		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Table 4.7. ANOVA table for comparison of fungicide treatments on urediniospore production per lesion by *Puccinia hemerocallidis* 7 days after fungicide application

Source	d.f.	Mean square	*F* value	*P* value
Replication	2	24627.10	2.40	0.1166
Fungicide[a] (F)	4	1473.16	0.14	0.9638
Main-plot error	8	6242.74	0.61	0.7609
Stage[b] (S)	2	25170.36	2.45	0.1118
F*S	8	9089.05	0.88	0.5461
Sub-plot error	20	10273.37		

[a] Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon. See Table 4.1 for formulations and rates.
[b] Rust lesion development stages 1 through 3 as illustrated in Figs. 4.1 through 4.3.

Figure 4.1. Lesions on daylily leaves caused by *Puccinia hemerocallidis* at the first stage of development. Lesions are not erumpent or sporulating.

Figure 4.2. Lesions on daylily leaves caused by *Puccinia hemerocallidis* at the second stage of disease development. Lesions are erumpent and just beginning to sporulate.

Figure 4.3. Daylily rust lesions on daylily leaves caused by *Puccinia hemerocallidis* in the third stage of development. Lesions are sporulating abundantly.

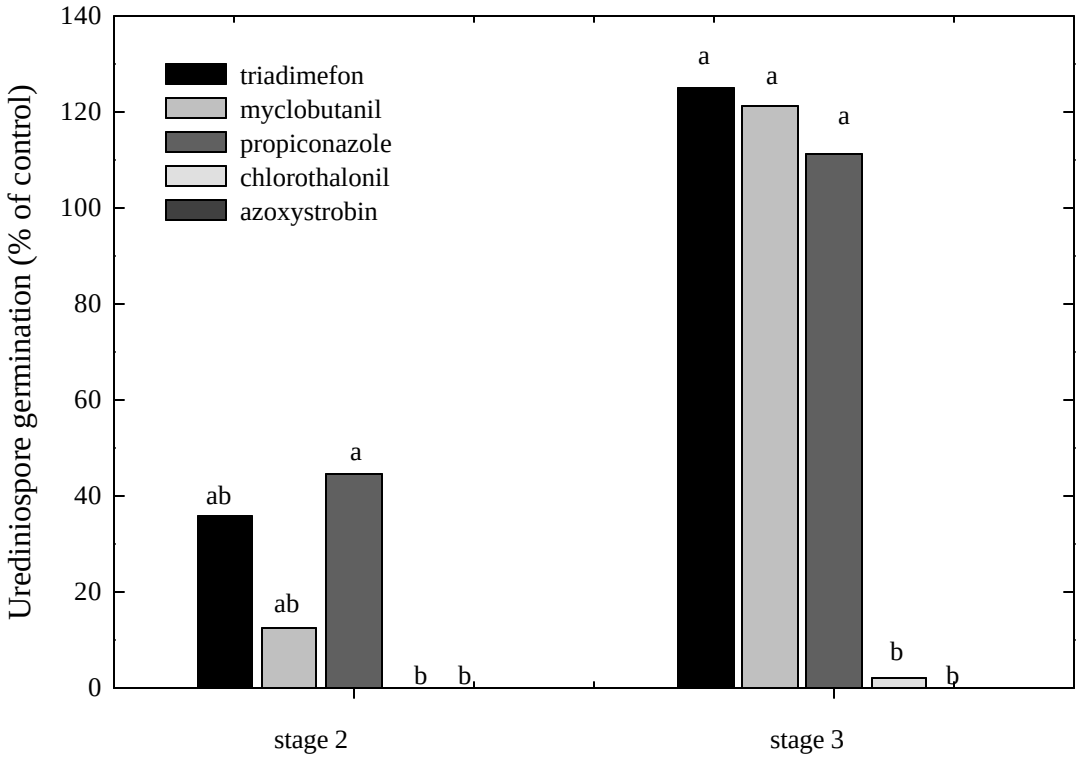

Figure 4.4. Effect of fungicide application on urediniospore germination of *Puccinia hemerocallidis* at stages 2 and 3 of lesion development 1 day after treatment application to infected daylily plants. Germination is shown as a percent of control for each infection stage. No sporulating lesions were present at stage 1 of lesion development (lesions present but not erumpent). At stage 2 of development, lesions were erumpent and just beginning to sporulate. Lesions at stage 3 of development sporulated abundantly. Values with the same letter are not significantly different within each lesion development stage based on Fisher's Protected LSD test (P = 0.05).

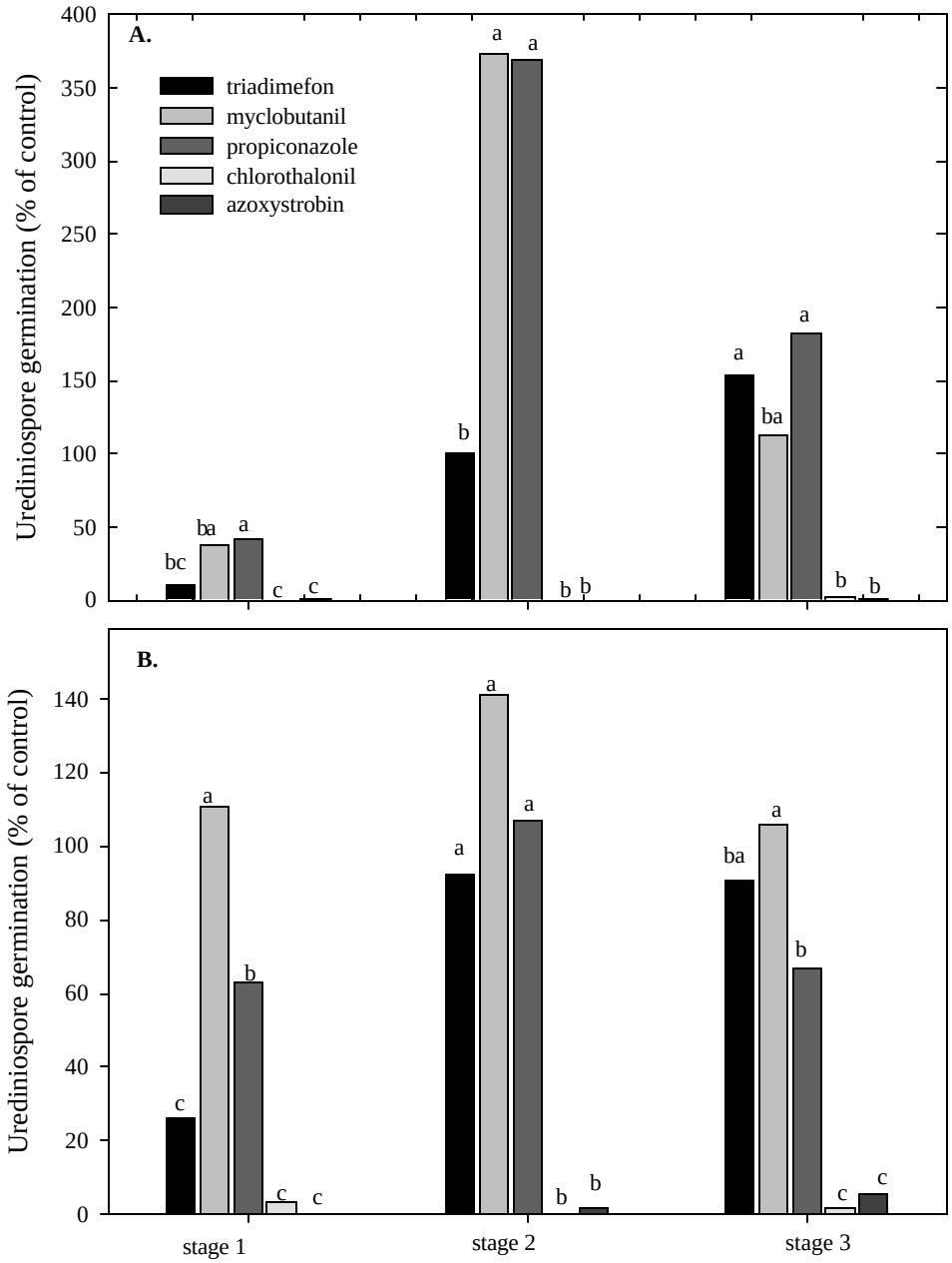

Figure 4.5. Effect of fungicide application on urediniospore germination of *Puccinia hemerocallidis* at stages 1, 2 and 3 of disease development 3 (A) and 7 (B) days after treatment application to infected daylily plants. Germination is shown as a percent of control for each lesion development stage. At stage 1 of lesion development lesions are present but not erumpent. Lesions at stage 2 of development were erumpent and just beginning to sporulate. Lesions at stage 3 of development sporulated abundantly. Values with the same letter are not significantly different within each lesion development stage based on Fisher's Protected LSD test ($P = 0.05$).

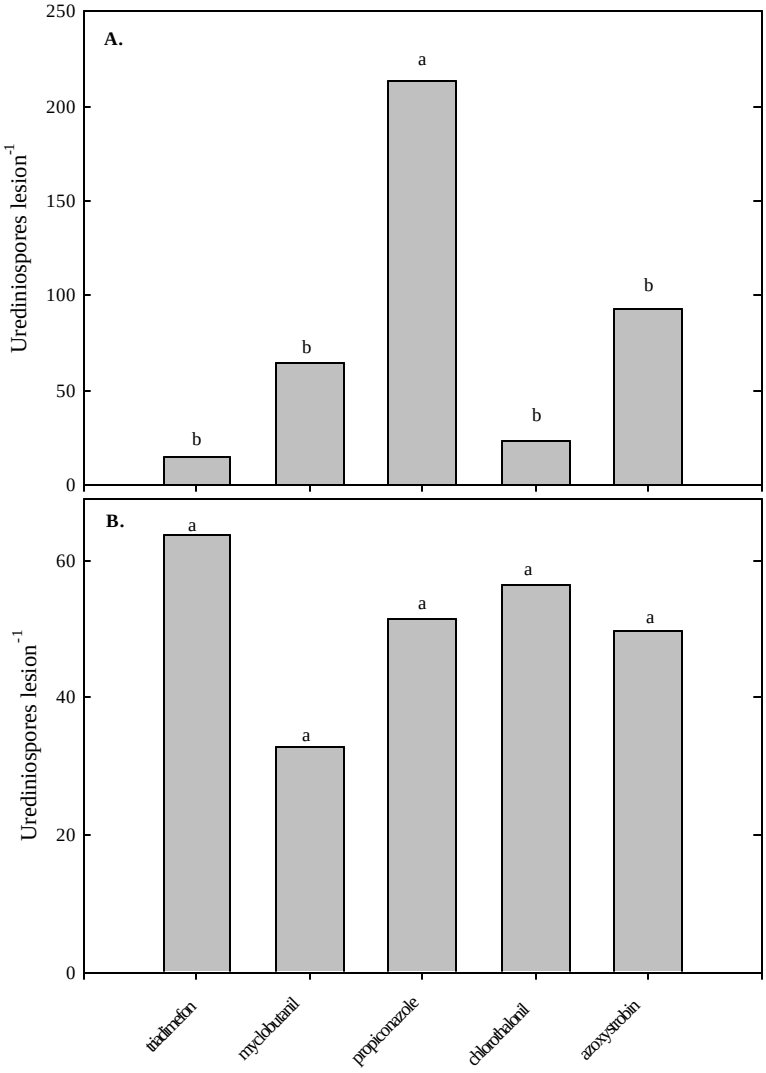

Figure 4.6. Effect of fungicide application at stage 2 (A) and 3 (B) of lesion development on urediniospore production per lesion by *Puccinia hemerocallidis* 1 day after application to infected daylily plants. Values are shown as a percent of control. Lesions at stage 2 of development were erumpent and just beginning to sporulate. Lesions at stage 3 of development sporulated abundantly. Values with the same letter are not significantly different within each lesion development stage based on Fisher's Protected LSD test ($P = 0.05$).

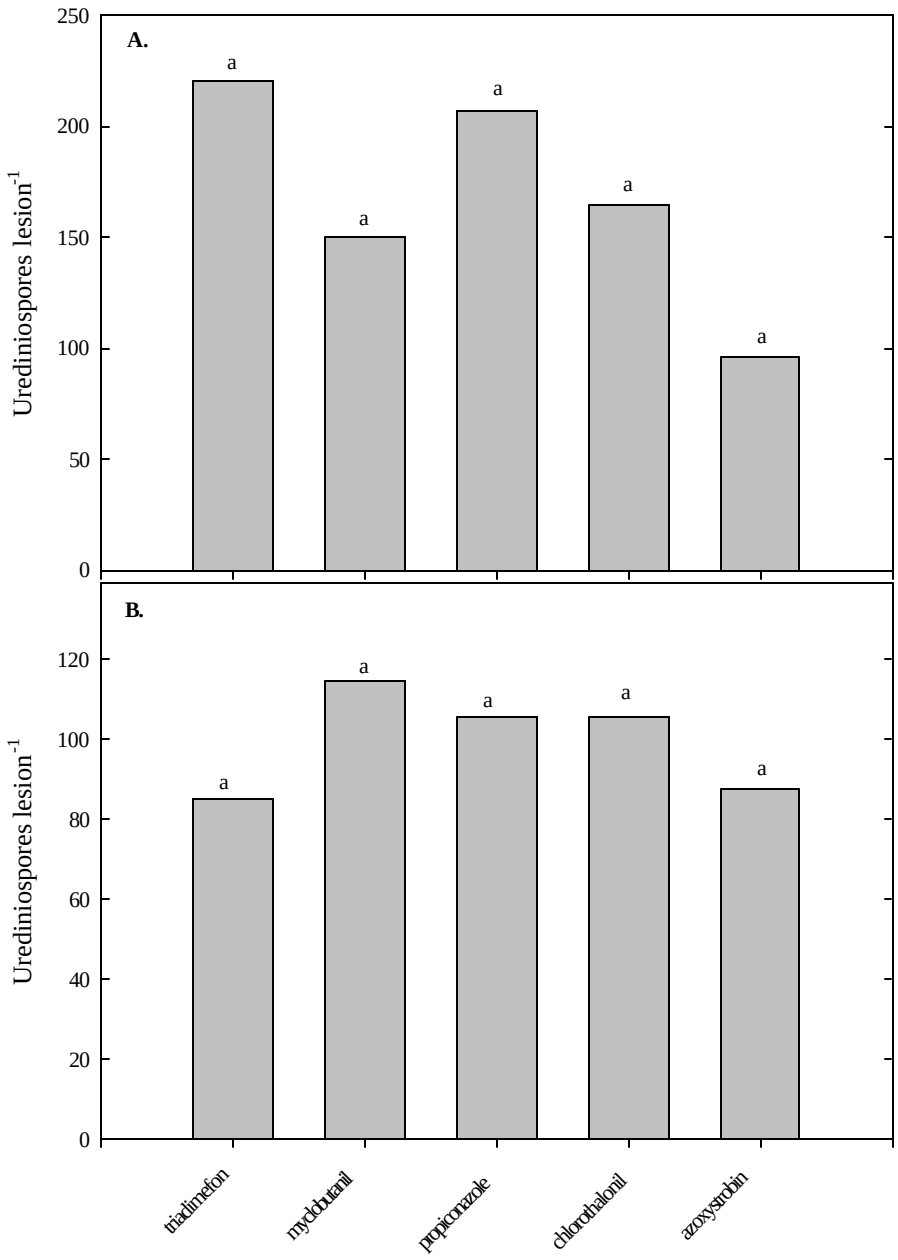

Figure 4.7. Effect of fungicide application on lesion development on urediniospore production per lesion by *Puccinia hemerocallidis* days 3 (A) and 7 (B) after application to infected daylily plants. Values are shown as a percent of control. Values with the same letter are not significantly different within each lesion development stage based on Fisher's Protected LSD test ($P = 0.05$).